UNDERWATER AND HYPERBARIC PHYSIOLOGY IX

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UNDERWATER AND HYPERBARIC PHYSIOLOGY IX

PROCEEDINGS OF THE NINTH INTERNATIONAL SYMPOSIUM ON UNDERWATER AND HYPERBARIC PHYSIOLOGY

Sponsored by

The Undersea Medical Society
The U.S. Office of Naval Research
The U.S. National Oceanic and Atmospheric Administration
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Frontispiece: Kenshun Sato, President of Asahi Seuken, at the age of 18.

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ACKNOWLEDGMENTS

The success in organizing the Ninth Symposium was in a major way attributed to the diligent work and contributions of the Japanese Society of Hyperbaric Medicine, but more especially to the support provided by Professor Kinsaku Sakakibara and Dr. Hideyo Takahashi, both of Nagoya School of Medicine. It was their unselfish dedication to this effort that sustained our efforts in the planning and the final culmination of a successful symposium, miles from the Society's office.

We particularly wish to thank Admiral Motohiko Matsuda, MC, JN (ret.), Tokyo; Dr. Christian Lambertsen, Philadelphia; Dr. Suk Ki Hong, Buffalo; and Dr. George Hart, Long Beach, for their technical advice and suggestions during the formative stages of meeting preparations. The staff of the UHMS, guided initially by Dr. C.W. Shilling and later by Ms. Jane Artigliere, are gratefully acknowledged. It was Ms. Artigliere's devoted attention to the details of meeting management that helped to free the Program Chairman and the Society's officers from the concerns for the timely coordination of all meeting functions.

All of the detailed work of editing and managing preparation of the proceedings has been diligently carried out by Ms. Ann Barker, while the difficult task of setting the type has been done by Mrs. Ellen Mahajan. For this devoted work on the manuscript requiring such close attention to detail, we are deeply indebted.

Finally we wish to offer our sincere thanks to Dr. Mark Bradley, President (1986), for his encouragement, advice, and support. His interest and support were essential.

This brief acknowledgment cannot recognize by name the large number of individuals who contributed to this meeting; our appreciation to all who assisted is gratefully acknowledged.

PREFACE

This compendium of papers presented at the Ninth International Symposium on Underseas and Hyperbaric Physiology comprises a broadened spectrum of interest which follows the natural evolution of the diving and hyperbaric medicine community worldwide. The name of the symposium was modified to reflect the extension of high pressure physiology and medicine from the undersea environment to dry chamber environments of high oxygen partial pressure involved in wound healing, therapy of osteomyelitis, burns, and other medical disorders. Clinical papers were encouraged in both diving medicine and hyperbaric oxygen therapy, and chronic effects of diving were addressed. At the same time, the basic physiology traditional to this symposium was maintained to provide a basic forum for scientists throughout the world to communicate their research.

In seeking a location for the Ninth Symposium, it became apparent that our Far East colleagues, especially in Japan, represented a vital and important group of scientists and physicians with whom closer communication was needed. The well-established history of breath-hold diving research, the large national program in medical hyperbaric oxygen therapy (137,000 treatments in 1985, 158 hospital facilities), and the commitments to ongoing research in diving and hyperbaric medicine all favored Japan as the choice for the Ninth Symposium. It was clear as the Symposium proceeded that the choice was well made. Our host, the Japanese Society for Hyperbaric Medicine (JSHM), under the excellent guidance of Professor K. Sakakibara, proved to be a catalyst needed to make the Symposium successful. Support from the JSHM was beyond expectations. The excellent social activities, the precise operation of the program, and the unseen but ever-present work of the JSHM organizing committee produced an excellent meeting.

The proceedings begin with Admiral Matsuda's foreword derived from his keynote address. The theme is well established even at the beginning—combining the sciences of undersea and hyperbaric physiology to enhance knowledge and improve applications of pressure and oxygen in both operational and clinical settings.

The papers herein bespeak the state of the art in all aspects of undersea and hyperbaric medicine and physiology. It is our hope that these data are quickly superceded by new knowledge, but while we wait, the proceedings provide an accurate assessment of our science during this brief interval in the history of undersea and hyperbaric science.

Alfred A. Bove M.D., Ph.D.
Arthur J. Bachrach, Ph.D.
Leon J. Greenbaum, Jr., Ph.D.

Previously published symposia in this series include (sponsored by the University of Pennsylvania and the Office of Naval Research): Proceedings of the Underwater Physiology Symposium, National Academy of Sciences—National Research Council, Washington, D.C., 1955; Proceedings of the Second Symposium on Underwater Physiology, National Academy of Sciences—National Research Council, Washington, D.C., 1963; Underwater Physiology: Proceedings of the Third Symposium on Underwater Physiology. Williams & Wilkins, Baltimore, Maryland, 1966; Underwater Physiology: Proceedings of the Fourth Symposium on Underwater Physiology, Academic Press, New York, 1971, Sponsorship of the Fourth Symposium was joined by the National Oceanic and Atmospheric Administration and the Undersea Medical Society. Underwater Physiology V: Proceedings of the Fifth Symposium on Underwater Physiology, Federation of American Societies for Experimental Biology, Bethesda, Maryland, 1976, and Underwater Physiology VI, Proceedings of the Sixth Symposium on Underwater Physiology, Federation of American Societies for Experimental Biology, Bethesda, Maryland, 1978, were sponsored by the University of Pennsylvania, the Office of Naval Research, the Undersea Medical Society, and the National Oceanic and Atmospheric Administration. The publication of the Proceedings of the Sixth Symposium represented the transfer of direct responsibility for the series from the University of Pennsylvania to the Undersea Medical Society. Underwater Physiology VII: Proceedings of the Seventh Symposium on Underwater Physiology, was planned and published by the Undersea Medical Society in 1981 at the request of the sponsors: the University of Pennsylvania, the Office of Naval Research, the Undersea Medical Society, and the National Oceanic and Atmospheric Administration. Underwater Physiology VIII: Proceedings of the Eighth Symposium on Underwater Physiology was planned and published by the Undersea and Hyperbaric Medical Society in 1983 and 1984, respectively, and sponsored by the University of Pennsylvania, the Undersea Medical Society, Inc., the U.S. Office of Naval Research, and the U.S. National Oceanic and Atmospheric Administration.

FOREWORD

An Overview of Diving, Hyperbaric Physiology, and Medicine in Japan[1]

The 9th International Symposium on Underwater and Hyperbaric Physiology opened on 16 September 1986. It was my great honor and pleasure to be selected as the keynote speaker. Since this international symposium, which has a history of more than 30 years, is being held for the first time in the Asian region, I would like to briefly review the history of this symposium and of diving and hyperbaric medicine in Japan.

The First International Symposium on Underwater and Hyperbaric Physiology was held in Washington, DC, 10-11 January 1955, and was chaired by Dr. C. J. Lambertsen. Dr. Lambertsen, Captain A. R. Behnke, and Dr. Hermann Rahn presided over the sessions on Decompression and Bends, General Respiratory Problems, and Oxygen Toxicity, respectively.

The chief editor of the proceedings was Loyal G. Goff, while the sponsors were the Office of Naval Research (ONR), the Panel on Underwater Swimmers (Chairman: Dr. C. J. Lambertsen), the Committee on Underwater Warfare (Chairman: Dr. Eric A. Walker) of the National Academy of Sciences/National Research Council (NAS/NRC).

The Second Symposium was held in Washington, DC, 25-26 February 1963, and the chairman of the planning group was Dr. Lambertsen, who, with Leon J. Greenbaum, Jr., Ph.D., edited the proceedings. The ONR and Mine Advisory Committee of the NAS/NRC sponsored the symposium.

The Third Symposium was also held in Washington, DC, 23-25 March 1966. Dr. Lambertsen again chaired the Planning Group and edited the

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[1]Adapted from Dr. Matsuda's Keynote Address at the Opening Plenary Session of the 9th International Symposium on Underwater and Hyperbaric Physiology, held 16-20 September 1986, Kobe, Japan.

proceedings, which were published in 1967. Sponsors were the Committee on Undersea Warfare of the NAS/NRC and the ONR. The session on Recent Naval Experiences in Extending Useful Diving Depths was organized, during which the state of the navy of each participant country was reported. Lt. Kunihiko Uchida, MC, JMSDF, reported "Recent Underwater Physiological Experiences in the Japanese Navy."

The Fourth Symposium was held in Philadelphia in 1969, and the chairman of the Planning Group was Dr. Lambertsen. The contents of the symposium became more substantial and the membership of the Advisory Group became more international. Dr. Lambertsen edited the proceedings, and the sponsors were the University of Pennsylvania, the ONR, and the Undersea Medical Society (UMS). Academic Press published the proceedings in New York and London in 1971.

The Fifth Symposium was held at Freeport in the Bahama Islands in 1972. The chairman of the Planning Committee was Dr. Lambertsen. In addition, the first business meeting of the European Undersea Biomedical Society was conducted at this symposium. Dr. Lambertsen edited the proceedings, and the sponsors were the University of Pennsylvania, the UMS, the ONR, and the National Oceanic and Atmospheric Administration (NOAA). The Federation of American Societies for Experimental Biology published the proceedings in 1976.

The Sixth Symposium was held in Coronado, California, 6-10 July 1975, and Dr. Lambertsen held the chairmanship of the Planning and Editorial Group. The results were presented on the 60-m simulated dive of the *Seatopia* series, which is one of the cooperative programs between the Japan Marine Science and Technology Center (JAMSTEC) and the University of Hawaii. The editors of the proceedings were Dr. C. W. Shilling and Ms. M. W. Beckett. The sponsors were the University of Pennsylvania, the UMS, the ONR, and the NOAA.

The Seventh symposium was held in Athens, Greece, 6-9 July 1980. The chairman of the Governing Board was Dr. A. J. Bachrach. This symposium was organized as a satellite symposium for the 1980 Congress of the International Union of Physiological Sciences, held in Budapest, Hungary, in the same year. At this symposium, the results of the 300-m simulated dive of the *Seadragon* series were reported by JAMSTEC through the panel on Diving Physiology and Technology of the Marine Resources Engineering Coordinating Committee (MRECC), which is the United States-Japan Cooperative Program on Natural Resources (USJNR). This 300-m simulated dive was a cooperative research program conducted by JAMSTEC, the State University of New York at Buffalo, and the University of Hawaii. The proceedings were published in 1981 by the Undersea Medical Society, and were edited by Dr. A. J. Bachrach and Ms. M. M. Matzen. The sponsors were the University of Pennsylvania, the UMS, the ONR, and the NOAA.

The Eighth Symposium was held in St. Jovite, Quebec, 15-19 June 1983, and the chairman of the Governing Board was Dr. John M. Hallenbeck. The proceedings, edited by Dr. Bachrach and Ms. Matzen, were published in 1984.

The sponsors were the University of Pennsylvania, the UMS, the ONR, and the NOAA.

The Ninth Symposium opened on 16 September 1986, for 5 days, under the new name of the 9th International Symposium on Underwater and Hyperbaric Physiology. The chairman of the Governing Board was Dr. Alfred A. Bove. An HBO session and a clinical diving medicine session were newly added. The symposium was sponsored by the UMS, the ONR, the NOAA, the Naval Medical Research and Development Command (NMRDC), the U.S. Air Force School of Aerospace Medicine, the University of Pennsylvania, and the Japanese Society of Hyperbaric Medicine, which extended its cooperation to this symposium in the role of the Local Organizing Committee and national host, as well as cosponsor.

The symposiums from the first to the sixth were all organized in the United States. The seventh was held in Greece and the eighth in Canada. More than 30 years have passed since the first symposium to the current ninth.

The first symposium was held in 1955, 10 years after World War II. Around that time, the technology for underwater activities expanded from primarily military purposes to civilian applications. Diving technology was being developed for use in deep waters as well as in shallow waters. However, since systematic diving technology and scientific data were not available at that time, the main topics for discussion at the symposium involved the physiological results recognized at that time, a consensus on the direction to be taken in solving the various physiologic problems, encouragement of research activities related to these problems, and research proposals concerning underwater technology.

It was reported that several people from the universities, industry, and from both Canada and the United Kingdom participated. Drs. Lambertsen, Behnke, Rahn, Lanphier, and others had enthusiastic discussions.

At the second symposium, information was exchanged not only among the U.S. Navy, universities, and industry, but also among the participants from the United Kingdom, France, Sweden, and Switzerland. In this way, internationalization was making progress. The sponsors for the first, second, and third symposiums were the Committee on Undersea Warfare of the NAS/NRC and the ONR. The founder of the symposium series and the chairman of the Planning Committee for the first to the sixth symposiums was Dr. C. J. Lambertsen. An Advisory Group was organized which became the International Symposium. Over the years, symposium sponsorship has shifted from primarily military support to a mix of military and civilian sponsorship, and reflects the growing importance of nonmilitary diving.

DIVING, HYPERBARIC PHYSIOLOGY, AND MEDICINE IN JAPAN

The characteristic diving in Japan is breath-hold diving which has been practiced since ancient times. Japan is the land of the Ama divers who have been in existence for at least 2000 years. The First International Symposium on Breath-Hold Diving and the Ama of Japan was held in Tokyo in 1965, under

the leadership of Professor H. Rahn of Buffalo and Professor T. Yokoyama of Keio University, Tokyo. Even today, approximately 15,000 male and female divers are engaged in this type of diving in Japan, harvesting abalone, shellfish, seaweed, and other marine resources. Many Japanese scientists have conducted extensive research on these divers in the past and have contributed much to the understanding of the various physiological problems associated with breath-hold diving. Since all the important findings from these studies are already published, I will not spend time on this subject, except to point out the great contribution made by Professor Gito Teruoka.

More than 50 years ago (1932), Professor Teruoka published a paper entitled "Die Ama und ihre Arbeit" in *Arbeitsphysiologie*, which not only described a most unusual working environment, but also provided the first description and the first careful measurements of the various aspects of breath-holding operations, including the instantaneous velocities of ascent and descent, and the pattern of alveolar gas exchange. Unquestionably, this paper is a classic in the field of diving physiology and stimulated many physiologists, including a young American student named Hermann Rahn, who decided to come to Japan 30 years later to conduct studies on these divers, thus resurrecting our interest in breath-hold diving.

The history of modern diving began around 1857 during construction of the docks in Nagasaki Harbor. The technology was imported from Great Britain. Ten years later, in 1867, Mr. M. Masuda, who had just returned from The Netherlands, carried out repairs to the hull of H.M.S. *Harachy* (Great Britain) by diving under the ship with the use of a "helmet." This was the first time for such an operation in Japan.

In 1872, the development of diving apparatus was begun by the Ship Bureau of the Japanese Imperial Navy. However, satisfactory results could not be obtained and nothing was put to practical use. In 1913, the Mask Diving Apparatus developed by Mr. I. Ogushi was completed. In 1924, Mr. Y. Kataoka succeeded in salvaging gold ingots from the sunken ship *Yahata Maru* from a depth of 70 m by using the Mask Diving Apparatus. In 1933, Mr. K. Asari developed a new apparatus, i.e., an air-storage device installed in the mask. This apparatus became the Asahi Senken Underwater Breathing Apparatus. In 1937, an Open-Circuit Breathing Apparatus carrying a compressed air cylinder (or scuba) was completed.

Along with the development of the diving technology, medical problems, in particular the prevention and medical treatment of decompression sickness, began to emerge.

According to "History of Hyperbaric Medicine Research in Japan," by Professor H. Kita, research carried out before World War II involved physiological studies on breath-hold divers, and the prevention and medical treatment of decompression sickness related to helmet and mask dives. In addition, prevention and medical treatment of caisson disease developed in the period 1900 to 1910 (the late Meiji era), during bridge construction across the Oryokjuko River which runs between the Korean Peninsula and the Chinese mainland, and in the period 1920 to 1926 (the late Taisho era), during bridge

construction across the Sumida River in Tokyo. In the Showa era, an epidemiologic study of decompression sickness caused by working in compressed air for the excavation of the Kan-Mon Tunnel was the main subject of research.

In the Japanese Imperial Navy, labor, physiologic, and hygienic studies on underwater and salvage workers were conducted until the end of World War II. After World War II, the safety of persons engaged in diving and caisson work was supervised by the Ministry of Labor. Since then, Dr. Ichiro Nashimoto, who was one of the staff of Professor H. Kita in the Department of Hygiene, Tokyo Medical and Dental University (now professor of hygiene, Saitama Medical College) and Dr. Y. Mano (now assistant professor of public health, Tokyo M&D University) have been engaged in epidemiologic studies of decompression sickness, its etiology, medical treatment, prevention, and related subjects. On the other hand, Drs. O. Shigeto, K. Hayashi, M. Kawashima, and M. Kito of the Kuyshu Rosai Hospital have carried out research on the medical treatment and prevention of decompression sickness as well as pathologic studies. In particular, their research on aseptic osteonecrosis and osteomyelitis is well known.

In 1954, the Japanese Maritime Self-Defense Force (JMSDF) was established. At the same time, the necessity of submarine and diving medicine became important. Many related personnel were dispatched to the U.S. Navy for education and training in deep-sea diving technology. The Japanese pioneer medical officers in diving who participated in this program were Dr. K. Uchida (Lt. MC, JMSDF) and Dr. H. Nakayama (Lt. MC, JMSDF, now professor of hygiene, Tottori University).

In 1967, an underwater medical laboratory was established at the JMSDF Regional Hospital, Yokosuka; I was commanding officer of the hospital. Facilities for 250-m simulated dives and a 10-m training tower were constructed. Experimental studies on submarine medicine, deep-sea diving medicine, and saturation diving were carried out. The results were reported at the 4th International Congress on Hyperbaric Medicine held in Sapparo in 1969.

In 1978, the Underwater Medical Laboratory of JMSDF Hospital, Yokosuka, became an independent JMSDF Undersea Medical Center. The commanding officer is now Captain H. Ohiwa, M.C.

In the early 1960s, undersea activities in France and the United States stimulated the interest of scientists and engineers in Japan. In 1965, the Science and Technology Agency (STA) of the Japanese government provided a grant for comprehensive studies of the recent advances in the field of diving with the intention of launching similar experiments in Japan.

Using the grant, a group of scientists of the Tokyo Medical and Dental University conducted a successful chamber saturation diving experiment in 1967 to a depth of 10 m. In 1968, the STA signed a contract with the Japan Association of Underwater Exploration to perform engineering studies to construct an undersea habitat system, which consisted of a habitat, a support ship equipped with a deck decompression chamber, and a personnel transfer capsule.

In 1971, the newly established Japan Marine Science and Technology Center (JAMSTEC) took over the project with the intention of conducting sea experiments. It was at this time that the project was christened *Seatopia*. Two habitat missions were planned, one at 30 m and the second at 60 m, which would lead to the ultimate goal of four aquanauts living at a depth of 100 m for one month.

In 1974, a 500-m Undersea Simulation and Training Facility was completed at JAMSTEC, which became an excellent hyperbaric chamber complex not only for conducting the US-Japan Cooperative Diving Research Program, but also for providing Japanese scientists and engineers with the opportunity to perform man-rated simulated diving experiments.

As a component of the United States-Japan Joint Cooperative Program on Development and Utilization of Natural Resources (UJNR), the Panel on Diving Physiology and Technology was organized and the first joint meeting of the panel was held in October 1972 in Tokyo.

One resolution adopted at the meeting called for an exchange of scientists for the purpose of conducting experiments of mutual interest. Subsequently, formal arrangements were made between the two countries, and I am happy to say that this international cooperative program turned out to be highly successful. Under the cooperative program, six saturation dives were conducted jointly by the scientists from both countries.

All of these dives, with the exception of one that was conducted in Honolulu, Hawaii, were hosted by JAMSTEC, which has an excellent hyperbaric chamber complex, as noted. The primary focus of the cooperative diving research program was placed on studying the effects of a prolonged exposure (up to 17 days at 18.6 ATA in *Hana Kai II*) to a hyperbaric helium-oxygen environment on major physiologic functions, such as energy balance, heat balance, fluid balance, and cardiovascular and respiratory functions, HPNS and physiologic performance, circadian rhythms, water immersion, and maximal work capacity.

The most significant results obtained from past cooperative saturation diving experiments were published in the proceedings of the sixth and seventh underwater symposium and in *Undersea Biomedical Research*. These results provide basic information needed for the development of countermeasures to protect the safety of divers as well as for establishing safe dive profiles (e.g., the depth and duration of dive) and environmental parameters inside the hyperbaric chamber.

JAPANESE SOCIETY FOR HYPERBARIC MEDICINE

The Japanese Society of Hyperbaric Medicine was established in 1965. The society is made up of scientists engaged in research on the medical treatment and prevention of decompression sickness caused by diving and caisson work, and by physicians who engage in hyperbaric oxygen therapy. Later, groups of chest surgeons, heart surgeons, anesthesiologists, physicians, and otorhinolaryngologists jointed the group.

According to Dr. K. Sakakibara, professor of hyperbaric medicine, University of Nagoya, the first report on hyperbaric oxygen therapy in Japan was made in 1963 by Dr. S. Furuta, University of Tokyo, and his study group. At that time, I was the director of the Neurology and Psychiatry Department, Self-Defense Forces Central Hospital in Tokyo, and was at the same time a research member. Several times, I provided information related to hyperbaric physiology to Dr. Furuta for study.

In 1964, Professor J. Wada of the Sapporo Medical College and his study group reported on hyperbaric oxygen therapy. Research for hyperbaric oxygen therapy in Japan was begun by these two groups. In 1965, Professor K. Sakakibara and his study group conducted similar studies which are detailed in a report entitled "The History and Future Aspects of Hyperbaric Oxygen Therapy in Japan." This report was presented by Professor Sakakibara at the commemorative speech for the 20th Anniversary of the Japanese Society of Hyperbaric Medicine, which was held in Okinawa in 1985.

Finally, I would like to briefly introduce the University of Occupational and Environmental Health (UOEH), Kita-kyushu, National Defense Medical College (NDMC), Tokorozawa and Research Institute for Environmental Medicine (RIEM), Nagoya University.

The UOEH was established at Kita-kyushu in 1978, where a number of hyperbaric physiological studies have been done, as well as those jointly conducted with JAMSTEC. The 3rd International Symposium of the UOEH on Hyperbaric Medicine and Underwater Physiology was held in 1983, and the proceedings, edited by Professors K. Iraki and S. Matsuoka, were published the same year.

The NDMC was established at Tokorozawa, Saitama, in 1973. NDMC dispatched graduate students to the Undersea Medical Center for training and education in submarine and diving medicine.

The RIEM was established in 1946 and has also conducted a number of endocrinological researches on man under the hyperbaric helium-oxygen environment together with JAMSTEC.

I have provided a brief overview of diving, hyperbaric physiology, and medicine in Japan. This symposium has been opened in the Asian region for the first time in its history. In closing, I sincerely hope all will benefit fully from the symposium, and will strengthen the understanding and relationships among all of us involved in this unique area of medicine and physiology.

Kobe, Japan
September 1986

M. Matsuda, M.D., D. Med. Sci.

Opening Remarks at 9th International Symposium on Underwater and Hyperbaric Physiology

Kentaro Takagi, M.D.

Mr. Chairman, Dr. Bove, ladies and gentlemen.

I was a physiologist and I am now a Senator of the National Diet; retired early from active research work about 10 years ago. So it is a great pleasure and honor for me to be given such an opportunity to provide an opening remark at this international symposium attended by excellent researchers the world over.

During the years 1940 to 1945, just in the war time, I was involved in producing a respiratory mask of the "intermittent positive pressure breathing type" for pilots flying at stratosphere altitudes. The mask we developed was uncomfortable for the pilot. At the present time, we can fly to higher altitudes more comfortably without a special mask, and even ascend to cosmic space by rocket or space shuttle equipped with adjustable pressure cabins, and we can work even on the surface of the moon using a space suit.

As to underwater physiology, a symposium on Ama, the Japanese diving women, was opened in Tokyo in 1965 as a satellite symposium of the International Physiological Congress. I was the chairman of this symposium. I remember Professor Sir Adrian and Dr. Rahn and others attended the symposium. The manuscript was first published as *Underwater Physiology Vol. 1.* Early in this century, caisson disease was discovered and its research took place in Europe. As is well known, in 1935 Haldane and Priestley discussed the hyperbaric diving helmet and mechanism of caisson disease in their famous book, *Respiration.*

In the case of the Ama, the main themes of the research were breath-holding time, breaking point, and direct influence of water pressure and temperature on the body. The limit of diving was at least 10 to 15 meters depth and about 1.5 minutes average diving time. Here, I would like to show some figures of my old researches on Ama and some results on the breaking point of breathholding. Figure 1 shows the Ama on the boat equipped with

water or wet suit which serves to protect the cooling of the body from cold water. Figure 2, cited from the book of Dr. Rahn, shows goggles of various types. The upper left one (a) is the most primitive goggle which has the defect of the eyes being pushed out by the pressure of the water and sometimes damage in the ear. To the right (b) is the goggle with the small balloon which served to balance pressure inside and outside the goggle, preventing the eyes from projection. The goggle on the lower right (c) can control the pressure by the expired air through the tube from the mouth. The lower one (d) is the improved mask used at present. The goggle covers the eyes and the nose, allowing the pressure inside the goggle to be adjusted by air from the nose. The mask protects the eyes and ears from damage simultaneously.

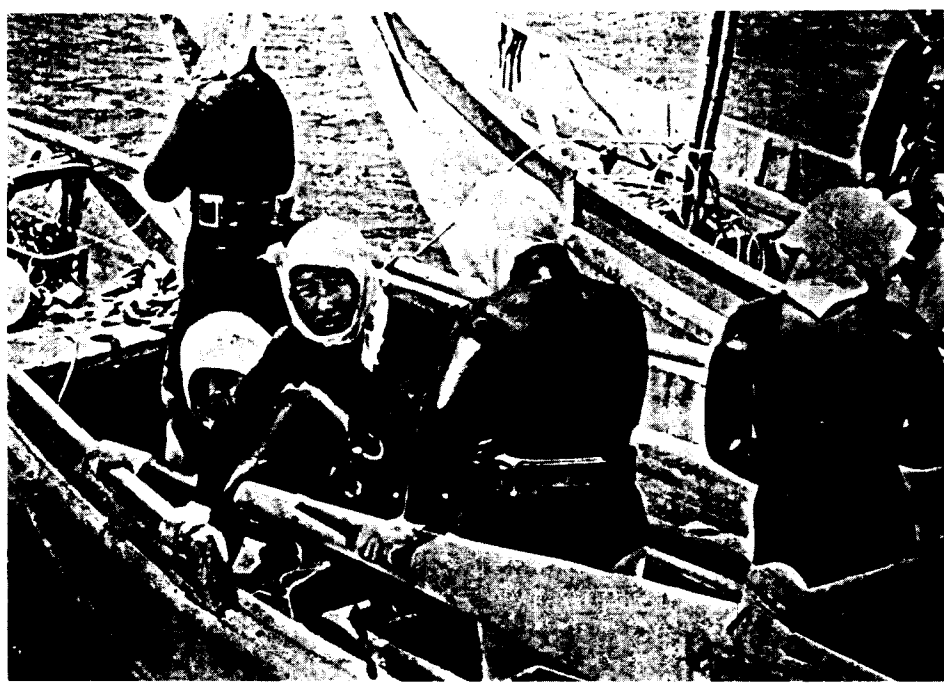

Fig. 1. Japanese diving women (Ama) on a boat with wet suit.

Figure 3 shows Funado-type diving. When the Ama jumps into the sea from the boat, her body can sink rapidly to the bottom of the sea, due to the weight of an iron block attached to her body. She can have enough time to pick up many more shells that the Kachido-type diver. When she feels stifled, she pulls the life rope bound around her body, and her husband immediately pulls up her body by the rope through the pulley firmly attached to the side of the boat.

The most important factor to determine the breath-hold breaking point is the concentration of CO_2 in the blood, which increases rapidly at first stage, then increases almost linearly. At a certain Pco_2, the breaking point occurs

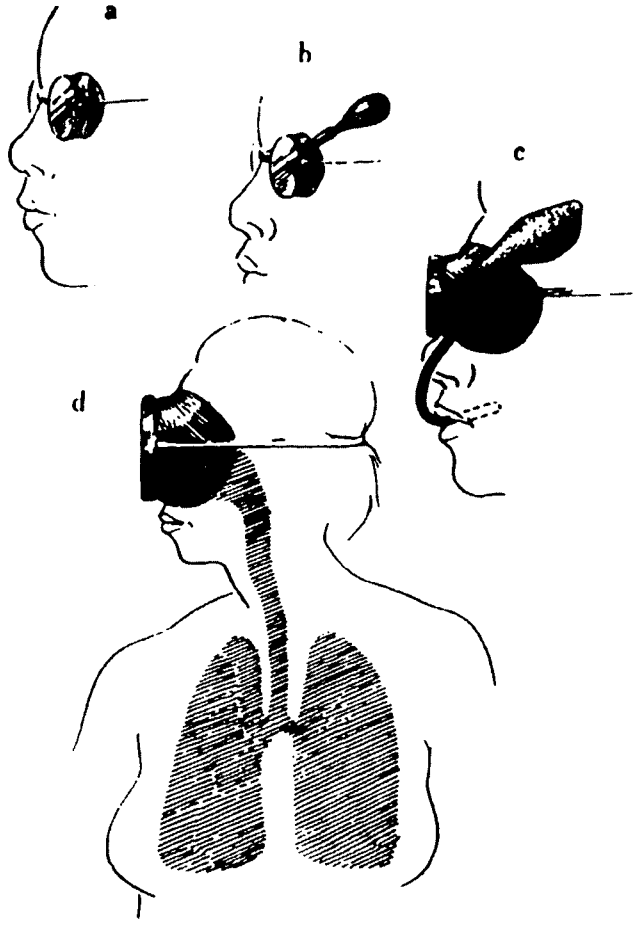

Fig. 2. Various kinds of goggles (Rahn 1965). a, Most primitive goggle without pressure regulating balloon; b., goggles with pressure regulating balloon; c, goggle with pressure regulatory balloon and mouth-pipe; and d, goggle including the nose.

(Fig. 4). This is the usual process. However, if the subject inspires not ordinary air, but air with higher CO_2 before the dive and the breath is held, then the holding time becomes shorter as expected, but P_{CO_2} at the breaking point is not the same as in ordinary breath holding. Repeating such experiments disclosed that the determining factor of breaking point is not only the P_{CO_2}, but also the duration of P_{CO_2} during the breath holding.

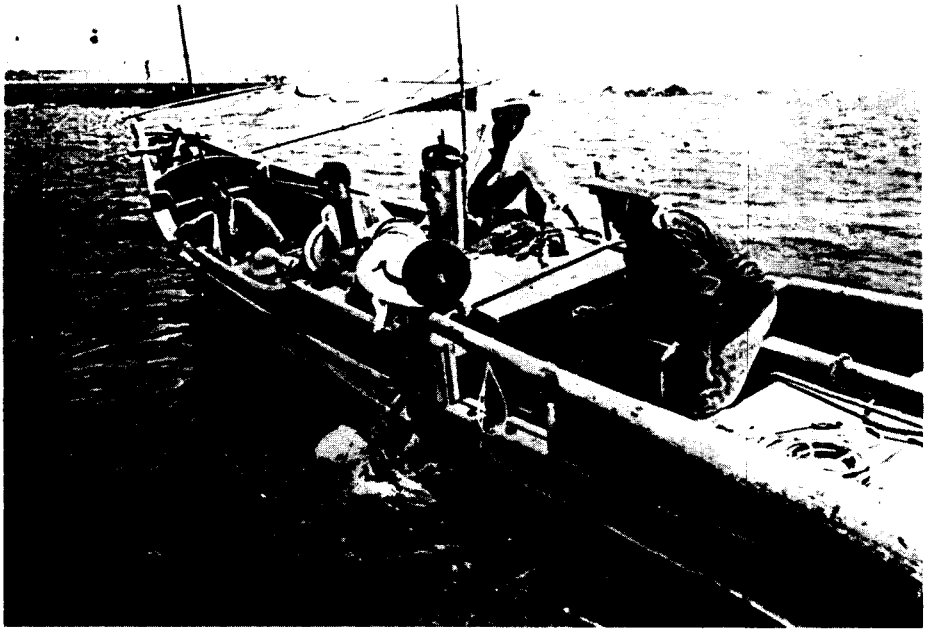

Fig. 3. Funado-type diving, illustrating pulley attached to the side of the boat.

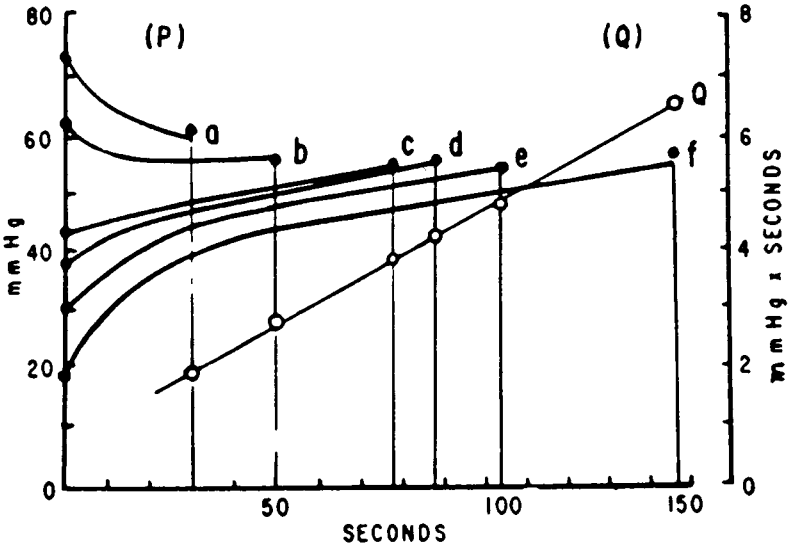

Fig. 4. Calculated alveolar PCO_2 changes, measured PCO_2 at breaking points, and Q = t (Q = integrated PCO_2 value) curve in breath holding. CO_2 concentrations in inhaled gas were: a, 16.0%; b, 12.8%; c, 6.7%; d, 5.5%; e, 3.2%; and f, 0.0%.

Another interesting fact was the change of heart rate when the face was immersed in water of various temperature (Fig. 5). The heart rate decreased more distinctly in the trained subjects that in the untrained, and the colder the water the more effective the heart rate. In this figure, the curves at right show the change of heart rate of a trained subject, and left curves show that of an untrained subject. We are reminded of the decrease of heart rate of diving animals when they dive in the water.

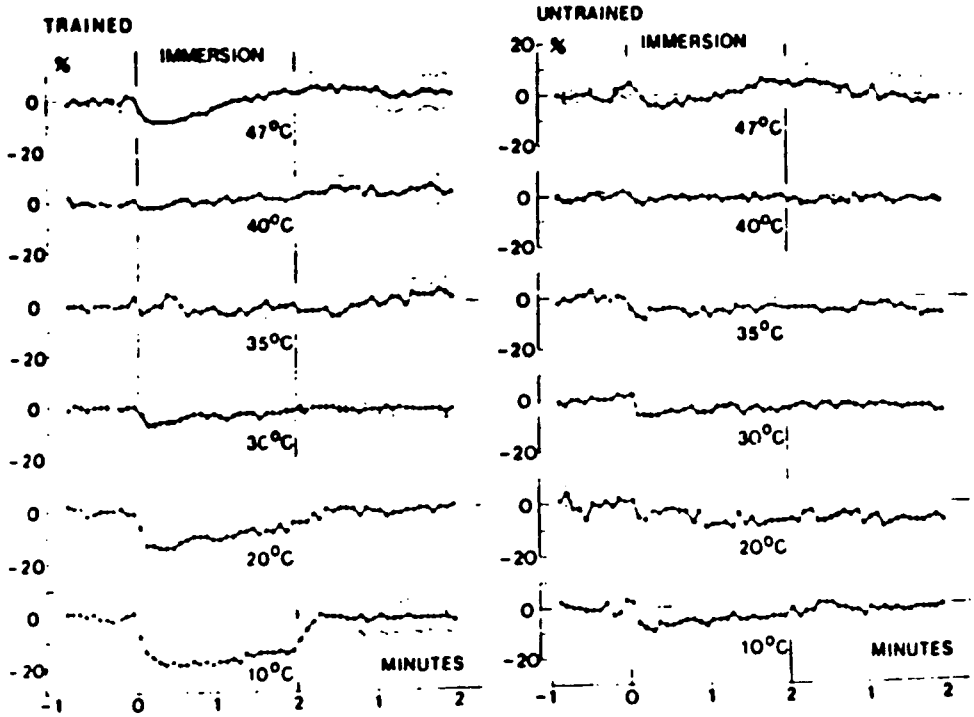

Fig. 5. Average responses of heart rate to nonapneic facial immersion in water of varied temperature. Heart rates are shown in percent changes from the mean control rate before immersion. Standard deviations are illustrated above and below the tracing of the mean heart rate. Beginning and the end of facial immersion are indicated by the 2 vertical lines. Left, trained subjects; right, untrained subjects.

The diving suit has developed, as well as the use of scuba, pressurized submersibles, and hyperbaric oxygen therapy, but we meet now other obstacles to overcome. The ideal method to dive into hyperbaric environments is neither by skin diving nor in a hyperbaric boat, but in a submarine in which the environment is at atmosphere. Like the pressure cabin in the cosmic aeroplane or rocket, the boat or the suit has a normal atmosphere in it, equipped with TV camera for observation and magic hand for picking up material.

The research which began with the Ama and primitive diving apparatus has now developed into clinical applications like hyperbaric oxygen therapy, and into exploration of marine resources. However, there remains some unsolved and troublesome problems.

The sea occupies two-thirds of the surface of the earth and there are enormous, even unknown resources that are important for mankind. We have a dream and a hope in the sea. The future of this research field is very wide and important, like cosmic research. I am sure the symposium will be successful and I wish further prosperity and development of this symposium. Finally, I would like to express my cordial gratitude to Dr. Sakakibara, Chairman of the Local Organizing Committee of the symposium, and his collaborators for their excellent arrangement. Thank you for your attention.

SESSION 1: BREATH-HOLD DIVING AND NEAR DROWNING

9th International Symposium on Underwater and Hyperbaric Physiology
Undersea and Hyperbaric Medical Society, Bethesda, MD, 1987

BREATH-HOLD DIVING: ALVEOLAR O_2 AND BLACKOUT

H. Rahn

Historical accounts of breath-hold divers can be traced back more than 2000 years, describing the activities among the sponge divers of Greece, pearl divers in the Persian Gulf and India, and the seafood divers of southern Korea and Japan (Fig. 1). In each region the skills developed independently but are basically similar, namely, head-first descent in shallow or deep dives, the latter being assisted by a weight. Ascent from deep dives was also assisted by pulling up the diver with a rope. As Davis (1) wrote, breath-hold diving was also a recognized profession in naval warfare, divers being employed to destroy boom defenses in harbors during the siege of Syracuse (415 B.C.) and of Tyre (333 B.C.), and according to C. J. Lambertsen (personal communication) the Frogmen of the U.S. Navy's Underwater Demolition Teams operated as breath-hold swimmers throughout World War II. Even today, breath-hold diving is still part of the training of underwater demolition teams.

Breath-hold diving has become a popular sport throughout the world for spear fishing, underwater hockey games, and competition in depth records. Although breath-hold diving is still practiced in Greece for harvesting sponges, it is now in decline, as is the harvesting of pearl shells in the Persian Gulf and in the Pacific around Thursday Island, Australia, and the Tuamotu Archipelago, French Polynesia. On the other hand, in Japan and Korea breath-hold diving for the harvest of seafood flourishes. Today there are some 13,000 full-time divers in Japan. About 65% are males, responsible for 75% of the annual harvest of all divers in Japan (2).

Estimates today are that some 16,000 Ama are operating in South Korea. These are all women, 70% of them residing on the Island of Cheju, 60 miles south of the southern tip of Korea. The geographic distribution of the Japanese and Korean Ama has been mapped by Kohara (3), suggesting that male divers of Japan cannot successfully compete with women divers in waters north of the 25°C August isotherm line.

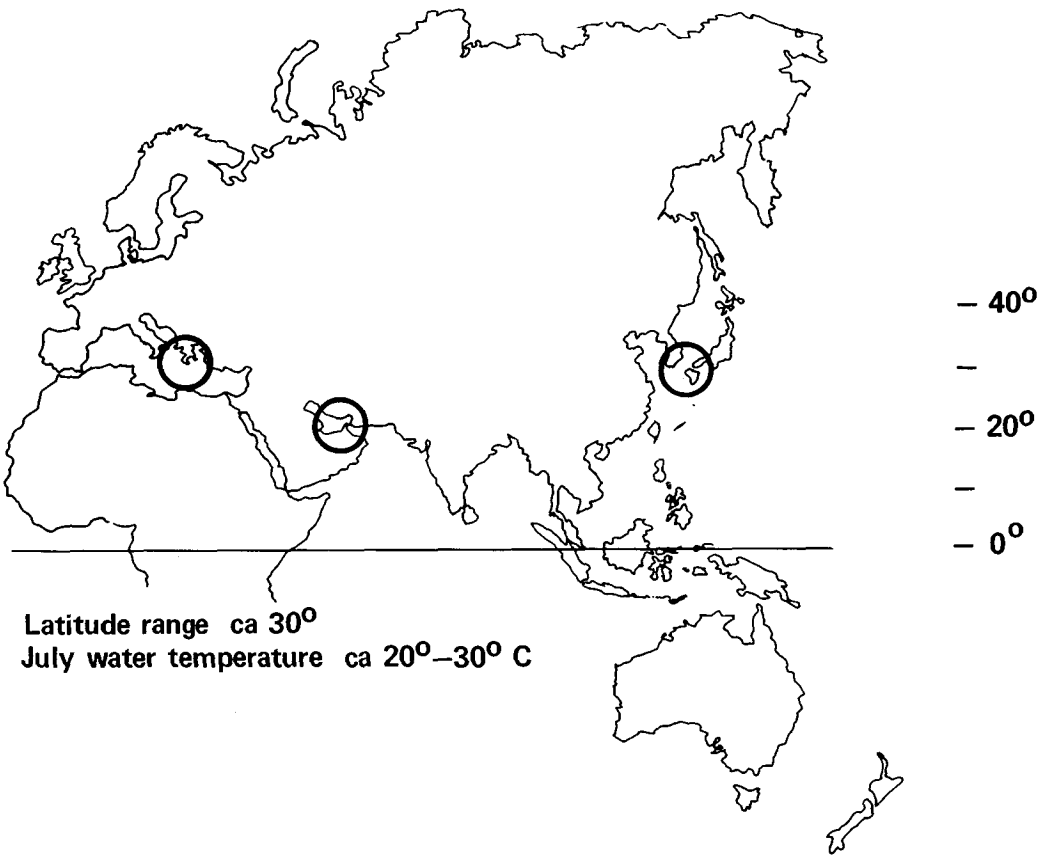

Fig. 1. Areas (circles) where professional diving activities date back some 2000 years. Sponge harvest in Greece, pearl and pearl shell harvest in Persian Gulf and India, and seafood harvest in Korea and Japan.

Diving Pattern

It becomes important to ascertain the typical diving patterns to understand the causes of underwater blackout, loss of consciousness, and drowning. This includes rates of descent and ascent, bottom time for harvesting, recovery time at the surface, and working shifts which have been described for Korean and Japanese female divers (4-6) and male Japanese divers (2). One must also consider the rate of body cooling during a work shift and the importance of protective clothing in providing insulation against cold stress, a topic considered elsewhere in this symposium.

Figure 2 illustrates two principal diving techniques employed by female divers. The unassisted diver rests on a float and dives to depths of about 5 m.

This is the common mode for all divers in Korea and many divers in Japan (Cachido). The assisted diver (Funado) is found only in Japan. She dives from a boat with a 15-kg weight to about 20 m and is pulled back up by her male assistant. The typical diving patterns for the Cachido and Funado are shown in Fig. 3 where total harvesting times on the bottom are about the same for both, namely, 25% of the work shift. These patterns are based on cotton-suit divers. During the last 10 to 15 yr divers have adopted wet suits, and Fig. 4 shows the diving pattern of wet-suited Cachidos in Korea, diving to 5 and 10 m in the summer (7). Diving patterns of the Japanese male divers have been described recently by Shiraki et al. (2). These men wear neoprene wet suits with 4-kg counterweights and on a typical summer day spend 4 h in the water performing 175 dives with a total bottom time of 67 min.

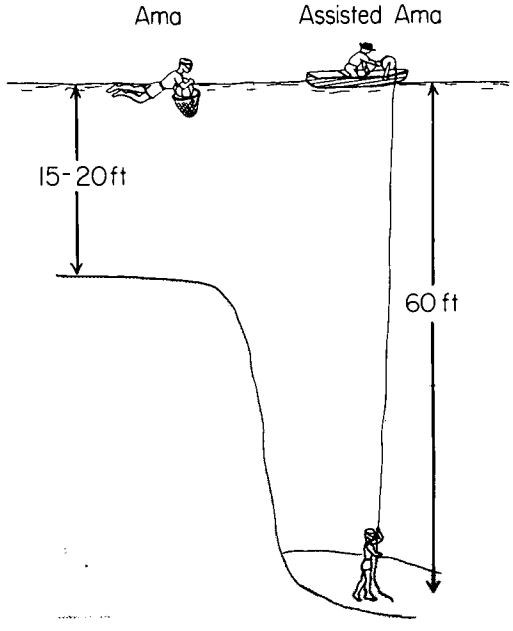

Fig. 2. Diving techniques of female Ama of Japan and Korea. Unassisted divers (Cachidos) in Japan and Korea on left, assisted divers (Funados) of Japan using weights for descent and ropes for return [from Hong et al. (5)].

Breath-Hold Diving Accidents

Although no reports are available of fatalities in Korean and Japanese divers, they are believed to be rare. This may not be surprising, considering that divers are full-time professionals who belong to local fisheries unions that regulate diving seasons, harvest areas, and are concerned about family welfare. The divers train their successors in safe diving techniques following century-old traditions and emphasize efficiency of harvesting techniques rather than competition.

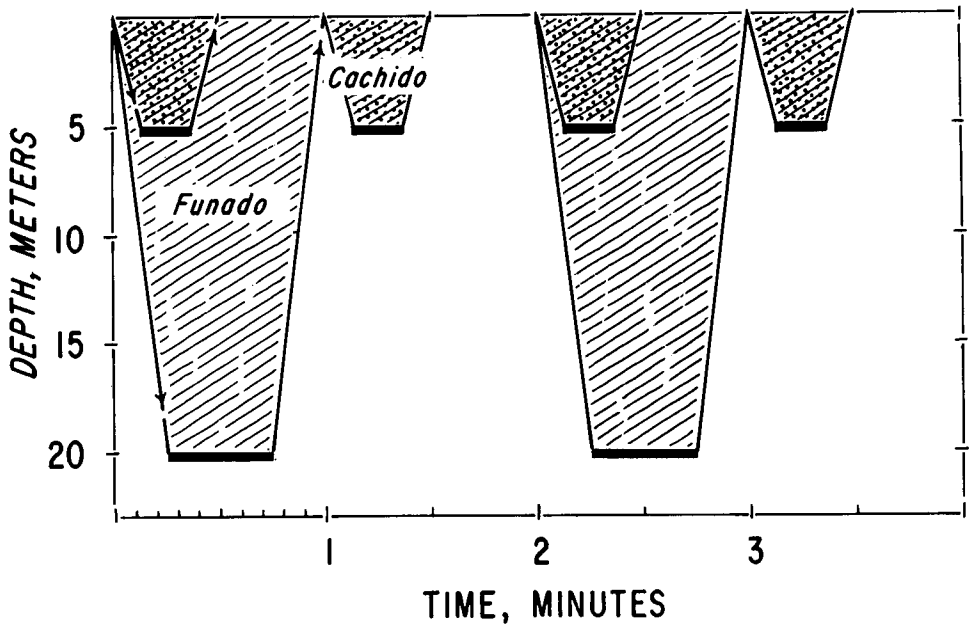

Fig. 3. Typical diving pattern in summer of Cachidos and Funados wearing cotton suits [from Hong et al. (5)].

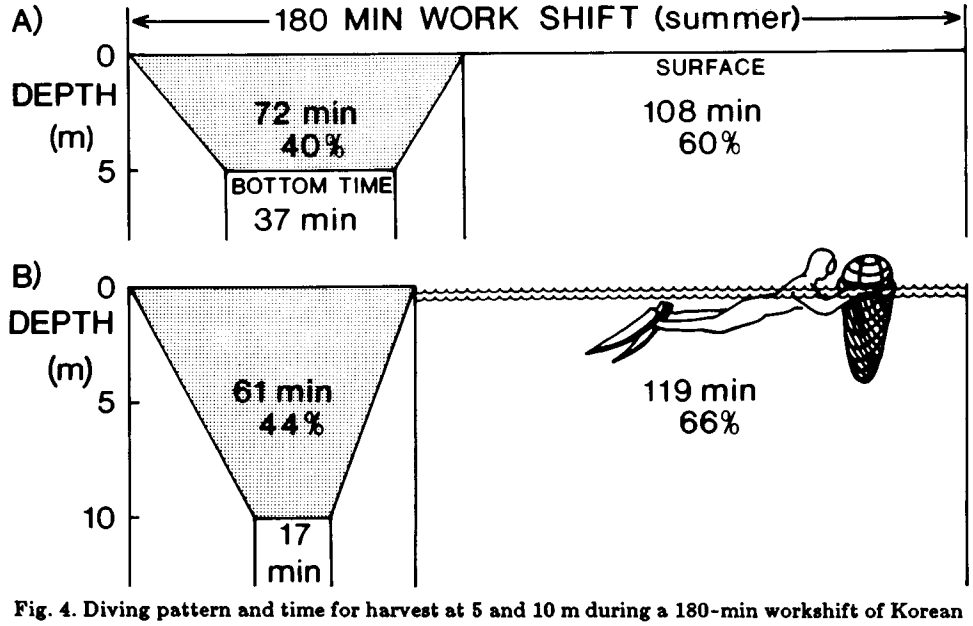

Fig. 4. Diving pattern and time for harvest at 5 and 10 m during a 180-min workshift of Korean Cachidos wearing wet suits, fins, and counterweight [from Park et al (7)].

On the other hand, in the more competitive areas of breath-hold diving where mostly males participate, such as underwater distance swimming, spear fishing, depth records, and the annual pearl-shell diving rendezvous in the Tuamotu Archipelago, diving accidents have been reported. A list of such accidents is shown in Table 1 (8-11).

Table 1
Breath-Hold Diving Accident Reports

Dive	Outcome	Reference
Distance swimmers (USA), 1-2 m	58 Cases loss of consciousness	(8)
	35 survived	
	(average age 18)	
	23 died	
Spear fishers (South Africa), 20 m	13 deaths since 1968	Landsberg (pers. com.)
(Mediterranean) Estimates of deaths	Means/year	(9)
Spain	5	
France	20	
Italy	10	
Yugoslavia	10	
Greece	10	
Pearl shell divers (Tuamotu Archipelago)	47 (of 235) affected (1 d)	(10)
	34 - vertigo, nausea, mental anguish	
	6 - partial paralysis	
	3 - temporary unconsciousness	
	2 - mentally affected	
	2 - died	
Submarine-escape tank divers, 20 m	Decompression sickness	(11)
	4 cases	

Factors that can lead to blackout, loss of consciousness, and drowning during breath-hold diving and even to decompression sickness (10, 11) (Table 1) can be analyzed in terms of changes in O_2, CO_2, and N_2 stores of blood and tissues leading to hypoxia, hypocapnia, and hypercarbia as well as accumulations of N_2 stores during repetitive diving.

Performance and Alveolar Gas Composition

The question is what are the limits of alveolar gas composition for normal motor and mental functions? These have been described in detail based on multiplication tests, choice reaction times, and visual discrimination tests during various degrees of hypocapnia, breathing air at sea level (12), and hand-steadiness and visual discrimination tests during various degrees of hypoxia, hypocapnia, and hypercarbia performed in a high-altitude chamber breathing air (13). The results of more than 300 individual tests expressed in *regions of performance* on the alveolar O_2-CO_2 diagram have been replotted in Fig. 5. The combinations of O_2 and CO_2 tensions which yield arterial O_2 saturations for 55 and 80% are shown as isopleths. If one assumes that these performance levels, obtained during acute exposure, are applicable to mental and motor performance during breath-hold diving, they become useful in assessing the performance level when alveolar gas samples are obtained during a dive or immediately after surfacing [for further details see Rahn, (14)].

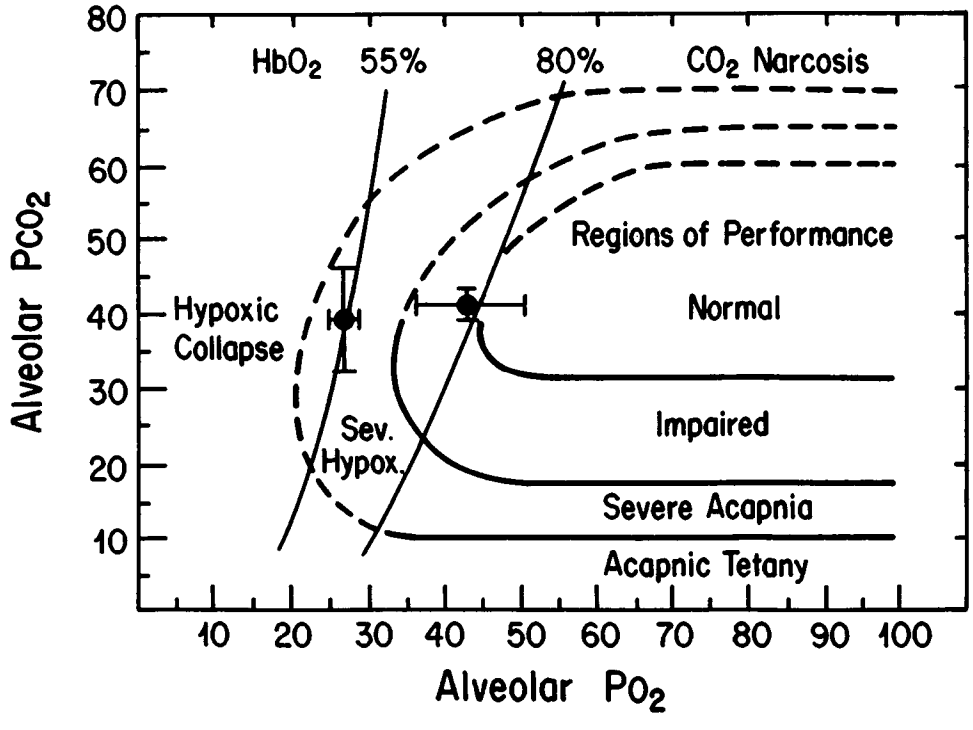

Fig. 5. Regions of performance plotted on the O_2-CO_2 diagram and redrawn from the data of Otis et al. (13) and Rahn et al. (12). Also shown are the mean values and SD of alveolar composition for two groups of divers immediately upon returning from dives ranging from 10 to 27 m, (see Ascent Blackout). Isopleths for calculated arterial O_2 saturations of 55 and 80% are indicated.

Breath-Hold Blackout

Craig (8) reported 58 cases of loss of consciousness during breath-hold diving in swimming pools in young people who had competed for distance records. He pointed out that these could be traced to excessive hyperventilation before the dive delaying the onset of the urge to breathe, followed by severe hypoxia and unconsciousness. A general interpretation of these observations is shown in Fig. 6, where the heavy line depicts the limits of *regions of impaired performance* as shown in Fig. 5. On the right are displayed the blood and tissue CO_2 stores at the mixed venous P_{CO_2} level (15). The metabolic CO_2 production (leaving the faucet) is removed by ventilation, resulting in a lung P_{CO_2} of 40 Torr at rest, with an exchange ratio, $R = 0.8$. When the breath is held at this stage of eupnea, the *top arrow* indicates the alveolar pathway, terminating near the performance border. With excessive hyperventilation, blood and tissue CO_2 stores are lowered, lung P_{CO_2} falls to 20 Torr, and now the alveolar breath-hold pathway follows the *lowest arrow*. The CO_2 stimulus for breaking is attenuated and the alveolar oxygen continues to fall below 30 Torr, resulting in severe hypoxia and eventually hypoxic collapse (*see* Fig. 5). This has been referred to as breath-hold blackout (16) or shallow water blackout (17).

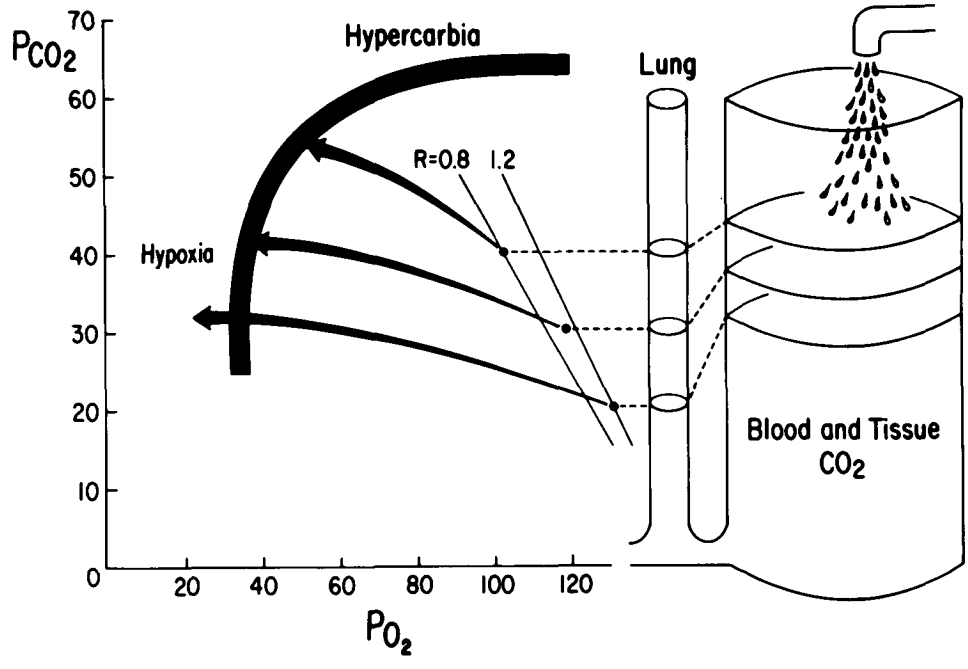

Fig. 6. Breath-hold pathways (arrows); at top during eupnea and below after hyperventilation, plotted on the O_2-CO_2 diagram. Right, blood, tissue, and lung CO_2 stores of the body and how they can be changed by hyperventilation. Metabolic CO_2 production is pictured as water emerging from the faucet. For details see text.

Alveolar Gas Composition During Deep Dives

When breath-hold dives penetrate to various depths, the alveolar composition changes drastically due to the compression of lung gases where O_2, CO_2, and N_2 initially pass into the blood and tissues. Figure 7 shows the alveolar composition shortly after arrival at various depths where the number in *circles* indicate the depth, meters, at which the alveolar sample was obtained. The average composition at the surface just before the dive (start) is also shown. As the depth increases so do the CO_2 and O_2 tensions. Gas tensions at 7.6, 15, and 27 m (18), at 8.2 m (5), at 10 m (19), and at 18.5 m (20).

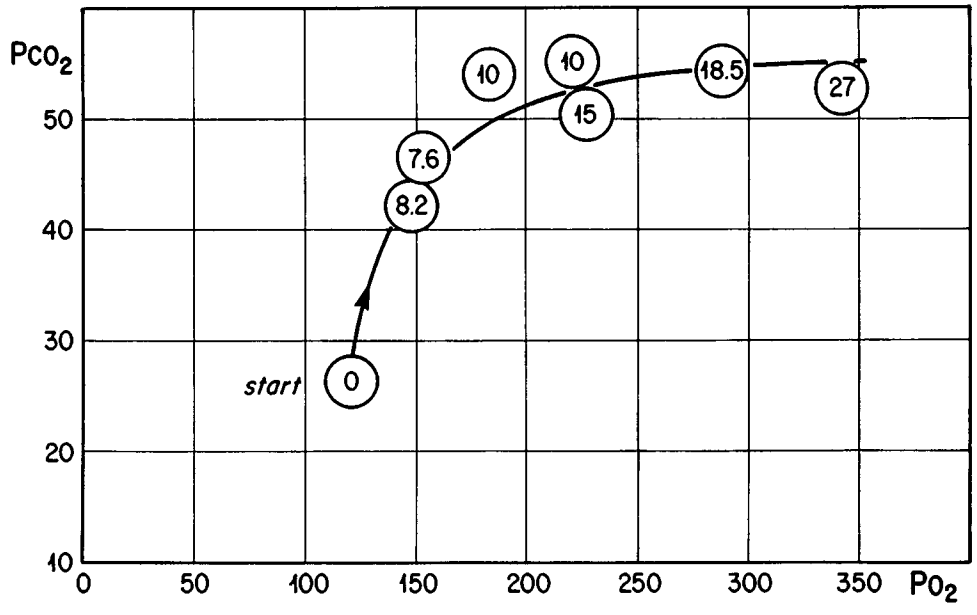

Fig. 7. Alveolar gas composition shortly after arrival at various depths (meters) (circles). The average starting composition just before the dive is also shown. Sources of data are given in the text.

Figure 8 shows the alveolar pathway at 10-s intervals during a simulated breath-hold dive of 1-min duration to 10 m in a pressure chamber at an oxygen consumption rate of 320 and 580 ml/min (19). Descent and ascent are shown by *broken lines*. The 20-s period at the bottom is shown by a *solid line*. Note that the lower rate of oxygen consumption is associated with a higher P_{O_2} and lower P_{CO_2} at depth as well as after return to the surface.

To simulate the alveolar composition of the Japanese Funado during her repetitive 20-m dives as shown in Fig. 3, a computer program was developed to follow the O_2, CO_2, and N_2 tensions and volumes in the lung, blood, and tissues (21, 22). In Fig. 9 are shown the alveolar O_2 and CO_2 tensions every

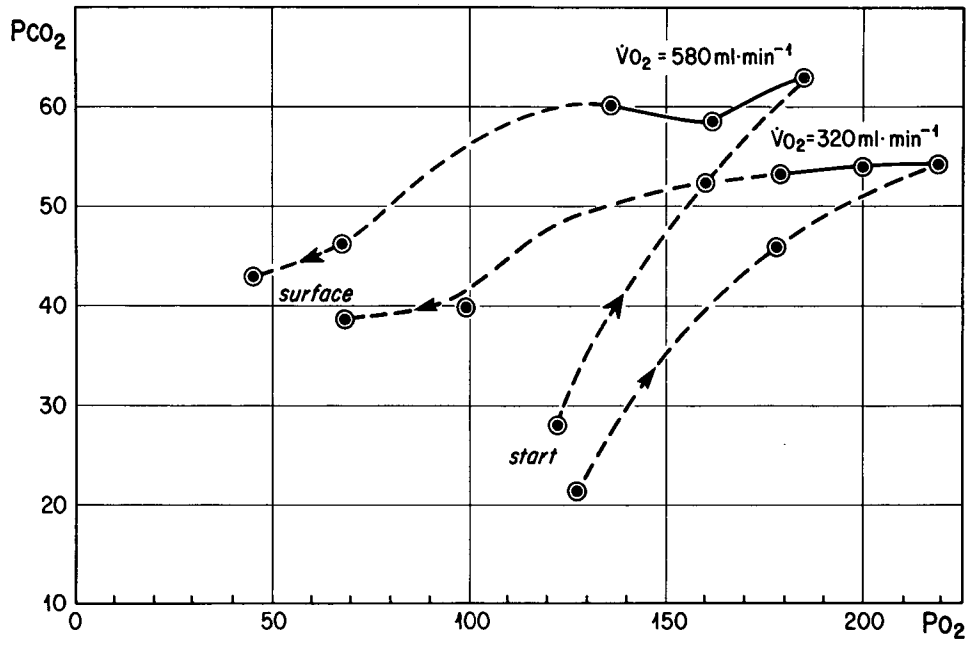

Fig. 8. Alveolar pathway at 10-s intervals of a simulated dive in a pressure chamber to 10 m at two different rates of oxygen consumption. Descent and ascent are shown as broken lines, bottom time is shown as solid line. [Replotted from Lanphier and Rahn (19)].

5 s during a 20-m dive lasting 1 min at an O_2 consumption of 800 ml/min. Descent and ascent times each lasted 15 s, and bottom time was 30 s. The pattern of this simulated 20-m dive is similar to that of the 10-m dive observed in Fig. 8.

Ascent Blackout

A previous computer analysis of a 100-m breath-hold dive, simulating the 105-m record of Mayol of France and the 94-m dive of Maiorca of Italy, showed that for an O_2 consumption of 360 ml/min the maximal alveolar P_{O_2} reached was 800 Torr. The alveolar P_{O_2} remained above 100 Torr for most of the ascent time but toward the end fell rapidly below the mixed venous P_{O_2}, inducing an O_2 reversal in the lung (17, 21).

Note that throughout the 10-m dive (Fig. 8) and the 20-m dive (Fig. 9) alveolar O_2 was maintained above 100 Torr during most of the dive, even during the beginning of ascent. It is only during the last phase of lung expansion that the P_{O_2} falls rapidly and on occasion may fall below the mixed venous level and induce an O_2 reversal.

What are the alveolar O_2 and CO_2 tensions at the end of a deep dive? Elsewhere (22) we have assembled these values from the reports of Teruoka (4), Hong et al. (5), Lanphier and Rahn (19), Schaefer (23), Paulev and Naera

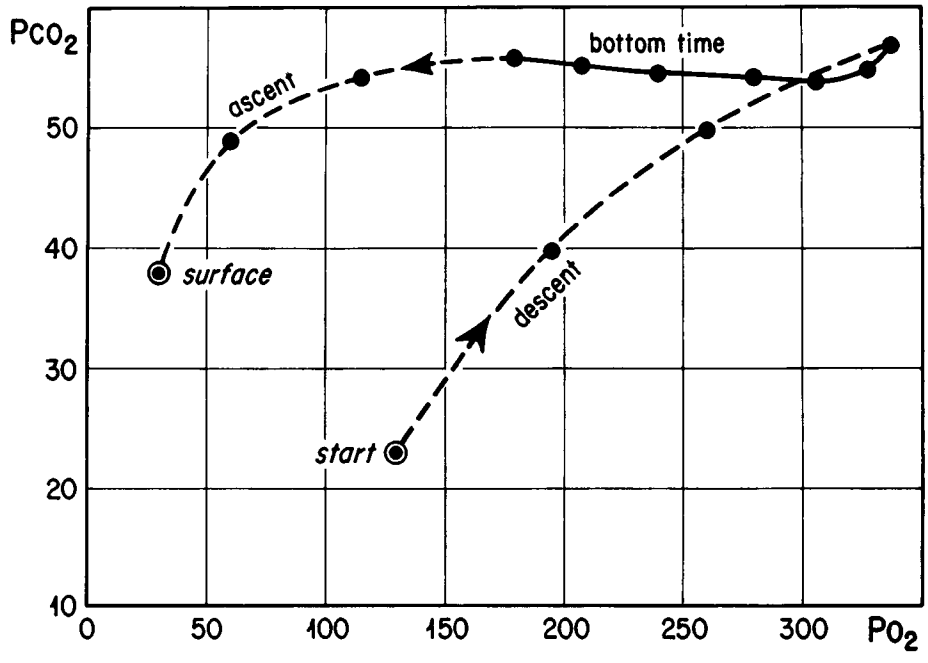

Fig. 9. Alveolar pathway at 5-s intervals during a 20-m dive simulating the diving pattern of the Funado shown in Fig. 3. These values were generated from a computer program (see text).

(20), and Craig and Medd (24), which together represent 86 dives. The average diving times for each group ranged from 45 to 90 s, and depths from 10 to 27 m. The average alveolar P_{O_2} = 45 Torr, SD 8; and the alveolar P_{CO_2} = 42 Torr, SD 1. This average alveolar composition is shown in Fig. 5 on the border of the *normal and impaired performance* area, and the calculated arterial O_2 saturation is 80%. It suggests that alveolar O_2 tensions above 35 Torr at the end of a dive are relatively safe and performance only slightly compromised. It is of interest to note that in spite of variations in diving time, diving depth, and rate of O_2 consumption the alveolar P_{CO_2} values were remarkably similar, namely, a mean value of 45 Torr with a SD of 1 Torr.

On the other hand, several cases of near loss of consciousness were reported by Cross (10) for the Tuamotu divers (*see* Table 1), by Paulev and Naera (20) and Schaefer and Carey (18) for their submarine-escape diving tank instructors, and by Lanphier and Rahn (19) for their subjects in the pressure chamber when they attempted 80-s breath-hold dives at a higher work load than 590 ml O_2/min. An underwater movie (presented at the Physiology of Breath-Hold Diving Workshop, October 1985, at Buffalo, NY) of the ascent of Maiorca in his attempt to establish a depth record for breath-hold diving showed him losing consciousness a few meters below the surface.

From the 86 dives reported above we have selected 11 dives where alveolar O_2 values after reaching the surface were below 30 Torr. These dives

ranged in time from 50 to 90 s and in depth from 10 to 27 m. The average alveolar P_{O_2} = 27 Torr, SD 1, whereas the average alveolar P_{CO_2} = 40 Torr, SD 7. These values are also shown in Fig. 5. They lie in the *severe hypoxia performance* area. The calculated arterial O_2 saturation was 55% and is similar to the 58% value recorded by ear oximeter by Lanphier and Rahn (19) after 80 s, at the end of their most taxing breath-hold dives. It has been suggested (17, 19), that when alveolar P_{O_2} values of less than 30 Torr are observed, such values most likely fall below the mixed venous O_2 level and indicate a temporary reversal of the O_2 flux across the lung. The length of time that such low O_2 values are maintained undoubtedly determines the degree of performance and whether ascent blackout will occur.

When breath-hold dives are performed at depths greater than 5 m they are apt to provide the diver with false signals as to his eventual performance. Impairment before the dive due to acapnia of hyperventilation is reversed rapidly because during descent lung compression quickly raises alveolar P_{CO_2} above the mixed venous level and finally reaches values about twice that of the initial P_{CO_2} (Figs. 7 and 8). For similar reasons, O_2 tensions rise quickly and are maintained above normal throughout most of the dive, even during the first part of ascent.

The danger comes during the last part of ascent when maximal rate of lung expansion occurs. Whether O_2 tensions will fall below the mixed venous level will depend on the degree of lung volume expansion and to what extent the length of the dive and the rate of oxygen consumption have depleted the lung O_2 reserves before the time of ascent. During breath-holding at the surface, both rising CO_2 and falling O_2 tensions are signals to the respiratory center that force a person to break his hold. During a deep dive, up to the time of ascent the diver depends only on the stimulus of P_{CO_2} (which is high) and the stimulus from a compressed lung volume (deflation stimulus) to terminate his dive. However, as Lanphier and Rahn (19) and Craig and Hartley (25) have pointed out, even these stimuli may become attenuated by the fall in P_{CO_2} and the expanding lung volume once ascent is initiated and may actually produce a temporary relief just at the time when alveolar O_2 falls most rapidly.

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ENERGY EXPENDITURE BY THE DIVING AMA IN JAPAN

T. Yokoyama

The Ama makes breath-hold dives to collect shellfish, sea weeds, and bottom fish at various depths. They have to overcome various physiologic stresses, in their working environment, e.g., hypoxia during dives, water pressure, body heat loss, abnormally large drag resistance in water, and abnormal gravity. Their simple and primitive method of breath-hold diving, their traditional working costumes, as well as their diving gear do not protect them sufficiently from the stresses that they must tolerate. Although they recently began to wear wet suits, only a few of them use swimming fins and to conserve the natural fish resources, none is allowed to use scuba.

Pattern of Breath-Hold Diving

The Ama in Japan exhibit three major types of mechanics in diving (Fig. 1).

Unassisted Ama: An unassisted Ama (Cachido), compatible with Hae-Nyo in Korea, actively dives without assistance to depths of 5 to 10 m. The unassisted dive is one of the most primitive patterns of Ama breath-hold diving. The duration of a dive on routine, repeated dives is between 20 and 40 s. Ama must actively overcome positive buoyancy as well as drag resistance on descent and while on the bottom. During ascent they are only partly assisted by buoyancy. Relatively large oxygen consumption restricts the working depth as well as the bottom time on the breath-hold dive.

Partially Assisted Ama: A partially assisted Ama descends passively, carrying the counterweight, but actively swims up on her own effort, taking advantage of buoyancy. The counterweight gives higher speed for descent and minimizes energy expenditure to swim down.

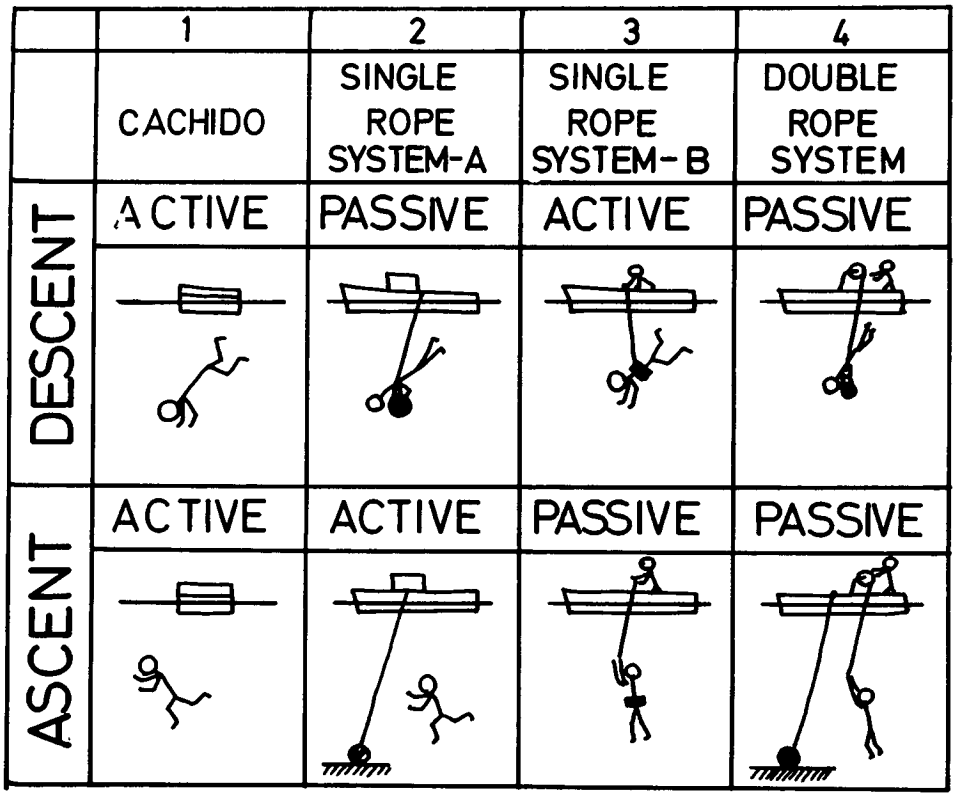

Fig. 1. Diving patterns for the Ama of Japan.

Assisted Ama (Funado): An assisted Ama (Funado) descends to a depth of 15 to 25 m with an average velocity of about 1.2 to 1.5 m/s. She carries on descent a counterweight of 12 to 15 kg, which provides for proper negative buoyancy and appropriate descent velocity without consuming extra energy. The Ama releases the weight when she reaches the bottom. As soon as she finishes her job on the bottom the partner on board pulls her up to the surface. In some Ama villages, a winch driven by an inboard engine is used to pull up the Ama and the counterweight. The mean speed of descent of assisted Ama is 2.5 times faster than that for unassisted Ama. The mean speed of ascent for assisted Ama was three times faster than that for unassisted Ama. Assisted Ama do not consume their excess energy for either descent or ascent and becomes active while looking for harvest on the bottom. Bottom time is extended, in addition to diving deeper, because less energy is used for descent and ascent.

Dynamics of Underwater Movement

To discuss the dynamics of underwater movement one has to know the

drag resistance of the Ama's body under any given conditions. Craig obtained data on the force necessary to tow a subject with a boat and data from experimental dives using various sizes of counterweights and descending in the foot-first position (personal communication). Using these data we calculated the mechanical work to be exerted on the Ama dives, taking into consideration the effect of compression of intrathoracic gas volume on buoyancy.

EQUATIONS FOR DIVES

Unassisted Dive

Descent:

$$m \frac{dv}{dt} = F + mg - R \qquad (1)$$

Ascent:

$$m \frac{dv}{dt} = F - mg - R \qquad (2)$$

Assisted Dive

Descent:

$$(m+m') \frac{dv}{dt} = (m + m')g - R \qquad (3)$$

Unassisted dive of Cachido: A Cachido swims down to 5 or 10 m by her own effort, overcoming positive buoyancy as well as drag resistance. The equation of her movement is given in Eq. *1*, in which F indicates the force she has to apply to descend at a given velocity, indicated by our standard diving schedule. If one can assume the descending velocity to be constant, the force F could be obtained through this differential equation. By integrating the force with time, one can also compute the work necessary during her unassisted descent.

For her ascent she also has to work to obtain indicated ascending velocity. The equations also show the differential indicating her movement during unassisted ascent.

Oxygen Debt

Oxygen is mainly consumed during breath-hold diving to perform any extra muscular work for underwater movement and to protect body temperature loss, in addition to maintain fundamental function of the Ama's body. Oxygen consumption used for these purposes is not linearly additive, so that in practice one cannot reliably calculate or measure oxygen consumption to meet net muscular work for underwater movement. We made measurements on various types of Ama during their work shifts and recorded the extra oxygen consumption immediately after their dives to calculate oxygen debt produced during a dive, which is shown in Fig. 2. The abscissa is depth that the Ama reached, and the ordinate, extra oxygen consumption (oxygen debt). *Solid*

circles represent the data for the Cachido, *open circles* that for the Funado, and *cross marks* for the partially assisted Ama.

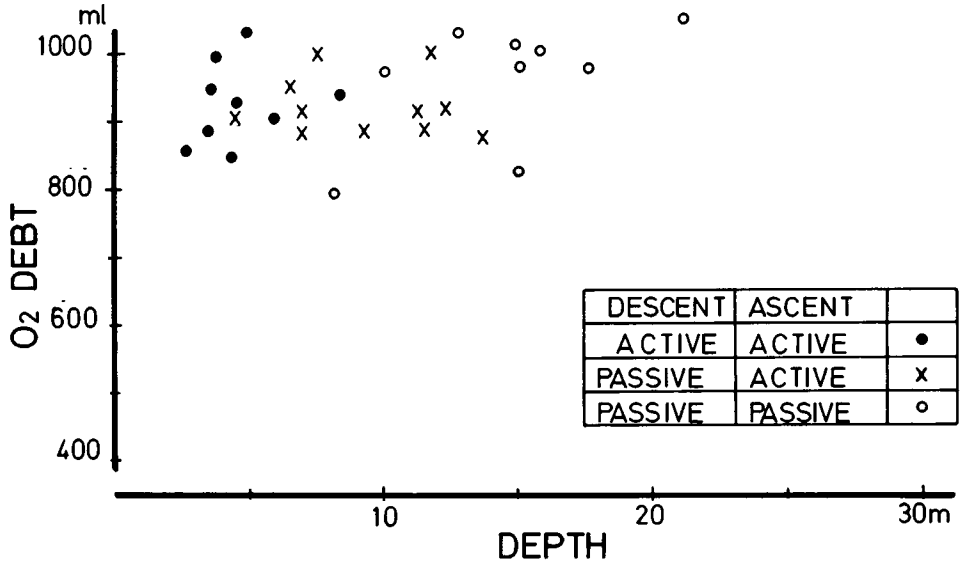

Fig. 2. Oxygen debt on a dive. Abscissa: depth, ordinate: oxygen debt.

Depending on the patterns of dives, the depth which the Ama could reach was different and therefore the data plots for Cachido, partially assisted Funado, and fully assisted Funado are partially separated by depth. But the oxygen debt produced during the Ama's dives ranged from 800 to 1200 ml and we found no consistent difference in oxygen debt in terms of the patterns of dives.

Work Efficiency

We can estimate the excess work to be done by the Ama during her descent, on the bottom, and on ascent. We can also measure the oxygen debt during her dives. The oxygen debt includes an Ama's fundamental oxygen consumption and excess oxygen consumption accounted for by heat loss during water immersion, as well as the oxygen consumption to meet her muscular work needs during the dives. We also know each component of oxygen consumption is not additive. But we can roughly estimate the Ama's overall working efficiency. An example is shown in Table 1. A Cachido reached a depth of 5 m during her dive of 30 s, and in this particular dive she produced an oxygen debt of 994 ml while her muscular work was estimated to be 44.6 kg/m. Thus, her efficiency of energy expenditure was estimated to be 2.5%. Data for a Funado were obtained on a 15-m dive for 50 s. Overall efficiency for the Funado's energy expenditure was 1.4%. We can estimate the work of

Table 1
Work Efficiency of Cachido and Funado

	Energy Expenditure, Single Dive	
	Cachido	Funado
Depth, m	5	15
Diving time, s	30	50
Work, kg/m	44.6	25.7
Excess $\dot{V}O_2$, ml	994	873

the Funado's partner to pull up the Ama from the bottom to the surface using the equation of movement, and we can measure the oxygen debt produced during this maneuver (Table 2). We found the work efficiency for the partner to be approximately 24.3%, which was consistently high as compared with that for the diving Ama. In addition to pulling the Ama to the surface, the partner has to pull up the counterweight, which is released by the Funado on the bottom. Thus, in total the partner consumes almost the same amount of oxygen as the Funado consumes during her dive.

Table 2
Work Efficiency of a Partner to Funado

	Work Efficiency to Pull Up Ama		
Depth, m	Work, kg/m	Excess VO_2, ml	Efficiency, %
12	212	404	25.5
13	229	446	24.3
15	264	550	23.0
Mean			24.3

Time Study on the Diving Ama

An Ama usually works two or three shifts of repeated dives a day and each shift usually continues for 20 to 90 min, with wide variation. The duration of a shift depends on various environmental conditions, the most important being water temperature.

Observations were conducted at Chikura in the Chiba area to study the Cachido's diving activities during one shift with water temperature of 24.4°C in midsummer and 18.2°C in early April. Dives in water of 18.2°C were worked out on an experimental basis for the present observations.

Five female Cachido with cotton diving costumes and five male Cachido

who wore only swimming trunks were studied. The depth of water was approximately 6 m. Figure 3 shows the data obtained on a male and a female Ama on each series of dives, and Table 3 indicates data as the mean obtained on five subjects of each group.

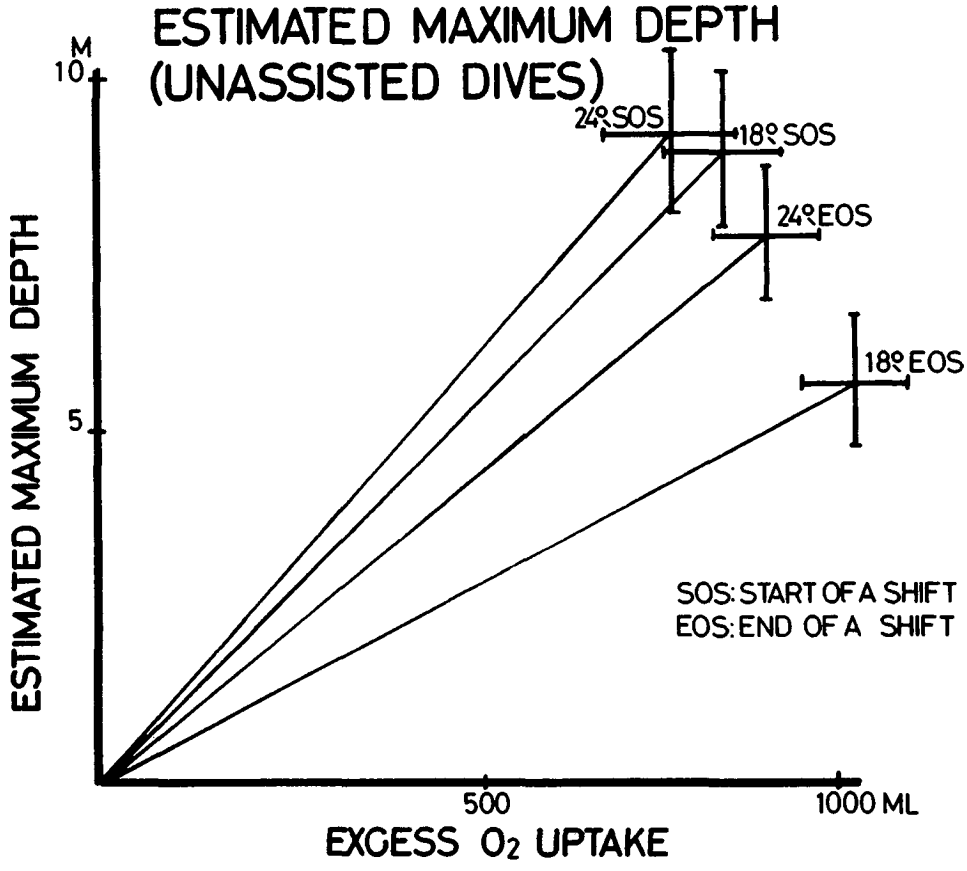

Fig. 3. Effect of water immersion on repeated Ama's dives.

Duration of a shift in water of 24.4°C was 36.8 ± 4.8 min for the male and 37.4 ± 4.0 min for the female while in water of 18.2°C (14.6 ± 2.5 min for the male and 28.6 ± 2.2 min for the female). No significant difference in duration of a shift was found between male and female Ama in water of 24.4°C, but there was a consistent difference in the duration of a shift in water of 18.2°C, as shown in Table 3. After repeated dives in a shift, the duration of a dive gradually became shorter. The effect of water temperature, the duration of the initial dive in a shift, plus a change of duration of a dive in a shift under different water temperatures of 24.4°C and 18.2°C were compared. Shortening the duration of a dive during a shift was more

Table 3
Effect of Water Immersion

		Male Ama		Female Ama		Female Ama
No. of Ama		5		5		3
Water temperature, °C		24.4	18.2	24.4	18.2	20.7
Clothes		trunks	trunks	cotton clothes	cotton clothes	wet suits
Duration of a shift, min		36.8 ±4.8* (31-44)**	14.6 ± 2.5 (11-18)	37.4 ± 4.0 (32-43)	28.6 ± 2.2 (26-32)	43.7 (41-46)
Duration of a dive, s	B	30.6 ± 5.6 (24-38)	25.4 ± 3.2 (21-30)	32.2 ± 4.0 (27-38)	26.8 ± 3.3 (22-31)	30.4 (29-34)
	E	25.2 ± 4.0 (20-31)	25.4 ± 3.2 (13-19)	26.4 ± 3.8 (22 -32)	18.2 ± 4.0 (12-23)	30.0 (26-34)
Bottom time, s	B	12.6 ± 3.0 (11-19)	7.8 ± 2.2 (5-11)	13.4 ± 3.0 (10-18)	8.6 ± 2.7 (5-12)	11.0 (10-12)
	E	7.4 ± 2.5 (4-11)	0.4 ± 0.5 (0-1)	8.2 ± 1.3 (7-10)	4.2 ± 1.5 (2- 6)	9.7 (8-11)
Surface time, s	B	20.4 ± 3.8 (16-26)	23.6 ± 3.4 (19-28)	21.2 ± 4.7 (16-28)	21.8 ± 4.5 (17-28)	20.0 (18-22)
	E	31.2 ± 4.1 (26-36)	41.8 ± 3.4 (38-46)	21.2 ± 4.7 (24-35)	38.2 ± 3.2 (34-42)	25.3 (21-29)

* Figures shown as ± are mean ± SD. ** Figures in parentheses refer to the range. B = the beginning of a shift; E = the end of a shift.

significant in water of 18.2°C compared with that of 24.4°C. Female Ama in water of 24.4°C had a mean duration of dive of 32.2 ± 4.0 s at the beginning of a shift, which became 26.3 ± 3.8 s at the end of the shift. In water of 18.2°C duration of a dive at the beginning of a shift was 26.8 ± 3.3 s and became 18.2 ± 4.0 s at the end of the shift (Fig. 3).

Water temperature during a shift of repeated dives also had a significant effect on bottom time (Fig. 4). In water of 24.4°C, the female Ama had a mean bottom time of 13.4 ± 3.0 s at the beginning of the shift, but it decreased to 8.2 ± 1.3 s at the end of that particular shift. The mean bottom time for the male Ama under the same condition was 12.6 ± 3.0 s at the beginning, which decreased to 7.4 ± 2.5 s at the end of the shift. In water of 18.2°C, the bottom time for the female Ama decreased from 8.6 ± 2.7 s to 4.2 ± 1.5 s during the shift, and that for the male Ama changed from 7.8 ± 2.2 to 0.4 ± 0.5 s during the shift.

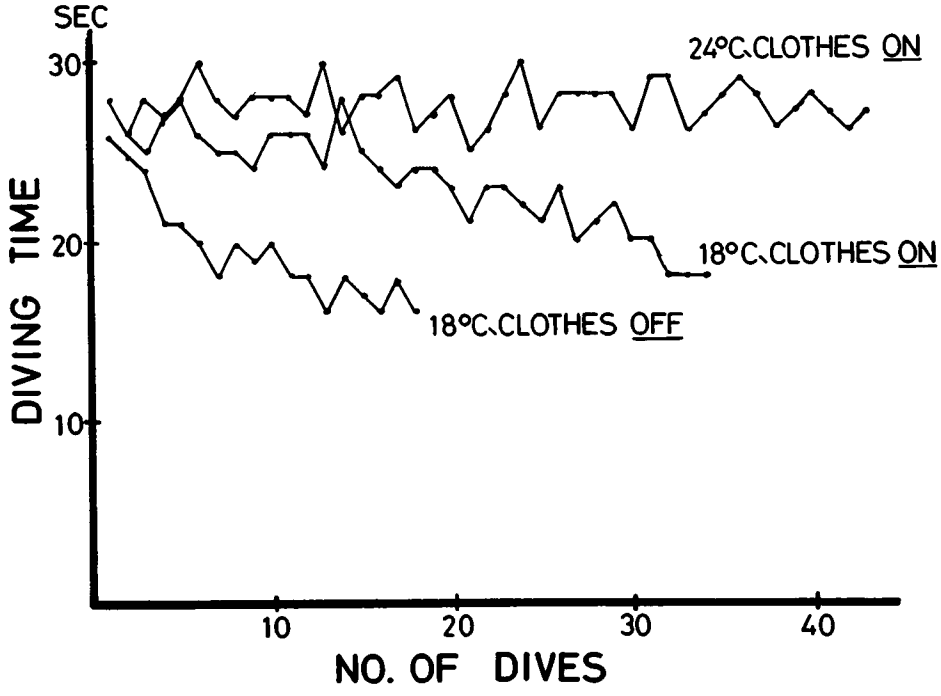

Fig. 4. Diving time, unassisted dives.

The time spent for descent as well as for ascent did not change significantly during the series of dives. The time spent for descent and for ascent showed no consistent difference related to temperature difference between 24.4 and 18.2°C.

Surface time, during which the Ama stays on surface between successive dives, is the time spent by the Ama to repay the oxygen debt developed during the preceding dive and also to look for harvest for the next dive (Fig. 5). There was no consistent difference of surface time between male and female Ama under water temperature of either 24.4 or 18.2°C. Consistently, we noticed prolonged surface times at the latter part of the shift, as compared with that at the beginning. To examine the change of surface time during a shift, the ratio of the surface time at the end of a shift against that at the beginning was calculated. In water of 18.2°C, the ratio was 1.63 ± 0.10, which was not consistently different from the ratio at 24.4°C. The male Ama in water of 18.2°C had a ratio of 1.63 ± 0.10, which was not consistently different from the ratio at 24.4°C. The male Ama in water of 18.2°C demonstrated a ratio of 1.96 ± 0.25. This ratio was consistently different from the ratio for the male Ama at 24.4°C.

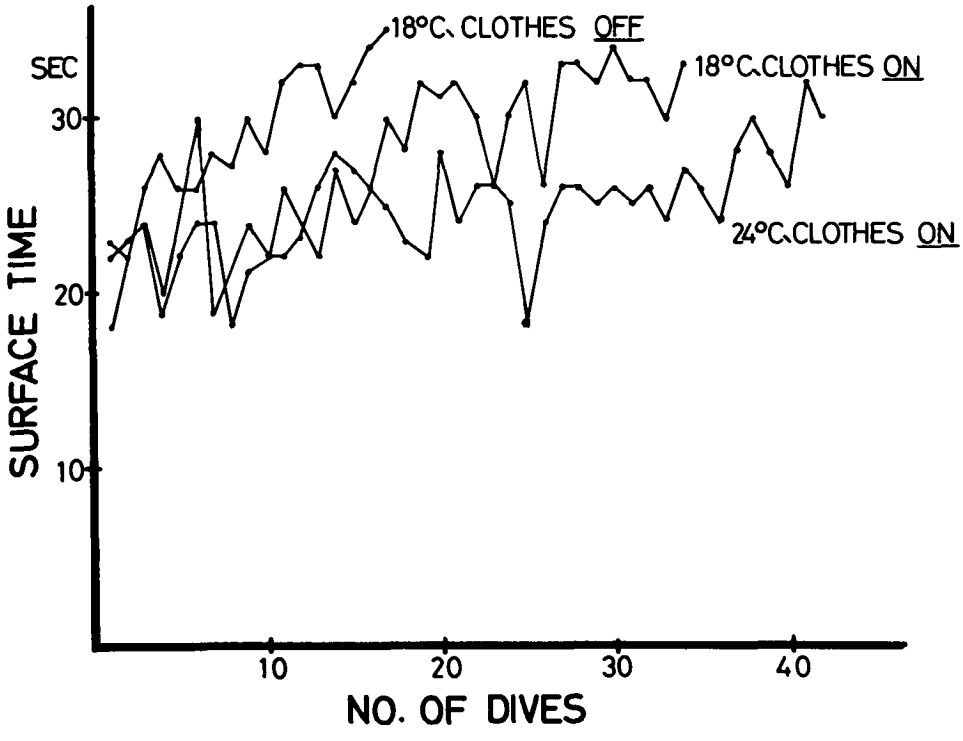

Fig. 5. Surface time, unassisted dives.

We conducted a time study on the Funado at Wagu in the Shima area (Fig. 6). Water temperature was 25.2°C on the surface and 22.6°C on the bottom and the depth was 18 m. An example is shown in Fig. 6. This shift of 35 dives continued for 96.7 min. During this particular shift the time for descent, as well as that for ascent, practically remained unchanged, showing a

mean vertical speed of descent of 1.66 m/s and that of ascent of 1.82 m/s. Mean bottom time for the initial 5 dives was 49.6 ± 2.1 s whereas that for the last 5 dives decreased to 37.6 ± 4.0 s. The mean surface time for the first 5 dives in the shift was 58.2 ± 16.0 s, which was increased to 104.4 ± 32.8 s for the mean of the last 5 dives. The Funado also demonstrated a tendency to decrease the bottom time and to increase surface time during the time course of the repeated dives, although these changes were rather slight as compared with those obtained on the Cachido.

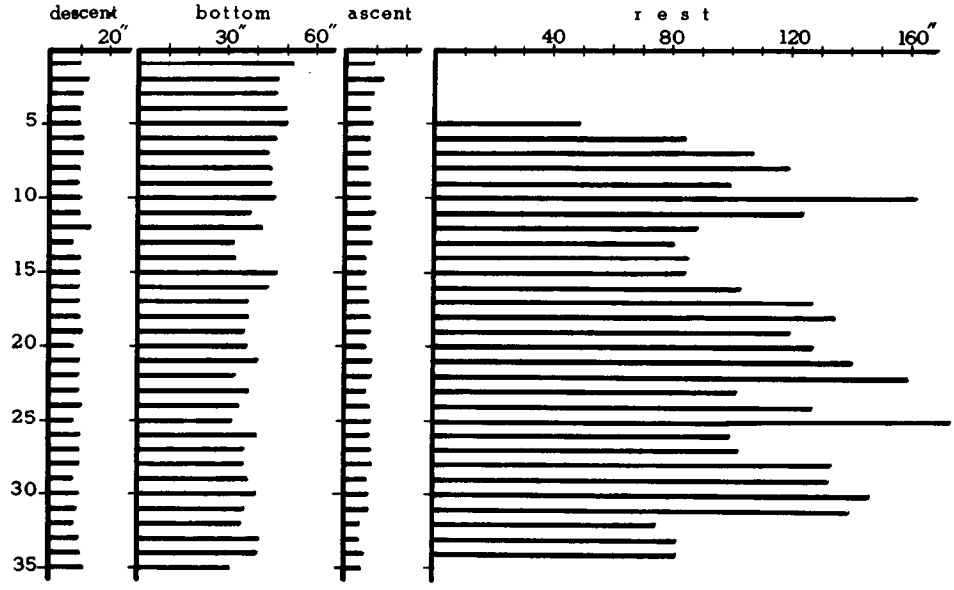

Fig. 6. Time study of the Funado's repeated dives on a shift.

Daily Energy Expenditure and Body Weight

We studied the daily caloric intake of the Ama. The daily energy expenditure for the Ama at Wagu of the Shima area was estimated to be 2800 ± 300 kcal; their daily food intake was 1780 ± 520 kcal for a day they made dives and 2180 ± 376 kcal for a day they were not able to work in the water. The daily food intake for the Ama at Chikura of the Chiba area was in the range of 2102 and 2870 kcal, giving a mean of 2327 kcal for 32 male Ama and of 1450 and 2364 kcal with the mean of 2150 kcal for 20 female Ama (Table 4). The loss of body weight during a diving season was 4.63 ± 1.84 kg (mean ± SD) for 24 males and 5.37 ± 0.83 kg for 18 females. Based on the estimated energy needed for dives and other energy expenditure for the Ama's daily life one could calculate the daily energy expenditure for an Ama's diving work. The Ama consumes 780 ml of oxygen for an unassisted breath-hold dive, and if an unassisted Ama makes 120 dives a day in two shifts, then the daily energy

expenditure used for repeated dives by an Ama is estimated to be 470 kcal. An additional oxygen consumption due to water immersion, which was estimated to be 110 kcal, has to be added. Thus daily excess energy expenditure for her diving job may be approximately 580 kcal. Usually the Ama works for a few hours in cultivating their own farm, and energy expenditure for this work may be estimated to be 270 kcal. Because the average female adult consumes energy of 2000 kcal a day, the unassisted Ama's daily energy expenditure would roughly be 2850 kcal. A decrease in the body weight during the diving season could well be explained by these estimated daily negative caloric balances, which are estimated to be -700 kcal/d. The assisted or partially assisted Ama makes dives differently from the unassisted Ama. But the total daily energy expenditure was estimated to be similar to that of the unassisted Ama.

Table 4
Caloric Balance of Ama in a Diving Season

Calorie Balance, Female Cachido, Chikura, Chiba	
Energy Expenditure	Food Intake
2000 kcal: Average female adult	2150 kcal
470 kcal: Diving work	
110 kcal: Water immersion	
270 kcal: Farm work	
2850 kcal: Daily total	2150 kcal: Daily intake
Balance: -700 kcal/d	
Body weight loss: 5.37 ± 0.83 kg/season	
780 ml oxygen/dive x 120 dives/d	

CONCLUSION

In the present paper we describe the mechanics of the diving Ama and body heat loss in relation to energy expenditure in their diving activities. We also compared energy expenditure for each type of diving pattern achieved by the Ama in Japan in terms of estimated muscular work, as well as measured oxygen debt for a dive. Although the oxygen consumption for a Cachido's dive was almost compatible with that for a Funado's assisted dive, the Funado was able to reach deeper than the Cachido and was also able to have a longer bottom time. This observation indicates that significant heat loss from the body restricts the Ama's diving work. The effect was found to be more evident in the male Ama than in the female. The female Ama could tolerate heat loss relatively better than the male Ama.

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HUMAN IMITATION OF MARINE MAMMALS AND ITS CLINICAL SIGNIFICANCE

Y-C. Lin

Human submergence without the use of artificial respiratory aids or man-made environments is, in effect, the imitation of diving mammals. Breath-hold (BH) diving involves head-out water immersion, BH or apnea, submergence, and underwater exercise. In the process, the diver encounters cold and increased ambient pressure. Every aspect of BH diving, as well as some combinations of its factors, has been shown to effect significant physiologic changes. Some of these changes have been proven clinically useful and are gradually finding their way into medical practice.

Diving physiology of marine or aquatic mammals has been reviewed extensively. Particularly relevant here is information on cardiovascular responses during diving (1-5). Humans also exhibit similar circulatory responses during BH diving, but to a lesser degree. Human responses have also been reviewed recently, though most of the information was derived from laboratory studies rather than from diving performed in natural settings (6-11). Rahn and Yokoyama (12) edited a comprehensive summary on human BH diving. This monograph and popular articles (13, 14) together with earlier work in humans (15, 16) boosted unprecedented interest in the physiology of BH diving in humans and stimulated studies worldwide, both in the laboratory and in the field.

This report pertains to the study of physiologic responses to two aspects of BH diving; namely, head-out water immersion and BH (apnea), and their clinical significance.

THE HUMAN AS A BH DIVER

Demography

Breath-hold diving is a widespread sport because it requires no equipment;

goggles or fins are optional. However, BH diving as a profession is found only in a few geographic locations. In Korea, divers are predominantly women (17), but in Japan about equal numbers of men and women are divers (18). BH diving as a profession has been declining steadily over the last 25 yr. Obvious reasons are changes in technology and job opportunities, depletion of resources (pollution and overharvesting), and the availability of man-made substitutes. If the current trend continues, BH diving as a profession could be extinct by the end of the century (17).

Professional BH Divers

Four groups of professional BH divers have been studied scientifically. These are the shell divers on the northern shore of Australia, pearl divers of the Tuamotu Archipelago, sponge divers of the Aegean Sea, and seafood collectors and pearl divers of Japan and Korea (Fig. 1). Now, the only significant number of professional BH divers are found in Japan and Korea. Most of the present knowledge about humans as BH divers in nature was obtained from Japanese and Korean divers.

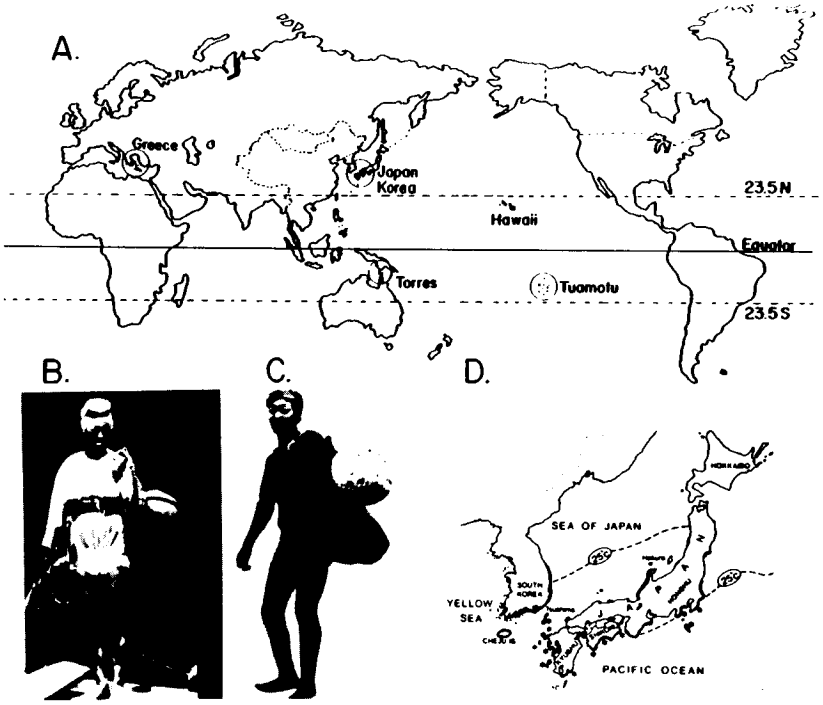

Fig. 1. A, prime locations of professional BH divers. B, C, traditional Ama with cotton suit and the modern Ama with neoprene wet suit, respectively (picture courtesy of Dr. Y. S. Park). D, in the present day, a significant number of BH divers can only be found in Japan and Korea [from Hong (8)]. Reproduced from Lin (77) with permission.

The assisted Ama, Funado, who descends rapidly with a counterweight, releases it, and works at the bottom, then is pulled up by a partner stationed on a boat, can reach 15 to 20 m depth with a total diving time averaging 60 s (19). The assisted Ama repeats another dive after 60 s of floating on the surface. In 1963, Hong and his colleagues (20) described diving patterns of unassisted diving women in Korea, called Kachido in Japan and Hae-nyo in Korea, on Cheju, an island off the southern tip of Korea. These divers descend and ascend on their own power. The average rate of descent and ascent in Korean women is symmetrical, approximately 0.6 m/s. Random observation of a large group of Ama indicated that they typically dive for 30-s duration to 5 to 7 m depth, repeating this 60 times/h. Exceptional Ama are able to perform sustained repetitive dives for around 60 s each. In 1932, Teruoka (19) reported a similar diving pattern in Japanese Ama.

The male divers of the Torres Strait archipelagoes dove for Trochus shells when buttons made from natural products were still in demand. Earlier, pearl shell diving was also popular. By the late 1950s, exhaustion of resources in shallow waters forced them to dive with surface-supplied compressed air. Similarly, scuba replaces BH diving in the Aegean Sea. According to Scholander et al. (16), most BH divers in Northern Australia can BH dive for 45 to 90 s. However, on occasion, a BH time as long as 150 s was recorded. Diving depth of 54 ft was recorded on occasion, but most dives were much shallower.

Male divers of the Tuamotu Archipelago were pearl divers. They dove regularly to exceed 30 m. According to Cross (21), many divers at the time of the survey (1957) were able to reach depths of 45 m. The maximum duration of a dive, from surface to surface, reported by Cross was 155 s. However, dives of 90 to 120 s were common, taking 30 to 50 s for descent and no more than 20 s for ascent, with a bottom time ranging from 30 to 60 s. To manage such long BH, the divers engaged in severe and prolonged hyperventilation before submergence. As human divers, this group encountered the greatest compression and decompression as well as other physiologic stresses (hypoxia, hypercapnia, and acidosis) compared to the aforementioned groups. Consequently, they are the only group that exhibited a high frequency of the decompression sickness known as taravana, literally "going crazy," suggesting CNS involvement. The symptoms ranged from vertigo, nausea, and paralysis, to death. Paulev (22) demonstrated in the laboratory that decompression sickness can indeed occur from prolonged repetitive BH dives when the product of depth and duration exceeds a certain limit. In contrast, decompression sickness is rare among Asian divers. This group exercises restraint and discipline on the diving time, depth, spacing between dives, as well as total diving duration per day (12, 18, 20, 23, 24).

Physiologic Constraints

Irimoto (23), studying daily work patterns of both male and female divers near Tokyo, made a most revealing statement regarding the nature of BH diving as a profession: "It can be said that the underwater gathering requires

quick judgment for accomplishing a series of minor activities, while being exposed to large changes in water pressure and restricted by the breath-hold time, though the relationship between man and the game is rather simple." Indeed, BH divers face the most pressing limitation of time underwater. The following studies illustrate this point clearly.

In repeated dives over 3 h, unassisted divers obtained only 37 min or 21% of the total work period at the bottom for the 5-m dives. The remaining time was spent on descent, ascent, and recovery on the surface. For the 10-m dives, the time available for collection is even more restricted. Only 17 out of 180 min, or less than 10% of the total dive time, is available at the bottom (17, 24).

A similar study was performed recently (18) on Japanese male divers at Tsushima Island, which is located between Korea and Japan. The male divers descend at about twice the rate of women, but the ascent rate is similar. Comparing a 5-m dive, total diving time was similar for man and woman, but bottom time was longer for the man (Table 1). Again, severe limitation of bottom time is evident. It seems that the evolutionary advantage of intelligence expressed as brain weight has placed the human at a disadvantage underwater (Fig. 2).

Table 1
General Pattern of Diving to 5- and 10-m Depths by Wet-Suit Divers

	Korean Women*		Japanese Men**
	10-m Dive	5-m Dive	5-m Dive
Single dive time, s	43 (100%)	32 (100%)	39 (100%)
Time for descent, s	19 (44%)	9.3 (29%)	8 (20%)
Time for ascent, s	12 (28%)	6.0 (19%)	8 (20%)
Bottom time, s	12 (28%)	16.5 (52%)	23 (60%)
Single surface time, s	85	46	42
No. of dives/h	28.1	46.2	44.4

* Park et al. (24). ** Shiraki et al. (18).

Another constraint is body heat loss in water. Even during summer, with water temperature nearing 25°C, body temperature falls continuously during immersion in water colder than 34 to 35°C. Exercise in water accelerates heat loss by reducing the insulation value of the body shell. With traditional attire (cotton suit), deep body temperature reaches 35°C in 1 h during the summer and in 0.5 h during the winter (25). Introduction of the neoprene wet suit

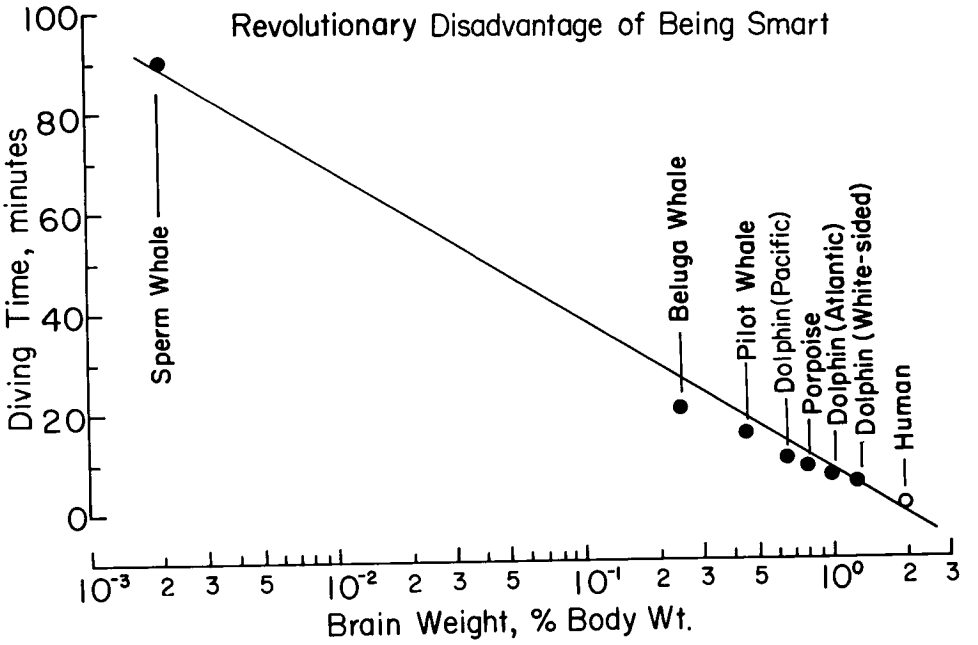

Fig. 2. Evolutionary disadvantage of being smart.

virtually eliminated the problem of hypothermia, both in summer and in winter, where water temperature is only 10°C (26). Instead of breaking work up into shifts for rewarming, as in the past, the divers now work one shift straight through in a work day. Use of the wet suit with its added buoyancy speeds up the ascent from 0.6 to 0.84 m/s, but descent rate remains the same (24).

HEAD-OUT WATER IMMERSION

As mentioned above, BH divers spend considerable time afloat on the water's surface. Head-out water immersion (HOWI), therefore, is an important component of BH diving. HOWI produces a variety of prominent physiologic effects, even in thermoneutral water (34-35°C). With temperature below 34°C, cardiovascular responses are exaggerated somewhat. The physiologic effects of water immersion have been reviewed recently, regarding respiration (27), circulation (28, 29), body fluid regulation (30-32), and temperature regulation (33, 34).

The effect of HOWI is prominent and clinically relevant. Briefly, the submerged tissues encounter: a) Hydrostatic compression which reduces venous capacitance in the lower extremities as well as displacing abdominal contents chestward; b) neutral buoyancy of human tissue which in water effectively eliminates gravity dependency; and c) a negative transthoracic pressure of about 20 cmH_2O which promotes redistribution of blood toward the upper body.

Through the combination of hydrostatic compression, neutral buoyancy, and negative pressure breathing, blood volume increases in the thoracic vasculatures, including the heart. Consequent to increase in central blood volume, a) low pressure mechanoreceptors are stimulated, b) pulmonary air space is invaded, and c) ventricular filling during diastole is facilitated. Therefore, the functional expressions of water immersion are diuresis, restrictive ventilation, and sustained increase in stroke volume and cardiac output.

Water immersion produces an unusual circulatory state, which is characterized by sustained increase in stroke volume. This change leads to varying degrees of increase in cardiac output. Calculation by weighted mean, according to the number of subjects used in each of the 8 studies, indicates stroke index increases by about 30% during immersion in 33 to 35°C water, over that in comfortable air, temperature 25 to 28°C. Heart rate decreases slightly in water, resulting in an increase of cardiac index by 24% (Table 2, Fig. 3). Results from other limited studies showed that the increased stroke volume is caused by an elevated preload of the heart as indicated by the increased central venous pressure and transmural pressure in water, and the reduced total peripheral

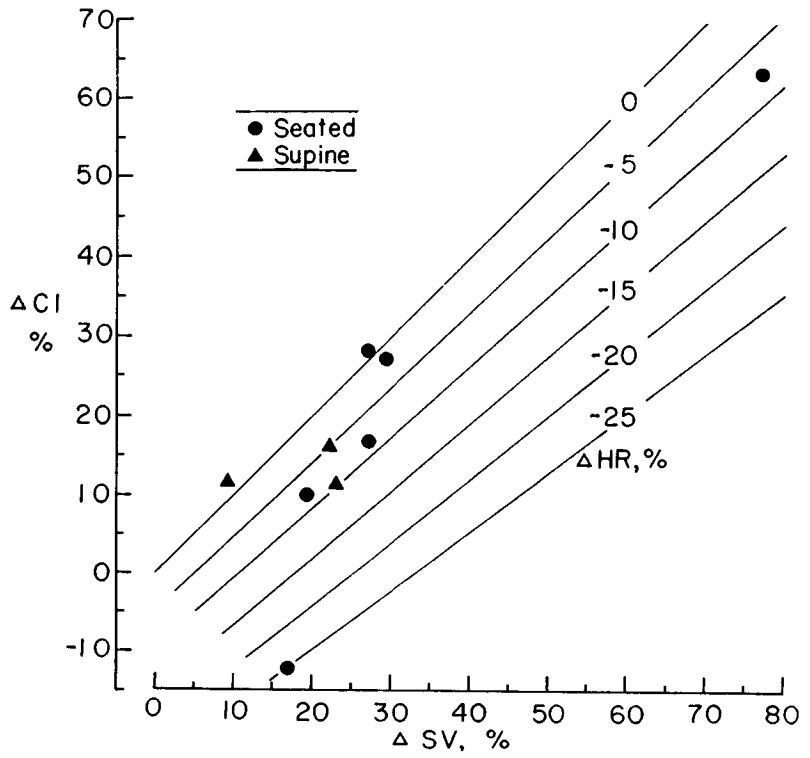

Fig. 3. Effect of head-out immersion on cardiac index (CI), stroke volume (SV), and heart rate (HR) expressed as percentage of change from their respective air controls. Reproduced from Lin (29) with permission.

Table 2
Effects of Head-Out Immersion on the Circulatory System

Measurements	Air	Water	Δ	*n*	Reports
Cardiac index, L/min/m^2	3.06	3.79	+ 24%	45	8
Heart rate, b/min	69.4	65.4	- 6%	45	8
Stroke index, ml/m^2	44.4	57.4	+ 29%	45	8
Central venous pressure, mmHg	- 1.4	15.8	+ 17.2	37	5
Esophageal pressure, mmHg	- 0.4	3.4	+ 3.8	5	1
Transmural pressure, mmHg	3.8	11.8	+ 8.0	5	1
Arterial blood pressure, mmHg					
Systolic	116.4	120.5	+ 3%	32	4
Diastolic	70.9	72.4	+ 2%	32	4
Mean	89.1	92.9	+ 4%	55	6
Total peripheral resistance mmHg(liter/min $m^2)^{-1}$	31.1	23.7	- 24%	19	3

For review, *see* Lin (29).

resistance (Table 2). In addition, we now know that cardiac output is redistributed preferentially toward the liver, fat, skin, and splanchnic region (28, 35). Of particular importance is the discovery of a five-fold increase in hepatic arterial blood flow in dogs during vertical HOWI. The clinical importance of this deserves serious attention.

Clinical Applications

The aforementioned circulatory changes form the basis for the following applications:

1. Head-out water immersion as a ground-based simulation for weightlessness. Together with bed rest studies, HOWI provides a significant data base to account for, and management of, physiologic changes during and immediately after space travel (36-39).

2. Head-out water immersion as a diuretic. Although the exact mechanism of diuresis remains unsettled (vagal-mediated cardiac mechanoreceptor-ADH response vs. the atrial natriuretic factor), HOWI has been shown to reduce excess body fluid in decompensated cirrhotic patients (32, 40, 41). Convertino et al. (42) demonstrated a suppressed plasma ADH in 10 heart-transplant patients, and 1 heart and lung-transplant patient during a -6° head-down tilt. This antiorthostatic posture, like HOWI, is a model of weightlessness.

3. Head-out water immersion promotes inert gas elimination by the sustained increase in cardiac output. Increase in cardiac output promotes inert gas elimination to a greater extent than increased respiration (43, 44). Balldin and Lundgren (45) have employed HOWI and demonstrated an increased N_2 elimination in man.

4. Head-out water immersion shortens decompression time by blood-flow redistribution to skin and fat tissues. Decompression from a deep saturation dive often takes days to accomplish. For example, safe decompression for a saturation dive to 300 m requires 12 d, and 700 m needs 31 d. Increasing blood flow to the slow compartments such as skin and fat should accelerate inert gas elimination and, hence, shorten the decompression time (44, 46).

5. Head-out water immersion increases blood flow to the liver. This property could be used for preferential delivery of drugs to or elimination from the liver. If blood flow to this organ were increased, such as during HOWI, the concentration of drugs in the general circulation could be reduced correspondingly for delivery of a drug to the liver at a given rate. This is especially important in the administration of drugs that are toxic. Similarly, HOWI, by increasing hepatic blood flow, could promote an accelerated rate of hepatic elimination of substances in the circulation. These possibilities are currently under test in the author's laboratory.

BREATH HOLD

The so-called diving reflex is a term used for describing circulatory changes during the act of breath holding. During submergence, profound bradycardia occurs in diving mammals, causing a precipitous fall in cardiac output. The intense peripheral vasoconstriction that follows the apnea matches precisely the reduced cardiac output and, consequently, the central arterial blood pressure of the predive level is maintained. The intensity and promptness of these responses are said to play an important role in conserving oxygen in natural divers. Humans also exhibit diving bradycardia, though somewhat attenuated (9-11, 15, 16). It is also known, however, that the circulatory responses in humans lack the intensity and promptness that are required for effective conservation of oxygen during diving (47). For this reason, humans are poor BH divers.

In humans, a typical circulatory response to apnea consists of bradycardia, not pronounced, but nevertheless a slowing of the heart (Fig. 4). Hypertension develops progressively in proportion to the duration of BH (10, 47), which differs from the response of marine mammals. Inasmuch as there is no evidence of increased cardiac output during BH (10, 47), we must conclude that vasoconstriction occurs in most vascular beds. The bradycardial response is by far the most frequently studied, both in the field and in the laboratory. Bradycardia develops promptly upon BH in humans, but is slow in achieving the maximal response. The BH-induced bradycardia may, in some instances, be astonishingly pronounced. Those indicated by an *a* in Fig. 5 are single subject records. The response is greatly exaggerated in men exercising while holding their breath. In some cases, the decrease may be as much as 90% of pre-BH heart rate (15, 16, 48-50).

Clinical Applications

Earlier investigators commented that the study of BH time may yield valuable information regarding temperament, will power, respiratory drive susceptibility to shock, integrity of cardiopulmonary function, and physical as well as mental fitness. Simple as BH and face immersion may be, lessons learned over the last 25 yr are plentiful and ready for harvest.

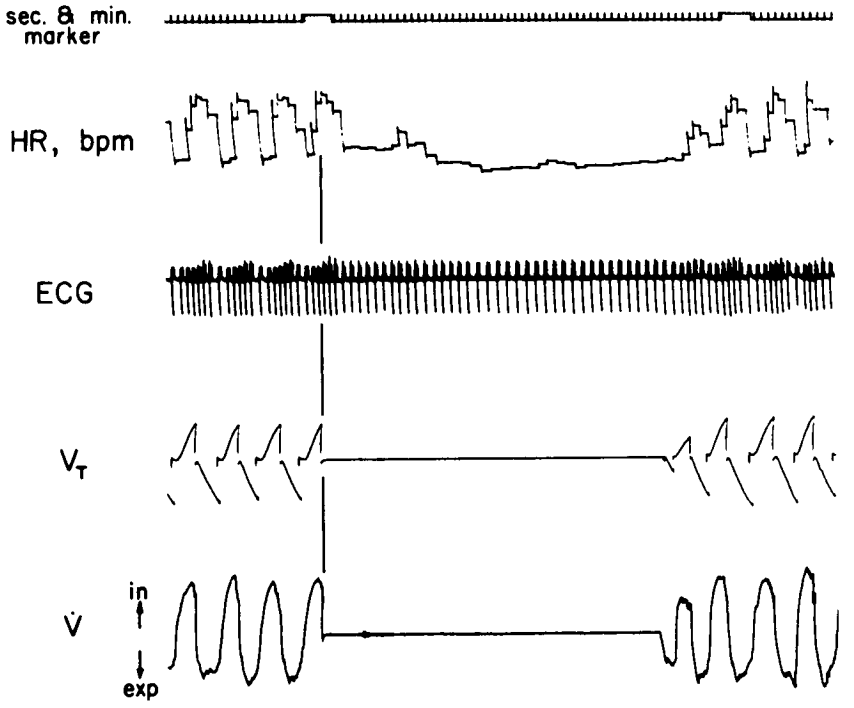

Fig. 4. Breath-hold bradycardia in humans. VT and $\dot{V}$ are tidal volume and respiratory flow rate, respectively. Bradycardia developed within three cardiac cycles after the cessation of respiration. Bradycardia intensified gradually reaching a maximum value after about 20 to 30 s of BH (78).

Paroxysmal Supraventricular Tachycardia (PSVT)

Experiments have shown that the elevation of vagal activity to the heart produces diving bradycardia as well as the BH-induced bradycardia, both in animals and in humans (10, 51), and since vagal fibers reach the supraventricular region of the heart, it should be possible to suppress supraventricular tachycardias by BH, which elevates vagal tone. Previously available methods for treating the paroxysmal supraventricular tachycardia are drugs such as digitalis, propranolol, or quinidine; electroversion; and increasing vagal tone. Vagal tone can be increased by vasopressor agents, carotid massage, Valsalva maneuver, eyeball compression, or gag reflex. All of these have been tried clinically for terminating PSVT, with some success. BH diving elicits vagotonic

action, causing bradycardia. Therefore, it is a logical choice for treating PSVT. Breath hold turned out to be the simplest and most effective way of producing increased vagal tone. Indeed, there are many reports showing affirmative results. Whayne et al. (52) reported that simulated diving abolished for several minutes the multiple premature ventricular contractions in one patient. Following this report, the diving reflex has been successfully demonstrated in terminating PSVT in infants (53-55), children (56), and adults (57-61). BH bradycardia can be effectively induced by face immersion in cold water (about 10°C) or BH with a cold towel applied to the face.

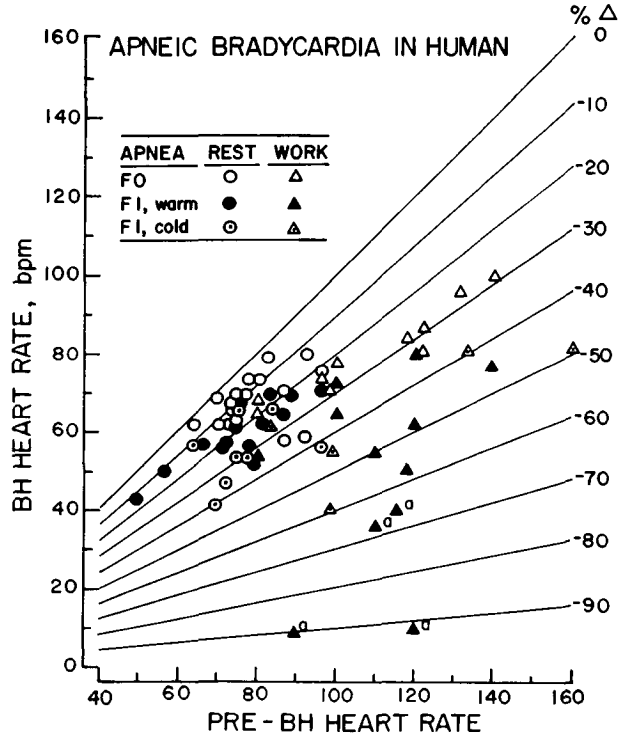

Fig. 5. Breath-hold induced bradycardia in humans. APnea at rest and during exercise is induced by BH alone (FO), face immersion in warm water (FI warm), or face immersion in water of less than 15 °C (FI cold). Data points are mean values, except those indicated by an a. Reproduced with permission from Lin (10).

Contraindications

The use of the diving reflex may not be hazard-free, however. Stimulation of the nerve pathways mediating the diving reflex may evoke serious or fatal arrhythmias in patients with preexisting organic cardiac disorders. Wildenthal and Atkins (61) cautioned that the first treatment should always be made under conditions of careful monitoring, preferably with emergency equipment available. They also indicated that contraindications for the application of diving reflex include: a) sick sinus syndrome, b) second-degree or third-degree heart block, c) concurrent propranolol treatment, and d) chronic reciprocating

atrioventricular junctional tachycardia. Though cautions are warranted where there is organic disorder in the cardiac conduction system, induction of BH-induced bradycardia seems safe even in myocardial infarct patients (62).

Near Drowning

The distinctive gas exchange pattern that was learned during a diving cycle, both in the field and in the laboratory (47, 63), clarified the causes of shallow-water accidents. Compression keeps P_{O_2} high while descending and at the bottom despite decreasing O_2 concentration, but during ascent P_{O_2} falls drastically by the decompression effect. Arterial P_{O_2} approaches that of venous blood on surfacing in a 60-s dive. However, an accident may occur when a person hyperventilates seriously before diving. In the normal course of events, divers depend on hypercapnic stimuli as the signal for surfacing. Under such conditions, oxygen depletion occurs before the arterial P_{CO_2} can build up to a level that normally signals the desire to resume breathing. Blackout often sets in suddenly without warning during the ascent phase. Shallow-water blackout still occurs at a alarmingly high rate (approximately 7700/yr in the United States). This occurs despite repeated warning (9, 13, 64) and the publication of detailed scientific findings attributing it to the fault of hyperventilation (19, 63, 65). The perils of excessive hyperventilation should be emphasized in all swimming and diving programs.

Although there is no reason to believe that oxygen conservation during diving exists in humans (47), it is important to note that apnea occurs when a person falls accidentally into water. Cardiovascular and respiratory responses that follow immersion enhance the chance of revival even after a hopelessly long time underwater. The odds of revivability improve when conditions favor a quick chill of the body, such as cold water, a thin body, and very young age (small body mass). Hypothermia and diving reflexes enhance survivability. Hypothermia reduces metabolic requirements, and the reflex apnea prevents inhalation of water and causes redistribution of blood flow, minimizing the damage to the brain and the heart. We have now learned that successful revivals are possible after 38 min (66) and more than 40 min (67) submergence. The condition that was reported by Siebke et al. (67) seems to be the most favorable, i.e., small body and cold water. They reported the successful revival of a 5-year-old Norwegian boy who regained full cerebral function after having been under ice water for more than 40 min. The valuable lesson here is that aggressive cardiopulmonary resuscitation should be attempted in drowning incidents, especially when cold water is encountered.

Cardiac Risk

Exaggerated BH bradycardia, cardiac arrest and syncope, may develop in persons with underlying cardiac diseases. Wolf (68) speculated that diving bradycardia may be related to sudden death in an elderly man while face washing. Cardiac arrhythmia develops often in BH diving, especially in cold water (16, 69-71). Such findings are rare in laboratory investigations where

only BH or BH with face immersion is involved. The most common forms of arrhythmia are those associated with enhanced cardiac vagal activity, such as a shift in the normal pacemaker locus and altered A-V condition. Occasionally, idioventricular rhythm, premature atrial beats, and premature ventricular beats were also observed. The fact that arrhythmia is rare during face immersion in the laboratory, but is common in the field, indicates that whole body immersion and cold-induced vasoconstriction may be contributory factors. It is, therefore, prudent to obtain health clearance before starting BH diving.

Myocardial Ischemia:

Kjekshus et al. (72) reported a beneficial effect of vagal stimulation on experimental myocardial ischemia in dogs. Compared to the unstimulated controls, vagal stimulation reduced mean arterial blood pressure, heart rate, ST elevation, and left ventricular dP/dt, but increased cardiac output substantially. This represents ingenious application of diving physiology. Confirmation is required, however. Whether BH-induced vagal activation benefits patients with myocardial ischemia warrants further attention.

Integrity of the Autonomic Nervous System (ANS):

BH or face immersion produces elevated vagal tone to the heart and sympathetic activity to the peripheral vessels. BH, therefore, is a simple method for assessing the integrity of the ANS in disease states. Integrity of the ANS has been so tested in myocardial infarct patients while convalescing (62) and in diabetic patients (73, 74).

Sudden Infant Death Syndrome (SIDS) and Sleep Apnea:

The primary cause of SIDS remains unresolved. Apneic spells during sleep are a common problem in preterm infants. Bradycardia occurs promptly upon apnea,whatever the cause may be. Whether cardiac arrhythmias other than the sinus bradycardia are a factor in SIDS remains to be determined. Nevertheless, exposure of the face to cold air and pressure on the eyeballs have been used to test the chronotropic liability in infants at risk of SIDS (75, 76). These procedures cause trigeminal and vagal mediated bradycardia resembling that of the diving response. Exaggerated cardiac response after provocation signals risk. Sleep apnea and prolonged asystole occur in adults more often than previously suspected (79). In some instances death may result. Guilleminault et al. (79) suggest that subjects with daytime cardiac arrhythmia suspected of secondary to abnormal vagal tone should undergo a 24-h physiologic surveillance for delineating the pathophysiology of sleep-related arrhythmia.

Upper Airway:

Mucosal stimulation of the upper airways could induce apnea and diving responses. This maneuver, therefore, may place some patients at risk (*see* **Cardiac Risk** above). Such situations may arise during examination of upper airways for dentistry, laryngoscopy, bronchoscopy; irrigation of the nose,

sinuses, or pharynx; intubation; aspiration of nasal or tracheal secretions; and application of aerosol through upper airways. We have now learned that apnea may develop in such maneuvers, and thus, caution should be exercised in these procedures, especially in patients with underlying cardiac abnormalities.

SUMMARY

Humans can imitate aquatic mammals to some degree. Breath-hold dive research started from observations in natural settings. Details of the components of BH diving are investigated mostly in the laboratory environment. Several spin-offs from research in BH diving are worthy of note. Maneuvers as simple as apnea, face immersion, or head-out water immersion show promise for clinical application. Diuresis and redistribution of blood produced by immersion have been shown to be clinically useful, besides providing physiologic data useful to space travel. Other possibilities remain to be investigated. Results from investigations on apnea have been shown to be relevant in treating cardiac arrhythmias of supraventricular origin, and in understanding SIDS and sleep apnea. Research in BH diving physiology has clarified the cause of drowning, and thus prevention is made simpler, and it has also stimulated a search for improved methods of treating drowning victims.

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HYPERCAPNIC EXERCISE: A SCREENING TOOL FOR EXTREME VENTILATORY RESPONSES IN DIVERS: A LONGITUDINAL ANALYSIS

Y. I. Daskalovic, E. Eilender, and D. Kerem

This study is a continuation of an ongoing investigation (1, 2) of the known "CO_2 retention" phenomenon in experienced divers, attempting to develop a simple predictive screening test for ventilatory compatibility in diving candidates. Because only a weak correlation was found in the past between the standard rebreathing CO_2 response test and an existing tendency for CO_2 retention during exercise on air (2, 3), this time we have adopted a longitudinal approach and a hypercapnic exercise test for the same purpose. The purpose of our study was to investigate: a) Whether hypercapnic exercise, which simulates the combined stress of external and endogenous CO_2 load encountered during prolonged closed system O_2 diving, may be used as a screening tool for prospective divers. b) Whether retrospective analysis of diving candidates' (including dropouts) CO_2 responsiveness and hypercapnic exercise data can help to establish a predictive screening test for ventilatory compatibility. c) Whether retest data after a period of extensive diving, together with the retrospective data of the dropouts, may clarify the mechanisms responsible for CO_2 retention in divers.

METHODS

The subjects were 226 novice divers with basic scuba (air and some closed-circuit O_2 diving) experience. Ninety had dropped out of diving shortly after completing the initial test. Their mean physical characteristics are presented in Table 1.

Thirty-two of the divers were retested after 8 to 48 mo. of extensive diving (up to 10 h/wk of air and oxygen diving).

The experimental system was contained in a Lanphier-Morin type wet

Table 1
Physical Characteristics of Subjects

n	Novice Divers (136)	Dropouts (90)	Retest (32)
Age, yr	19.5 (0.7)	19.6 (0.6)	21.5 (0.5)*
Wt, kg	72.5 (7.3)	73.5 (7.5)	75.5 (7.1)*
Ht, cm	178.0 (5.9)	178.0 (5.5)	177.0 (5.6)
VC, ml	5302.0 (665)	5333.0 (776)	5350.0 (577)
$FEV_{1.0}/FVC$, %	84.0 (5.8)	84.8 (5.3)	82.7 (5.9)

Mean (± SD). *n* = number of subjects. * = Different from other two groups ($P < 0.05$).

compartment of a pressure chamber. The subject was submerged and seated on an electrically braked cycle ergometer while breathing through a standard nonrebreathing, two-way valve attached to specially reinforced breathing tubes. The breathing circuit, situated in the dry chamber, could be switched from a closed bag-in-box system to an open system that provided the different gas mixtures through a demand valve (2).

Monitored variables were end-tidal CO_2 concentration (ET_{CO_2}), mixed-expired O_2 and CO_2 concentrations, respiratory rate (f), and tidal volume (V_T). Volume was measured continuously by a turbine flow transducer (K.L. Engineering). Oxygen and CO_2 concentrations were measured by Applied Electrochemistry (SA-2) and Beckman (LB-2) rapid response analyzers, respectively. Expiratory ventilation per minute ($\dot{V}_E$), oxygen uptake ($\dot{V}_{O_2}$), and CO_2 production ($\dot{V}_{CO_2}$) were calculated from the above. The work of breathing through the entire system was 0.04 kg/liter at a ventilation of 40 liter/min.

PROTOCOL AND DATA ANALYSIS

All tests were performed in the morning after a light meal. The subject performed a standard pulmonary function test (FVC and $FEV_{1.0}$) in the dry chamber, and entered the wet compartment wearing a mask, wet-suit top, and weights. After 5 min of adjustment breathing O_2, he was switched to the bag-in-box system primed with 7.0 to 7.5% CO_2 in O_2. The rebreathing lasted for about 4 min, until ET_{CO_2} reached the 9% level. A 3-min recovery period was followed by a 5-min constant load exercise, breathing air, at a $\dot{V}_{O_2}$ of 1.5 to 2.0 liter/min (eucapnic exercise). After steady state was attained, exercise was continued for 3 more min breathing 6% CO_2 in O_2 (hypercapnic exercise). Some subjects could not complete either the rebreathing or the hypercapnic exercise tests, or both, and were labeled quitters.

The following steady-state derived values were used for defining the ventilatory response to exercise: 1. Pa_{CO_2} (P_{CO_2}), estimated from ET_{CO_2} values (4); 2. $\dot{V}_E/\dot{V}_{CO_2}$, the ventilatory equivalent of CO_2, representing the standardized ventilatory response to eucapnic exercise; 3. $\dot{V}_E/\dot{V}_{CO_2}/P_{CO_2}$, in

hypercapnic exercise.

Linear regression was used to compute the scaled CO_2 response slope ($\Delta\dot{V}E/VC/\Delta PCO_2$) for each subject. Linear regression tests were performed between the above slope and the different derived variables in the two modes of exercise. Individual data, including response slopes and derived variables, of each group of subjects were averaged; the group means were compared by the unpaired Student's *t* test between the drop-outs and the remaining divers, and between divers and retested divers; in addition, a paired *t* test was performed between the first test and the retest of 32 divers.

Multiple linear regression analysis was performed to formulate a prediction equation; one for dropouts, the other for low-CO_2 responders in rebreathing, and "CO_2-retainers" in eucapnic exercise. Divers and drop-outs were assigned values of 1.0 and 2.0, respectively. Individuals with CO_2 response slopes lower than 0.30 were arbitrarily defined as low-CO_2 responders. Subjects with PCO_2 values greater than 44.0 Torr in exercise on air were arbitrarily defined as "CO_2-retainers." Independent variables chosen for the prediction equations were those with high correlation coefficients with their respective dependent variables (Table 2).

RESULTS

There was no difference in most of the physical characteristics, including VC and $FEV_{1.0}$, of the divers in their initial test and retest, or in comparison with the dropouts; only their age and weight had increased with time (Table 1).

Select correlations between the various rebreathing and exercise variables are summarized in Table 1. Its main points are: a) eucapnic exercise response and chemical drive are poorly correlated (i.e., PCO_2 [ex. air] vs. $\Delta\dot{V}E/VC/\Delta PCO_2$); b) the standardized ventilatory response to hypercapnic exercise ($\dot{V}E/\dot{V}CO_2/PCO_2$) correlates well with both the resting chemical drive and the response to eucapnic exercise ($\dot{V}E/\dot{V}CO_2$ and PCO_2); and c) there is only a weak correlation between any individual variable and divers or drop-outs.

Of the various derived parameters which retrospectively differentiate divers from short-term dropouts, both hypercapnic and eucapnic exercise variables have a good resolution. However, the CO_2 response at rest is not sensitive enough for this purpose (Table 3).

An equation with multiple variables from both hypercapnic and eucapnic exercise produced the best prediction of dropouts in the upper 15% of the continuum from 2.0 (dropouts) to 1.0 (divers), among 160 individuals (Table 4*a*). Fifteen of 24 subjects (63%) were predicted as dropouts; in the lower 15%, 18 of 24 subjects (75%) were divers. Perfect prediction would have placed only dropouts in the upper 15%, and only divers in the lower 15% of the continuum.

An equation with multiple variables from the hypercapnic exercise test

Table 2
Pearson-Correlation Test on Derived Variables in all Subjects

		REB		EX. AIR			EX. CO_2		
		$\frac{\Delta VE/VC}{\Delta PCO_2}$	PCO_2	VE/VCO_2	f		PCO_2	$\frac{VE/VCO_2}{PCO_2}$	f
REB	$\frac{\Delta VE/VC}{\Delta PCO_2}$								
	PCO_2	(-) 0.11 0.04 (258)							
EX									
	VE	0.13 0.02 (222)	(-) 0.55 0.00 (223)						
AIR									
	f	0.14 0.01 (258)	NS	0.56 0.00 (227)					
	PCO_2	(-) 0.33 0.00 (189)	0.62 0.00 (191)	(-) 0.45 0.00 (192)	(-) 0.23 0.001 (192)				
EX									
	$\frac{\dot{V}E/\dot{V}CO_2}{PCO_2}$	0.32 0.00 (189)	0.47 0.00 (191)	0.50 0.00 (192)	0.46 0.00 (193)				
CO_2									
	f	0.24 0.00 (217)	(-) 0.32 0.00 (195)	0.30 0.00 (199)	0.57 0.00 (199)	(-) 0.29 0.00 (193)	0.38 0.00 (193)		
	D/DO	0.11 0.03 (256)	NS	0.24 0.00 (191)	0.22 0.00 (243)	NS	0.15 0.03 (157)	0.15 0.03 (164)	

Top number = correlation coefficient R; (-) = negative R; 0.00 = P value (2-tail probability); NS = $P > 0.05$; EX = exercise; numbers in parenthesis are numbers of subjects; P_{CO_2} in both exercise modes are arterial values estimated from ET_{CO_2} (4); D/DO = divers or dropouts.

Table 3
Differences Between Group Means of Divers and Dropouts

	Variable	Group	n	Mean	± SD	Δ	P value (Two Tail)
R		DO	70	0.60	0.25		
E	$\Delta\dot{V}E/VC/\Delta PCO_2$					0.03	NS
B		D	120	0.57	0.26		
E		DO	51	25.7	2.8		
X	$\dot{V}E/\dot{V}CO_2$					1.2	0.008
		D	83	24.5	2.3		
A		DO	67	25.5	7.5		
I	f					3.2	0.002
R		D	119	22.3	6.1		
		DO	29	50.4	3.3		
	PCO_2					-3.7	0.000
E		D	93	54.1	4.5		
X							
		DO	31	2.4	0.4		
	VT					-0.3	0.006
C		D	95	2.7	0.5		
O							
2		DO	29	0.65	0.2		
	$\dot{V}E/\dot{V}CO_2/PCO_2$					0.03	NS
		D	93	0.62	0.2		

DO = dropouts; D = divers; EX = exercise; volumes in 1 BTPS; ventilation in liter per minute BTPS; partial pressure in Torr; $\dot{V}CO_2$ in liter per minute STPD.

enabled the prediction of low-CO_2 responders and CO_2-retainers among 186 individuals (Table 4*b*):

a. *Low-CO_2 Responders.* ($\Delta\dot{V}E/VC/\Delta PCO_2 < 0.3$)—13 of the 26 low responders (50%) were predicted in the lower 15% of all subjects; 77% (20/26) were predicted in the lower 30%; 6 were missed.

b. *CO_2-retainers.* ($PCO_2 > 44$ Torr in ex. air)—60% (15/25) were predicted in the upper 15% of all subjects; 84% (21/25) were predicted in the upper 30%; 4 were missed.

Repeat longitudinal testing showed resting chemical drive ($\Delta\dot{V}E/VC/\Delta PCO_2$) to be unchanged over the period checked; a tendency to increase exercise CO_2 levels and decrease respiratory rate is apparent (Table 5). This trend was demonstrated both by paired and unpaired statistical analysis.

Table 4

a. Prediction Equation For Dropouts

$$\text{Pred} = 0.352 + 0.028\underset{\text{(EX. AIR)}}{\dot{V}E/\dot{V}CO_2} + 0.0097f + 019\ \underset{\substack{PCO_2 \\ \text{(EX. } CO_2\text{)}}}{\underline{\dot{V}E/\dot{V}CO_2}} + 0.0015f$$

$$(R = 0.26)$$

b. Prediction Equation for "CO_2-Retainers" and Low CO_2 Responders

$$\text{Pred} = a + bPCO_2 + c\ \underset{\substack{PCO_2 \\ \text{(EX. } CO_2\text{)}}}{\underline{\dot{V}E/\dot{V}CO_2}} + df$$

Prediction	a	b	c	d	R
"CO_2-Retainers"	26.64	0.37	-6.28	-0.114	0.68
Low-CO_2 Responders	1.93	-0.012	-0.295	0.0022	0.38

DISCUSSION AND CONCLUSIONS

Resting chemical drive and ventilatory response to exercise seem to contribute independently to ventilation rates and CO_2 levers during the combined stimulus of endogenous and external CO_2 load. In corroboration with previous findings (2, 3), the above two responses seem poorly related. Hypercapnic exercise per se, and its derived prediction equation, should therefore be the test of choice for screening diving candidates for possible anomalies in ventilatory response in prolonged closed-system diving. Although our prediction equation does not identify *all* CO_2 retainers and low-CO_2 responders, none of the *extreme* cases were missed.

It may be noted that although the so-called quitters were significantly higher CO_2-ventilatory responders (Table 6), their correlation with the various derived test variables was weak; their contribution to the prediction of drop-outs was minor as well. The reason for this may be that although 29% of the drop-outs were quitters, 15% of all divers were also quitters.

Another point has to be made about the *relatively* low accuracy of the drop-out prediction by our hypercapnic exercise test-derived formula. Inasmuch as our prediction can apply only to physiologic factors and there might have been several other factors that decided a diving candidate's future in the diving profession, i.e., health, discipline, social problems, etc., it seems reasonable that we had missed quite a few. On the other hand, strong-willed

Table 5
Test-Retest Difference between Group Means (Paired *t* Test)

	Variable	Group	*n*	Mean	± SD	Δ	*P* Value (Two Tail)
REB	$\Delta\dot{V}E/VC/\Delta PCO_2$	D	32	0.62	0.3	-0.09	NS
		RD		0.53	0.4	(0.04)	(NS)
EX	$\dot{V}E/\dot{V}CO_2$	D	24	24.5	1.9	-0.6	NS
		RD		25.1	3.2	(0.0)	(NS)
AIR	PCO_2	D	29	38.3	4.8	-3.7	0.003
		RD		42.0	5.3	(-4.6)	(0.000)
	f	D	29	22.8	5.6	0.9	NS
		RD		21.9	6.4	(1.3)	(NS)
EX	$\dot{V}E/\dot{V}CO_2/PCO_2$	D	16	0.59	0.2	-0.07	NS
		RD		0.66	0.1	(-0.05)	(NS)
CO2	PCO_2	D	16	53.1	2.9	-4.8	0.001
		RD		57.9	3.9	(-3.7)	(0.001)
	f	D	16	28.4	6.1	5.1	0.005
		RD		23.3	3.7	(3.9)	(0.001)

Numbers in parenthesis are unpaired *t*-test values; D = diver; RD = retest diver; EX = exercise; volumes in 1 BTPS; ventilation in liters per minute BTPS; partial pressure in torr; VCO_2 liters per minute STPD.

Table 6
Difference Between Group Means of Quitters and Nonquitters

Variable	Group	n	Mean	SD	Δ	P Value (Two Tail)
R E B $\Delta\dot{V}E/VC/\Delta PCO_2$	Q	51	0.62	0.25	0.09	0.011
	NQ	143	0.53	0.25		
EX AIR $\dot{V}E/\dot{V}CO_2$	Q	34	26.2	2.5	1.8	0.000
	NQ	118	24.4	2.4		
E X PCO_2	Q	31	50.2	3.2	-2.6	0.002
	NQ	112	52.8	5.8		
C O 2 VT	Q	33	2.3	0.4	-0.4	0.001
	NQ	113	2.7	0.7		

Q = quitters; NQ = nonquitters; EX = exercise; volumes in l BTPS; ventilation in liter per minute; partial pressure in Torr; VCO_2 in liters per minute STPD.

high responders could adapt to the ventilatory stresses and remain in the profession.

Natural selection seems to prefer low responders to hypercapnic exercise stimuli to remain in the diving profession (Tables 3 and 5). Extremes in this respect should at least be made aware of the potential risks of hypercapnia in closed-system diving. If their low response stems mainly from a very blunted resting chemical drive, the risks may be very serious.

The results of longitudinal testing obtained so far (Tables 3 and 5) show elements of both inherent (divers vs. dropouts) and acquired (retested divers) tendencies to hypoventilate during exercise. Extending the longitudinal axis and the number of repeat subjects may further help explain the CO_2 retention and low resting chemical drives of more experienced divers.

In conclusion, there now exists a reasonably good predictive test for detecting or screening diving candidates for both CO_2 retention and future success in the diving profession. This relatively simple test of hypercapnic exercise is easy to administer and its benefits should be unquestionable. We can now repeat, and with more conviction, our previous recommendation (2) that all closed-circuit diving candidates should be tested, and that the very low responders (after further verification by a resting rebreathing test) be

excluded from such diving. However, only on completion of our ongoing investigation of CO_2 retention and ventilatory response to exertion in real diving situations will we have optimal validation of our present findings.

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PHYSICAL AND PHYSIOLOGIC ADAPTATIONS TO BREATH-HOLD DIVING IN HUMANS: A REVIEW

S. K. Hong

Probably millions of people in this world are engaged in amateur breath-hold (BH) dives for recreation. They are usually equipped with a facemask (or goggles) and a snorkel and dive to a shallow depth for short periods of time. On the other hand, many thousands of professional divers go to deeper depths for longer periods for certain underwater activities. The latter group includes Navy divers, female divers of Korea (Hea-Nyo), male and female divers of Japan (Ama), sponge divers in Greece, and pearl divers in the Tuamotu Archipelago. These divers learned the art of diving through many years of hard training that increased both the depth of diving and the bottom time, with minimal risks.

Unlike a scuba diver, a BH diver usually stays underwater for less than 2 min. During this short period, however, divers are exposed to certain physical and physiologic stresses, including the hydrostatic compression of the thorax, hypercapnia (and attendant changes in acid-base balance), hypoxia, physical exertion, and body heat loss. As divers engage in BH diving over a long period, they develop specific physical and physiologic adaptations to each of the above stresses, which markedly improve their diving ability. The characteristics of these adaptation phenomena reported for human BH divers, such as professional male and female divers of Korea and Japan and U.S. Navy Escape Training Tank instructors, will be systematically reviewed in this presentation.

LUNG VOLUMES AND MAXIMAL RESPIRATORY PRESSURES

Various lung volumes as measured in four different groups of BH divers are summarized in Table 1. It is evident that vital capacity is significantly greater in all diver groups as compared to that in the paired control. However,

Table 1
Lung Volumes (ml, BTPS) of BH Divers

Subjects	RV		Exp. RV		Inspiratory Capacity		Vital Capacity		Total Lung Capacity		Reference
	C	D	C	D	C	D	C	D	C	D	
Navy divers, male	1465	1593	1405	1317	2489	3057	4654	5397	6102	6990	(2)
	NS		NS		$P < 0.02$		$P < 0.01$		$P < 0.01$		
Korean Ama, female	1040	1140	1100	1050	1960	2390	3060	3440	4100	4580	(1)
	NS		NS		$P < 0.005$		$P < 0.01$		$P < 0.025$		
Japanese Ama*, female			610	621	562	798	1540	1790			(7)
			NS		$P < 0.01$		$P < 0.01$				
Japanese Ama, male			1241	1781	2644	2893	3885	4674			(4)
			$P < 0.05$		NS		$P < 0.05$				

*Lung volumes are expressed in ml/m^2 body surface; C = nondivers (control); D = divers; NS = not significantly different.

residual volume measured in only two of the groups was not significantly different from the corresponding control value. Larger vital capacity observed in the divers was entirely due to larger inspiratory capacity, except in Japanese male divers. In the latter group, larger vital capacity was mostly due to larger expiratory reserve volume. Measurements of maximal inspiratory and expiratory pressures in the divers indicate that the former is significantly greater in divers compared to controls (1). This means that inspiratory muscle force at a given lung volume is greater in the diver.

To ascertain that these changes in the lung volume are due to diving activities, Carey et al. (2) conducted longitudinal studies in U.S. Navy divers and found significant increases in inspiratory capacity, vital capacity, and total lung capacity after 1 yr of diving training (Table 2). Similar longitudinal studies were carried out by Bachman and Horvath (3) in athletes engaged in either swimming or wrestling training for 4 mo. As shown in Table 3, the swimmers showed significant increases in inspiratory and vital capacities, whereas wrestlers showed no significant changes in any lung volumes after the designated physical training program. These studies suggest strongly that higher inspiratory and vital capacities observed in divers cannot be

Table 2
Effects of BH Diving Training for 1 yr on Lung Volumes [Data from (2)]

Lung Volume	Changes in Volume ml BTPS	*P* Values
Tidal volume	+ 154	< 0.05
Inspiratory rev. volume	+ 151	< 0.05
Expiratory rev. volume	+ 141	NS
Vital capacity	+ 454	< 0.001
Residual volume	- 113	NS
Functional respiratory capacity	+ 32	NS
Total lung capacity	+ 381	< 0.001

+ = increase; - = decrease; NS = statistically insignificant.

Table 3
Changes in Lung Volumes (ml BTPS) During 4 mo. of Swimming or Wrestling Programs [Data from (3)]

Lung Volume	Swimmers	Wrestlers
Respiratory volume	- 260 ($P < 0.05$)	+ 40 (NS)
Expiratory residual volume	- 50 (NS)	+ 60 (NS)
Functional respiratory capacity	- 310 ($P < 0.05$)	+ 100 (NS)
Inspiratory capacity	+ 360 ($P < 0.05$)	+ 10 (NS)
Vital capacity	+ 320 ($P < 0.05$)	+ 60 (NS)
Total lung capacity	+ 60 (NS)	+ 100 (NS)

+ = increase; - = decrease; NS = statistically insignificant

attributed to daily physical workload in the air environment but to aquatic surface activity per se.

When a diver is immersed in water to the neck during surface intervals between two successive dives, intrapulmonary pressure becomes negative with respect to pressure surrounding the chest wall. As a result, the diver has to engage in the so-called "negative pressure breathing" in which inspiration becomes difficult while expiration becomes easier than during normal breathing in the air environment (where a pressure gradient between environment and lungs does not exist). The average level of negative pressure breathing during head-out immersion is estimated to be -16 cmH_2O (4). Because the inspiratory muscles have to work against this pressure gradient during head-out immersion, they seem to be developed to a greater extent than the expiratory muscles. This probably accounts for increases in the inspiratory capacity and the maximal inspiratory pressure observed in divers.

Theoretically, the maximal depth of a BH dive is determined by the total

lung capacity:residual volume ratio. The larger the ratio, the deeper the maximal depth. However, residual volume in both Navy divers and Korean women divers was not different from that of control subjects (Table 1). Although residual volume decreased by 113 ml after 1 yr of diving training in the longitudinal study conducted by Carey et al. (2), this change was not significant. However, Bachman and Horvath (3) reported a significant reduction of residual volume (by 260 ml) after 4 mo. of swimming training; the latter change was also accompanied by a significant increase in total lung capacity:residual volume ratio. It thus seems that residual volume is also subject to adaptation to diving training. However, the mechanism underlying such a reduction in the residual volume as a result of diving (or swimming) training is not clear.

Residual volume is determined by the static balance of muscle and recoil forces in the chest wall (5). In this regard, it is of interest to note the report by Kobayashi et al. (6) who found a greater "body wall pliability" in the Japanese Ama. A larger compressibility of the chest wall could account for heretofore unexplained lowering of residual volumes reported in swimmers (3). However, once again we are at a loss to explain how the body wall pliability is increased in divers.

Maximal voluntary ventilation (MVV) is generally greater in divers as compared to controls. For instance, MVV in Korean Ama is approximately 30% greater than that of nondiving women of similar age and physical characteristics (1). The magnitude of this difference in MVV between the 2 groups is about the same as that in vital capacity, suggesting that no difference exists in relative overall airway resistance between the 2 groups. On the other hand, Tatai and Tatai (7) showed that the percentage increase in MVV for Japanese Ama is far greater than that in vital capacity, which suggests a relative lowering of airway resistance. To resolve this difference, one needs more information on the airway resistance than is presently available.

CO_2 ADAPTATION

During the descending phase of BH diving, the thorax is compressed, thereby decreasing lung volume while increasing partial pressure of gases in the lung. The resultant increase in alveolar CO_2 pressure eventually reverses the usual direction of the diffusion of CO_2, transferring CO_2 from the lung to the circulation. Consequently, blood CO_2 pressure continuously increases during descent and while on the bottom (8).

In response to repetitive exposures to such intermittent hypercapnia, most BH divers (e.g., Korean women divers and Navy divers) show a reduced sensitivity of their ventilatory system to a given hypercapnia (1, 9). In studies conducted in Navy divers, Schaefer (9) noted that high tolerance to CO_2 (as indicated by the reduced CO_2 sensitivity of the ventilatory system) was lost after a 3-mo. lay-off. In this connection, it is interesting that Honda et al. (10) failed to demonstrate a reduced CO_2 sensitivity in unassisted divers of

Japan (Cachidos) who dive to shallow depths (~5 m) only during warm seasons. On the other hand, Masuda et al. (11) found reduced CO_2 sensitivity in assisted divers of Japan (Funado), who are also seasonal divers but dive to deeper depths (~10 m). Obviously, Cachido divers of Japan are not exposed to hypercapnia long enough to develop any adaptation, because the diving pattern of these divers is identical to that of Korean Cachidos who dive all year round and exhibit a reduced CO_2 sensitivity. On the other hand, Funado divers of Japan, seem to have developed CO_2 adaptation because of their deeper diving depths which should amplify the degree of hypercapnia.

Schaefer (9) also reported other parameters of adaptation to CO_2 in the Escape-Training Tank instructors, which are characterized by the higher concentrations of CO_2 (both H_2CO_3 and HCO_3) in plasma and red cells, lower plasma pH, lower water content of plasma, lower plasma Na and K, and lower K and higher Na in red cells. These differences in blood chemistry between divers and nondivers may be related to repetitive exposure to respiratory acidosis. In addition, Schaefer reported that a high tolerance to CO_2 in divers seems to be associated with a reduced adrenergic and stress response to CO_2. For instance, in response to an injection of a cholinergic drug (Mecholyl), divers exhibited a significantly smaller fall in blood pressure than nondivers. This finding suggests that adaptation to hypercapnia may include a depressing effect on vascular cholinergic receptors. Evidence of an increase in CO_2 stores was also obtained after a 2-yr period of diving training in Navy divers, as indicated by the elimination of a greater amount of CO_2 during 1-h hyperventilation (9). Although these adaptive changes observed in divers in response to hypercapnic stress are interesting, neither the physiologic significance nor the underlying mechanism is entirely clear at present.

ADAPTATION TO HYPOXIA

The alveolar O_2 pressure is maintained higher than the predive level during descent and while working on the bottom because of chest compression. However, it decreases rapidly during ascent because of decompression of the chest, often subjecting the diver to an extreme hypoxia (8). In response to repetitive exposures to such hypoxia, the ventilatory response to low O_2 is considerably lower in both the Escape-Training Tank instructors (9) and the Japanese Funado divers (11). However, such hypoxic adaptation has not been demonstrated in Korean Cachido divers (1). Although it is not known why there is a difference in ventilatory response to hypoxia among different groups of BH divers, it is again likely that different diving patterns may explain the results. Both the Escape-Training Tank instructors and Funado divers routinely dive to a depth ranging from 10 to 20 m as compared to ~5 m for Cachido divers. Because the degree of hypoxia during ascent is greater as the depth of dive increases, it is possible that Cachido divers may not develop a sufficient hypoxia during ascent to induce adaptation.

Schaefer (9) also reported that the lower ventilatory response to low O_2

breathing in the Escape-Training Tank instructors was associated with the formation of a larger O_2 debt. The magnitude of this O_2 debt incurred during 33 min of breathing 10.5% O_2 in N_2 amounted to 1032 ml in the divers (vs. 348 ml in nondivers), which cannot be met by the O_2 reserves. On this basis, Schaefer speculated that the tissue oxidation decreased in the divers during hypoxia. Although the above finding represents another form of adaptation to repetitive exposures to hypoxia, the biochemical and/or physiologic basis for this phenomenon is not clear at present. It is possible that a shift to an anaerobic energy-yielding process might have occurred in the divers. In this regard, it may be of interest to note that the magnitude of O_2 debt incurred during maximal exercise was the same in both Korean women divers and nondivers (12).

DIVING BRADYCARDIA

A significant bradycardia develops during BH diving (13), which can be reproduced by BH face immersion in water (14). Using the latter technique, the degree of bradycardic response was compared between professional divers and nondivers. In two studies using Hawaiian male divers (15) and Korean women divers (unpublished data of B.S. Kang), a significantly greater bradycardia as compared to controls was observed in the divers. However, it is not clear at present what these observations mean. It is tempting to speculate that this bradycardia response to diving may be subject to operant conditioning.

COLD ADAPTATION

That the body cools much faster in water than in air of the same temperature is well recognized. This is because the specific heat of water is 1000 times and thermoconductivity 25 times greater than those of air. This rapid loss of body heat from the core to the surrounding water through the body surface is the dominant problem of the human diver. The subcutaneous fat thickness (SFT) is usually not high in human divers and thus their diving activity has been dictated by the sea water temperature unless they wear wet suits to avoid cold water stress.

Temperature of the sea in which Korean diving women are engaged in daily diving is approximately 25°C in the middle of summer and 10°C in winter (16). Thus, even in summer, water temperature is considerably lower than a thermoneutral level (34 to 35°C for average human subjects). Despite the presence of such a potential cold stress to which these women divers are daily exposed year round, they used to wear light bathing suits made of cotton throughout the year. Consequently, their daily diving work schedule was largely dictated by the sea water temperature. On a typical summer day, these divers take three shifts a day, each lasting approximately 40 to 60 min. On the other hand, they usually take one to two short shifts (each lasting 15-30 min) a day in midwinter. A linear relationship exists between the duration of a work shift

and sea water temperature, clearly indicating that the main factor limiting the duration of work period is cold water stress.

Comprehensive studies on thermoregulatory functions of traditional Korean women divers (cotton suit divers) were conducted during the 1960s to determine if cold adaptation occurs in humans. The following pattern was obtained [*see* a review by Hong (17)]: a) a consistent, reversible increase in basal metabolic rate (BMR) in winter when sea water temperature is lowest (i.e., metabolic adaptation); b) a very small (but significant) increase in O_2 consumption in response to exogenous norepinephrine in winter but not in summer, indicating that the development of nonshivering thermogenesis is not the main feature of cold adaptation; c) a lower critical water temperature (T_{cw}) at a comparable SFT in both summer and winter, indicating the elevation of shivering threshold (i.e., hypothermic adaptation, most probably representing habituation); d) a greater maximal body (thermal) insulation (I_{max}) at a comparable SFT in both summer and winter; e) evidence for a more efficient countercurrent heat exchange system in the limbs; and f) maintenance of the lower finger skin temperature and blood flow ($\dot{Q}_{finger}$) during immersion of one hand in 6°C water (i.e., vascular adaptation). To ascertain the conclusion that these changes in thermoregulatory functions, observed in traditional (unprotected) Korean women divers, truly represent cold adaptation, we repeated the same studies in contemporary wet-suit divers (18). The rationale for this new series of studies was that cold adaptation as developed in traditional divers should disappear gradually once cold water stress is removed by wearing wet suits. Field studies conducted in the open ocean indeed proved that contemporary wet-suit divers are not exposed to any cold water stress (19). Remarkably, contemporary divers (who started wearing wet suits from 1977) exhibited a gradual cold deadaptation with the following time course: BMR, 3 yr; I_{max}; $\dot{Q}_{finger}$, 4 yr; and T_{cw}, 5 yr. These findings, demonstrating the differential time course for loss of various cold adaptation phenomena, clearly indicate the complicated nature of cold adaptation process. Most likely, cold adaptation represents many components with different time constants.

Although the demonstration of cold adaptation in these divers is important in terms of human biology, it should be noted that adaptation to cold is rather ineffective as a real defense against long, slow cooling, particularly if accompanied by exercise (20).

SUMMARY

In response to repetitive exposures to a variety of physical and physiologic stresses over a prolonged period, human divers develop various types of adaptation. Although the exact mechanisms underlying these adaptation phenomena are not clearly elucidated at present, there is no doubt that the overall efficiency of BH diving is considerably improved by development of unique adaptations which may serve to blunt or circumvent diving-related stresses.

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SESSION 2: POSTER PRESENTATION: BREATH-HOLD DIVING AND NEAR DROWNING; THERMAL PROBLEMS

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HEAT EXCHANGE IN MAN DURING EXERCISE IN WATER AT A SIMULATED DEPTH OF 10 M

S. Sagawa, K. Shiraki, H. Yoshino, and N. Konda

The use of wet suits protects man against cold stress during dives and enables him to extend considerably the duration of diving work (1, 2). Because physical insulation of wet suits is lower at depth than at the surface due to compression of trapped air (3, 4), in a deep dive humans lose body heat at a higher rate than in a shallow dive. An indirect evaluation of heat exchange in water has been made by estimating metabolic heat production and changes in body temperature (5). However, no publications have reported the direct measurements of heat loss during exercise in man wearing wet suits at certain depths. It is possible to estimate directly the heat exchange from the surface of the skin and wet suits by using heat flow transducers attached to respective surfaces. A pressure regulation chamber (hyperbaric chamber) equipped with a water tank and an exercise device enables us to simulate exercise tests in a given depth of water. The combination of these two devices makes our experimental design practical for collecting the desired thermal data in which accuracy is often sacrificed in field observations. Therefore, the present experiment was designed to obtain direct measurements of heat loss from the surface of the body and the wet suits and to evaluate the thermal insulation of the whole body as well as of different regions of the body during rest and exercise at a simulated depth of 10 m. This experiment provides insight into the thermal problems of wet-suit divers in waters in which the temperature is below thermoneutral.

METHODS

Subjects

Six healthy male volunteers with average age of 29.3 yr participated in

this experiment. Physical characteristics of the subjects are summarized in Table 1. All subjects signed consent forms after being thoroughly familiarized with the experimental procedures. The surface area of the body was calculated from height and weight (6). The calculation of surface area with wet suits was measured by applying the linear formula of DuBois (7). Skinfold thicknesses of 10 different skin areas were measured with a Lange caliper, and mean subcutaneous fat thickness and adiposity were estimated according to the description given by Allen et al. (8).

Table 1
Physical Characteristics of Subjects

Subject	Age, yr	Height, cm	Weight, kg	S.A., m^2	MFT, mm	Body Fat, %
1	36	169	61.9	1.72	2.7	14.2
2	32	165	66.5	1.75	5.7	20.8
3	32	180	73.4	1.94	3.9	17.1
4	30	168	70.7	1.82	6.6	22.4
5	24	163	58.7	1.64	5.3	20.1
6	22	172	65.0	1.78	1.2	9.2
Mean	29.3	169.5	66.0	1.78	4.2	17.3
± SE	2.2	2.5	2.2	0.04	0.8	2.0

S.A. = Dubois area; MFT = mean subcutaneous fat thickness.

Experimental Protocol

Test Study

All water immersion experiments were performed in a stainless steel tank with dimensions of 0.66-m wide, 1.92-m long, 1.30-m deep placed in a hyperbaric chamber. Water in the tank was stirred continuously by a circulation pump at a rate of 87 liter/min. Water temperature was monitored continuously by a copper-constantan thermocouple and was maintained constantly at 25.0 ± 0.1°C during the experimental period. For the experimental sessions, each subject arrived at the laboratory at 0830. The subject was harnessed to skin thermocouples, heat-flow discs, esophageal probe, and ECG electrodes and then dressed in fitted neoprene wet suits (5-mm thick) and boots and gloves (4-mm thick) in the chamber where the temperature was constant at 25°C. Thermocouples and heat-flow discs were also attached to the surface of the wet suits, corresponding to that region of the skin and secured with a water-permeable surgical tape (Hogi Van, Japan). After sitting for 60 min in air (equilibrium period) the diver was immersed in water up to the neck and seated on a wide-meshed chair. At normal atmosphere (1 ATA), the subject rested for 40 min in water and then performed exercises by lifting each leg 70 cm for 20 min at a rate of 40 times/min with a 4-kg counterweight on each leg. Using this leg

exercise, we could provide the subject a workload of 2.5 times the resting metabolic rate. Ten minutes after terminating exercise, the chamber was pressurized to 2 ATA. Room temperature was maintained at 25°C throughout the experiment. At 2 ATA, the subject followed the same time schedule as that of 1 ATA.

Measurements

All temperature measurements were made with copper-constantan thermocouples. Esophageal temperature (T_{es}) was measured with a thermocouple swallowed to the heart level. Measurements of skin temperature (T_{sk}) were obtained by eight point thermocouples on the forehead, hand, forearm, chest, abdomen, thigh, leg, and foot. Wet-suit temperatures (T_{suit}) were measured on the surface of the wet suits corresponding to the skin region, except for the forehead. Mean skin ($\bar{T}_{sk}$) and mean wet-suit temperatures ($\bar{T}_{suit}$) below the neck were calculated using the area weighting factor (9). Heat flow from the skin surface (H_{skin}) and wet-suit surface (H_{suit}) was measured with a heat-flux transducer (Thermonetics, HA-13-18-PC) attached to the vicinity site of the thermocouple on the skin and wet suits. Each heat flux transducer was calibrated and found to be identical in both air and water. Mean heat flows from the immersed skin and wet suits (below the neck) were calculated as regional heat flow times in the respective regional areas. For regional areas, the same weighting factor as that for $\bar{T}_{sk}$ (9) was applied. Attachments of thermocouples and heat flow discs on the corresponding skin and the wet-suit surfaces enabled the estimation of direct heat loss and insulative values of the wet suits. Body temperature, heat flow, and heart rate were monitored continuously and stored every 15 s on a data logger (7V07, San-ei Sokki, Tokyo) to be analyzed by a computer (M243, Sord, Tokyo).

Oxygen consumption was measured by an open-circuit method. The expired gas was collected into a Douglas bag through a mouthpiece once every 10 min while resting and once every 5 min throughout the exercise. Metabolic heat production ($\dot{M}$) was calculated from $\dot{V}O_2$ and the respiratory quotient. Thermal insulation of the body (I_{tis}) and wet suits (I_{suit}) was calculated as follows:

$$I_{tis} = (T_{es} - T_{sk})/H_{skin} \quad (1)$$

$$I_{suit} = (T_{sk} - T_{suit})/H_{suit} \quad (2)$$

For calculation purposes, the average value of the last 5 min of resting or exercise was used.

Heat storage (S) was calculated as follows:

$$\dot{S} = 0.92 * \dot{M} - \dot{H} \quad (3)$$

where 0.92 is a constant given by assuming that 8% of the heat produced is

lost via the respiratory tract (10). H is the total heat loss from the skin (including the unimmersed head portion). The paired *t* test was used form comparing mean values for resting and exercise during the course of the experiment. Data are expressed as mean ± SE, with a value of $P < 0.05$ considered significant.

Control Studies

To test whether the thermoregulatory responses during exercise are modified by the time duration of previous water immersion, two series of control studies were performed separately at 1 and 2 ATA. In the first experiment, the subject, wearing a wet suit, was immersed up to the neck in water and sat without exercising on the chair for 60 min (time-control study). In the second experiment, the subject was immersed for the same period, but he performed the leg exercises during the last 20 min (control-exercise study). These two series of control experiments were carried out at 1 and 2 ATA with the same time schedule. In these control experiments, the following results were obtained (Table 2). a) Resting values of T_{es}, T_{sk}, H_{skin}, and H_{suit} reached plateau levels after 40 min of water immersion at 1 and 2 ATA. b) Exercise increased T_{es} to the same magnitude at 1 and 2 ATA. c) The difference in T_{es} and T_{sk} between the control-exercise study and time-control study (resting) showed a pressure dependent figure. d) The H_{skin} and H_{suit} during exercise showed the same trend at both atmospheres, and H_{skin} was influenced only by the exercise and pressure per se, but not by the time duration. Accordingly, we adjusted our experimental design to compare the effect of depth on thermal and insulative responses.

RESULTS

Body Temperature

Changes in body temperature during exercise in water at 1 and 2 ATA are summarized in Table 3. At the resting period, T_{es} at 1 ATA was identical to that of 2 ATA, but mean skin and all regional skin temperature decreased significantly ($P < 0.05$) at 2 ATA. T_{es} rose significantly ($P < 0.05$) during exercise at both environments; however, the rate of increase was significantly less ($P < 0.05$) at 2 ATA (0.013°C/min vs. 0.008°C/min). A significant rise in T_{sk} was observed during exercise at 1 ATA but not at 2 ATA. During exercise, T_{sk} of the chest and thigh significantly increased ($P < 0.05$) at sea level but not at 2 ATA. On the contrary, T_{sk} of the hand and foot reduced significantly ($P < 0.05$) in both environments. These indicated that the responses of skin temperature to the exercises were not uniform in the proximal and distal parts of the body.

Heat Loss

Typical change for a representative individual in heat loss from the body surface and wet suits is illustrated in Fig. 1. The H_{skin} was equal to H_{suit} in

Table 2
Comparison of Body Temperatures and Heat Flow During Rest and Exercise at Different Depths

	Sea Level			10-m Depth		
	Resting		Exercise	Resting		Exercise
	40 min	60 min	20 min	40 min	60 min	20 min
T_{es} (°C)	36.79 ± 0.05	36.66 ± 0.05	37.26 ± 0.07	36.82 ± 0.08	36.73 ± 0.07	37.22 ± 0.09
$\bar{T}_{sk}$ (°C)	32.4 ± 0.1	32.2 ± 0.1	33.1 ± 0.2	31.4 ± 0.1	31.3 ± 0.2	31.6 ± 0.2
H_{skin} (W/m^2)	77.3 ± 2.8	75.5 ± 5.7	86.3 ± 3.1	99.5 ± 2.8	90.6 ± 2.1	109.4 ± 2.1
H_{suit} (W/m^2)	70.0 ± 0.9	65.1 ± 1.8	68.2 ± 2.7	93.0 ± 3.9	83.5 ± 3.1	93.4 ± 2.2

Values are means ± SE of four subjects with last minute during rest and exercise.

Table 3
Changes in Body Temperature During Exercise At Different Depths

	Sea Level		10 m-Depth	
	Resting	Exercise	Resting	Exercise
T_{es} (°C)	37.08 ± 0.06	37.34 ± 0.09*	37.09 ± 0.16	37.25 ± 0.12*
ΔT_{es} (°C min)		0.013 ± 0.002		0.008 ± 0.002**
$\bar{T}_{sk}$ (°C)	32.9 ± 0.2	33.2 ± 0.2*	31.1 ± 0.2**	31.2 ± 0.3
$\Delta \bar{T}_{sk}$ (°C min)		0.015 ± 0.005		0.008 ± 0.007**
$T_{sk\text{-chest}}$ (°C)	33.8 ± 0.4	34.5 ± 0.4*	32.9 ± 0.3**	33.2 ± 0.5
$T_{sk\text{-forarm}}$ (°C)	32.8 ± 0.2	32.9 ± 0.5	30.3 ± 0.3**	30.4 ± 0.5
$T_{sk\text{-hand}}$ (°C)	30.1 ± 0.3	29.2 ± 0.5*	27.2 ± 0.2**	26.9 ± 0.3*
$T_{sk\text{-thigh}}$ (°C)	32.9 ± 0.5	33.9 ± 0.6*	30.7 ± 0.3**	31.2 ± 0.6
$T_{sk\text{-calf}}$ (°C)	32.1 ± 0.2	32.2 ± 0.2	29.8 ± 0.2**	29.8 ± 0.2
$T_{sk\text{-foot}}$ (°C)	29.7 ± 0.4	29.8 ± 0.6	27.3 ± 0.3**	26.7 ± 0.2*

Values are means ± SE at the last minute during rest and exercise; * $P < 0.05$ comparing values between rest and exercise; ** $P < 0.05$ comparing values between sea level and 10-m depth.

air; however, H_{skin} exceeded H_{suit} upon water immersion and the difference was stabilized during resting periods in water. Exercise in water increased the difference due to a further increase in H_{skin}. This would indicate an increase in the convective heat flow due to a flow of water between the skin and the wet suits during exercise. The data obtained from all subjects are summarized in Table 4. At 2 ATA, H_{skin} and H_{suit} increased significantly ($P < 0.05$) at both resting and exercising periods. The difference of H_{skin} and H_{suit} is defined as the convective heat flow due to the water layer flow between the skin and the wet suits (H_{water}). Exercise did not change H_{suit} in either environment; accordingly, the increased heat loss caused by exercise was entirely attributed to the H_{skin}. The hydrostatic pressure did not change the H_{water} at either resting or exercising periods.

Table 4
Heat Losses and Thermal Insulation During Exercise in Water

	Sea Level		10 m-Depth	
	Resting	Exercise	Resting	Exercise
	Heat flow (W/m^2)			
H_{skin}	82.5 ± 1.9	89.2 ± 3.0*	95.8 ± 3.1**	104.0 ± 4.0*
H_{suit}	72.8 ± 1.9	73.0 ± 2.8	87.8 ± 2.7**	89.2 ± 5.9
H_{water}	9.7 ± 1.8	16.2 ± 2.9*	8.0 ± 2.2	14.7 ± 3.9*
	Thermal Insulation ($^{\circ}C/W \cdot m^2$)			
I_{suit}	0.105 ± 0.002	0.109 ± 0.002	0.062 ± 0.001**	0.063 ± 0.003
I_{tis}	0.053 ± 0.004	0.048 ± 0.004*	0.065 ± 0.004**	0.059 ± 0.005*
$I_{suit} + I_{tis}$	0.158 ± 0.003	0.156 ± 0.004	0.126 ± 0.005**	0.122 ± 0.005
I_{tis}				
Chest	0.053 ± 0.008	0.043 ± 0.008*	0.048 ± 0.008	0.045 ± 0.011
Forearm	0.046 ± 0.003	0.047 ± 0.008	0.063 ± 0.002**	0.061 ± 0.007
Hand	0.064 ± 0.005	0.090 ± 0.001*	0.164 ± 0.027**	0.168 ± 0.034
Thigh	0.045 ± 0.005	0.033 ± 0.005*	0.055 ± 0.005**	0.046 ± 0.006*
Calf	0.055 ± 0.003	0.057 ± 0.003	0.077 ± 0.003**	0.075 ± 0.006
Foot	0.090 ± 0.008	0.102 ± 0.015	0.175 ± 0.025**	0.218 ± 0.019
Abdomen	0.042 ± 0.003	0.034 ± 0.001	0.038 ± 0.004	0.038 ± 0.007

Values are means ± SE of average at last 5 min. * $P < 0.05$ comparing values between rest and exercise. ** $P < 0.05$ comparing values between sea level and 10-m depth.

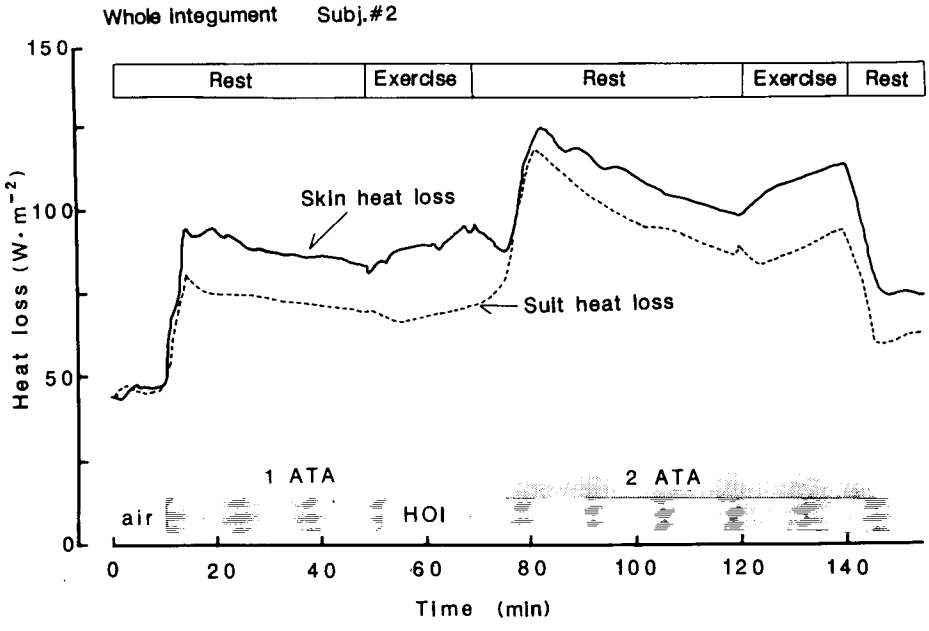

Fig. 1. A typical example of heat loss from the surface of the body (H_{skin}) and wet suits (H_{suit}) during rest and exercise in water. Solid line represents H_{skin} and broken line represents H_{suit}.

Insulative Value

Typical change for representative individual in overall I_{tis} and I_{suit} is illustrated in Fig. 2 and data of all subjects are summarized in Table 4. Overall I_{suit} was decreased remarkably ($P < 0.05$) by the increase of depth and was independent of the exercise at either atmospheres. The change of regional I_{suit} at different sites of the skin was similar to the overall I_{suit}. Although the overall I_{tis} was relatively stable during resting at 1 ATA, it increased gradually at 2 ATA. The exercise decreased overall I_{tis} in both environments. This indicated a progressive vasoconstriction due to an increased H_{skin} at this depth. Although the overall I_{tis} increased significantly ($P < 0.05$) as the depth increased, there was a considerable regional difference in I_{tis} at all skin sites. Insulation in the extremities (forearm, hand, thigh, calf, and foot) was significantly high at 2 ATA ($P < 0.05$), representing vasoconstriction in these areas; however, there was no change in the chest and abdomen. The exercise significantly reduced the overall I_{tis} in both environments ($P < 0.05$). Among the regions tested, I_{tis} in the chest and thigh was reduced significantly ($P < 0.05$), representing vasodilation during exercise. Conversely, the hand showed significantly increased I_{tis} ($P < 0.05$), suggesting vasoconstriction during exercise.

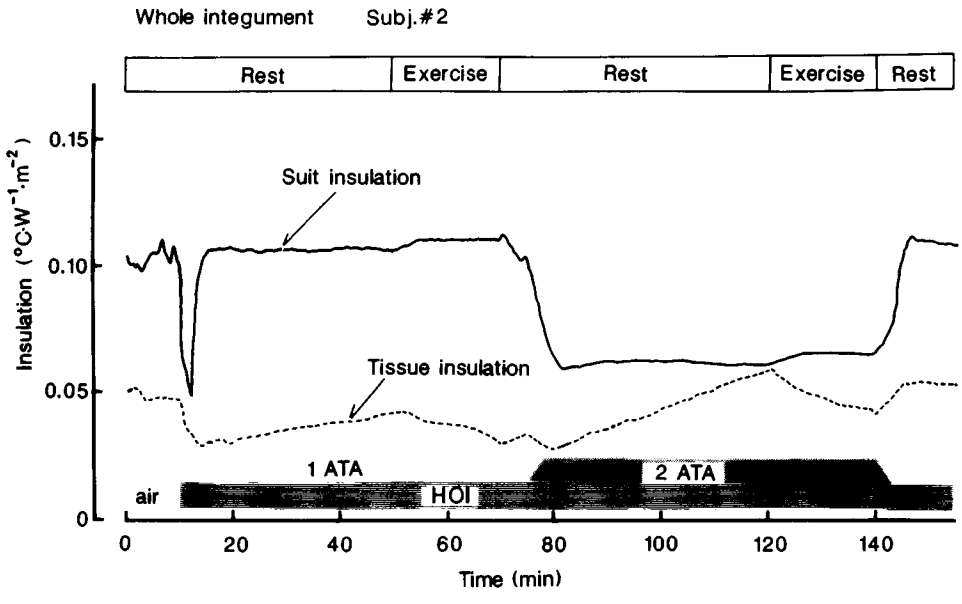

Fig. 2. A typical example of overall suit insulation (I_{suit}) and tissue insulation (I_{tis}) during rest and exercise in water. Solid line represents I_{tis} and broken line represents H_{suit}.

Thermal Balance

Thermal balance at the end of exercise in both environments is summarized in Table 5. $\dot{M}$ at rest was the same in both atmospheres and the increment of $\dot{M}$ during the exercise was of the same magnitude. An increased negative heat storage (S) without decreasing T_{es} at 2 ATA would indicate a pronounced vasoconstriction in accordance with the increased I_{tis}. The positive $\dot{S}$ during exercise was attributed to larger heat production in comparison to heat loss at this water temperature. The $\dot{S}$ during exercise was significantly ($P < 0.05$) less at 2 ATA. As $\dot{M}$ during exercise was almost identical in both environments, the smaller $\dot{S}$ at 2 ATA was entirely attributed to a high $\dot{H}$. This smaller $\dot{S}$ reflected the slower increase of body temperature during exercise in deep water as shown in Table 3 (0.013°C/min at sea level vs. 0.008°C/min at 10 m).

DISCUSSION

The use of wet suits is effective in protection of hypothermia in the open sea, but the insulative value of wet suits is totally dependent on the water depth (1). At 10-m water depth, we observed that mean I_{suit} decreased by 60% of the value at sea level. On the contrary, overall I_{tis} during resting

Table 5
Heat Exchange During Rest and Exercise at Different Depths

	Sea Level		10 m-Depth	
	Resting	Exercise	Resting	Exercise
$\dot{M}$ (W/m^2)	51.5 ± 2.2	126.5 ± 11.5	54.7 ± 4.2	135.4 ± 14.3
$\dot{H}$ (W/m^2)	84.5 ± 2.0	91.6 ± 3.6*	99.0 ± 3.8**	108.3 ± 4.5*
$\dot{S}$ (W/m^2)	-37.0 ± 2.4	24.7 ± 7.6*	-48.7 ± 2.1**	16.3 ± 9.2*

Values are means ± SE of average at the last 5 min. * $P < 0.05$ comparing values between rest and exercise. ** $P < 0.05$ comparing values between sea level and 10-m depth.

increased by 23% from the sea-level value, and thus total thermal insulation ($I_{tis} + I_{suit}$) was smaller than that at 1 ATA. This means that the physiologic mechanism, vasoconstriction reflex, does not fully compensate for the loss of I_{suit} at 2 ATA. The core temperature (T_{es}) would normally be expected to decrease in this situation; however, this was not the case in the present experiment. A relatively short period (60 min) in water at the present experimental temperature of 25°C may not be enough to cause hypothermia at 2 ATA. The same result has been reported in Japanese male breath-hold divers (11). As shown in Table 4, the extremities play a major role in increasing overall I_{tis}, but the role of the torso seems insignificant. That is, at 2 ATA, the insulative value of the hand and foot increased by 2.6- and 1.9-fold, respectively. The higher increase in I_{tis} in the extremities was observed by Cannon and Keating (12) when the subject wearing bathing trunks was transferred from a warm water temperature (35°C) to a lower water temperature (22°C). Thus, at depths as well as at sea level extremities of the body play an important role in thermoregulation. Exercise decreased the overall I_{tis} in both environments, and regions responsible for the reduction were mainly in the thigh and chest (Table 4). This fact agrees with the data of Park et al. (13), who reported a higher I_{tis} in the hand and foot during exercise in shallow dives.

The I_{suit} did not change during exercise in either environment because the I_{suit} is dependent only on the bubble size in the suit material (neoprene) (4). In this experiment, we could measure the heat loss separately through the skin (H_{skin}) and from wet suits (H_{suit}) by direct measurement. The difference between H_{skin} and H_{suit} (H_{water}) was defined as convective heat flow due to water movement under the suit. Wolff et al. (14) estimated that H_{water} at the trunk accounted for 45 and 60% of skin heat loss during rest and exercise, respectively, although systemic measurements of H_{water} were not made. Their calculation of H_{water} seems too high because direct measurement of H_{water} in the present experiment only accounted for 10 and 18% of mean H_{skin} during rest and exercise, respectively. A lower water temperature (10°C in their

experiment) in comparison to the present experiment (25°C) may explain the discrepancy. Experimental depths at a lower water temperature are needed to elucidate the problem. It is of interest that the magnitude of H_{water} during exercise was identical in both atmospheres. This fact is important because it suggests that the increment of heat loss by a given exercise in water does not depend on the depth.

Exercise accumulated less heat at depth, which resulted in significantly less increase in T_{es} and $\bar{T}_{sk}$ during exercise at 2 ATA (Table 3). This smaller $\dot{S}$ during exercise was attributed to an increased H_{skin}. The increased H_{skin} was attributed to the reduced I_{suit}. There was an increase in I_{tis}, hence the vasoconstriction, preventing an increase in H_{skin} at 2 ATA; the I_{tis} is not enough to prevent the increase in H_{skin} because the fall in I_{suit} is so large and the sum of I_{suit} and I_{tis} is smaller than that at 1 ATA. Thus, at the depth the same effect is expected as if the diver were exposed to a lower water temperature than the actual water temperature.

In summary, the results of the present experiment may suggest that the thermal response of a diver with wet suits is not affected qualitatively by pressure per se, and changes in his body temperature during exercise in water are very much dependent on the depth of water, hence the I_{suit}, providing that metabolic heat production is identical.

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COOLING IN COLD WATER: PREDICTIVE MODELING

M. M. Winsborough, D. L. Reeves, and A. J. Bachrach

The relationship between subcutaneous adipose tissue (SAT), surface area-to-mass ratio ($A_D \cdot wt^{-1}$), and cooling has been investigated by a number of workers.

Nunnelly et al. (1) used computer modeling to predict the relationship among SAT, immersed clothing insulation, water temperature, and time in producing a fall in core temperature to a critical value (34°C). The change in blood distribution with falling core temperature was taken into account in their model, which clearly demonstrates the relationship between SAT and cooling. Veicsteinas et al. (2) used both surface and deep skin thermistors to record changes in regional and overall insulative capacity at rest and at exercise during cold immersion. At rest they found that fat accounted for only 10 to 15% of the total body insulative capacity. During exercise the insulative contribution of fat against overall heat loss rose to 40%. The difference in response was attributed to the differing roll of muscle in thermal insulation at rest and at exercise. Toner et al. (3) measured the metabolic cost of both shivering and exercise in cold-immersed males and females in addition to the interaction between SAT and $A_D \cdot wt^{-1}$ on cooling in their subjects. In agreement with Veicsteinas et al. they were able to demonstrate that at rest the larger body mass of the men in their study (at comparable SAT levels) provided a more effective insulative barrier to cooling than the smaller body mass of the females, but that exercise eliminated any differences between the 2 groups in thermal response. The conclusion of Sloan et al. (4) that cooling is linearly related to 1/SAT corrected for body mass in young swimmers of both sexes, has recently been challenged by Mittleman et al. (5), who found a bell-shaped relationship between SAT and cooling. This effect was attributed by the authors to shivering thermogenesis. The work reported here supports the conclusions of both Toner et al. (3) and Sloan et al. (4), and may provide an

explanation for the differences between the conclusions of Sloan et al. (4) and Mittleman et al. (5).

MATERIALS AND METHODS

Subjects

Seven young, healthy male subjects participated in this study. Their physical characteristics are given in Table 1. All were volunteers who met the medical requirements of the study and gave their written consent to the experimental procedures involved. A medical officer examined each subject before and after cold immersion and closely observed the subject throughout all procedures.

Table 1
Physical Characteristics of Subjects

Subject	HT, m	WT, kg	A_D, m_2*	$A_D \cdot wt^{-1}$** ($cm^2 \cdot kg^{-1} \cdot 10^{-2}$)	Age	Percent Body Fat	Average Skinfold Thickness 15 sites, mm
7	1.70	65.5	1.76	2.69	34	11.2	5.87
2	1.78	81.82	2.0	2.44	32	9.5	10.33
4	1.94	91.82	2.26	2.46	24	9.4	11.17
3	1.70	64.45	1.76	2.69	29	10.1	12.20
1	1.80	80.90	2.03	2.51	36	9.0	12.80
5	1.78	80.68	1.98	2.45	33	19.5	14.66
6	1.71	61.36	1.69	2.75	26	4.5	21.86

* Surface area. ** Surface area-to-mass ratio.

Protocol

The experimental design of the study is described in detail by Reeves et al. (6). Therefore, a description of the physiologic procedures only will be given here.

An earlier study (7) was used to predict an initial water temperature (head-out immersion, in swim trunks only) that would reduce the core temperature of subjects by 1.5°C within 60 min. As in the original model, a common clo value for the fully vasoconstricted shell of an average man was used for

all subjects. (A unit of insulation which allows the heat transfer of one kilocalorie per square meter per hour with a temperature gradient of 0.18°C between two surfaces.) Shivering thermogenesis was taken into account to predict water temperature. The model proved reliable in practice and provided a simple and consistent cooling method for physiological and neuropsychological measurements. Dependent measurements were made pre- and postimmersion with the subjects seated adjacent to the cold tank, wearing a swim suit. Postimmersion, each subject received the added protection of a light cellular blanket. Dependent measures included selected neuropsychological tests (8) along with blood pressure (BP) (measured once), heart rate (H_r), and hand (T_{hd}) and rectal (T_{re}) temperature (recorded every 10 min). All physiological measurements were repeated immediately pre- and postimmersion, including grip strength and tapping rate. In addition, all subjects were reexamined by the medical officer immediately before and after cold immersion. Each subject then sat in the immersion tank in stirred cold water (Fig. 1) with the water temperature controlled between 10 and 14°C, dependent on individual surface area, until T_{re} fell 1.5°C below immediate preimmersion T_{re}. This took approximately 1 h from entering the cold water. H_r, T_{hd}, and T_{re} were monitored continuously during immersion with the data recorded by hand from digital displays at 5-min intervals during the slow-cooling phase and every minute during fast cooling. BP continued to be measured and recorded every 20 min unless an intermediate measurement was considered necessary. The subjects were encouraged to rinse out their mouths with either hot water or hot decaffeinated coffee during and after the cold stress, as frequently as necessary to control shivering. This protocol was repeated on separate days a) with the subjects immersed in water at 35°C, and b) with the subjects seated quietly in air, in the immersion tank room, for a time equal to the full experimental procedure.

Measurements

Body surface area was estimated, using height and weight, by the method of DuBois and Dubois (8). Lange calipers were used to measure the skinfold thickness at 15 sites: chin, triceps, forearm, calf, thigh, suprailiac, subscapular, chest (sternal notch level); midaxillary, midclavicular, immediately adjacent to the nipple; trunk (waist level); midaxillary, midclavicular, immediately adjacent to the umbilicus; umbilical: midaxillary, midclavicular. The mean of the summed skinfold thickness was used to estimate SAT and percent body fat (BF) by the method of Brozek et al. (9). T_{hd} and T_{re} were monitored by means of appropriate thermocouples. The skin thermocouple was taped to the dorsal surface of the hand that was not immersed in water. The rectal probe was inserted 10 cm into the rectum. Water temperature was measured using a thermocouple (connected to a Yellow Springs thermometer) placed at midwater level, well away from the sides of the tank. The immersion tanks were the product of hot-water habitats. The water was thermostatically controlled to ± 1°C set temperature and stirred continuously. All temperature data were hard

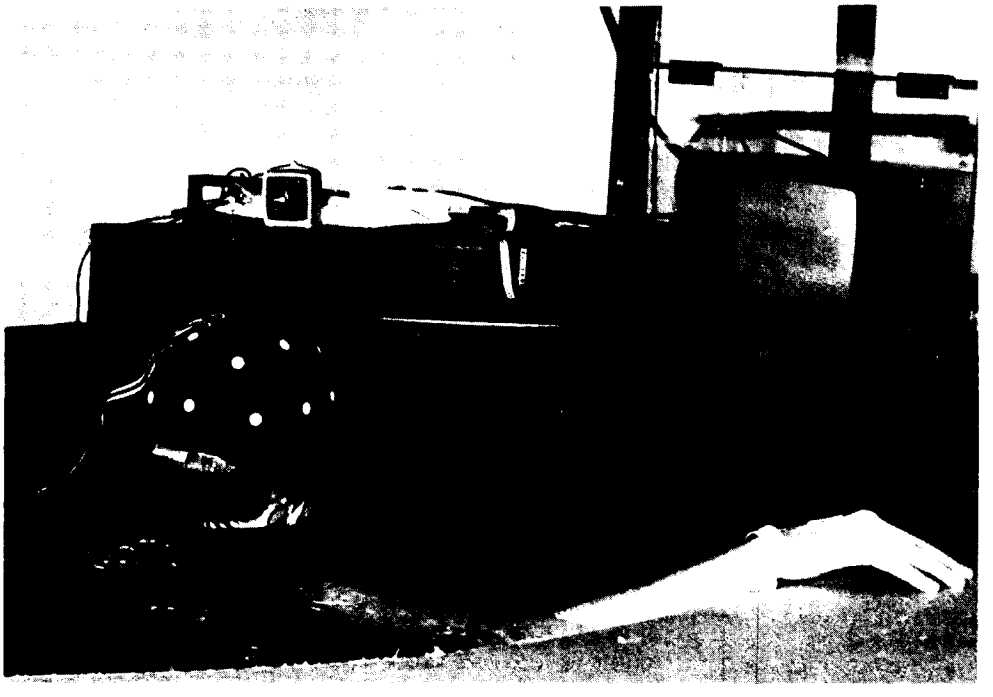

Fig. 1. An immersed subject carrying out the battery of neuropsychologic tests.

wired to Senortek thermometers, with digital displays from which data were recorded by hand. H_r was measured by sphygmomanometry. Grip strength was estimated using a Lafayette Instrument Co. Dynamometer. Rate of tapping was measured for 6, 15-s periods from which the meaned sum was taken.

RESULTS AND DISCUSSION

In the Naval Medical Research Institute study the rate of fall of T_{re} measured over total immersion time showed a linear relationship to 1/SAT $A_D \cdot wt^{-1}$ (Fig. 2). The equation of the line of best fit is: $y = A + Bx$

$$y + -0.099 + 10.4798x \qquad (1)$$

Regression coefficient (r) = 0.979, 95% confidence limits + ± 0.0336. Plotted in this way our data support the work of Sloan et al. (4), who reported a similar relationship in young swimmers of both sexes. An approach of this kind presupposes a linear fall in T_{re} with time, which was a valid approach under the experimental conditions of Sloan et al. (4), who were unable to measure core temperature in the open sea. It remains a valid and useful first approximation of the overall thermal response to acute cold-immersion stress.

However, interpretation of the thermal response to cooling is sensitive to the variables chosen and the way in which the data are expressed graphically. As a result, both Sloan et al. (4) and Mittleman et al. (5) seem to be correct in the interpretation of their data and neither is exclusive to the other.

When real elapsed time was used to analyze our data, as opposed to total elapsed time (4), the response to cooling was clearly multiexponential with two main components: a slow initial response related to SAT (Fig. 3), followed by a much faster, multicomponent secondary response (Fig. 4). The slow initial response to cooling could be described by an equation of the form:

$$y = A/(B + x)/y = 3.70/(8.58 + x)\, r^2 = 0.974 \qquad Fs = 185.46 \qquad (2)$$

The fast secondary response was best described by the expression: $y = Ax^N + B$.

$$y = 0.123x^{1.461} + 99.9 \qquad r^2 = 0.813 \qquad Fs = 330.106 \qquad (3)$$

In the fast cooling phase the skin and subcutaneous fat seemed to act as a fixed resistance with other components canceling out differences in fat thickness (Fig. 5). The response of subject 4 may be related to morphologic differences between this subject, who was very tall with very long limbs, and the rest of the group at median $A_D \cdot wt^{-1}$. For the same body mass and SAT he could be expected to lose heat at a faster rate than the other subjects because of a greater heat loss from his extended periphery. In addition, parallel experimentation within the laboratory suggests that there may also be a difference in the metabolic response to cold in this subject. However, both subject 4 and subject 6 cooled at a rate that was consistent with the overall experimental response (Fig. 4). Our data support the conclusion (2, 3, 5) that the thermal response to cooling is dependent on overall body composition rather than on any one facet of body composition. Toner et al. (3) also concluded that the skin and subcutaneous fat layers act as a fixed resistance to cooling at exercise. It appears from the work reported here that this may also be true at rest. A tighter fit to the mean for experimental (Fig. 4) purposes could have been obtained with the use of individual clo values and previously measured values for shivering thermogenesis, but for the purposes of the survival prognosis of accidental immersion victims, this work suggests that average values for these parameters can be used with some confidence.

A similar approach could be used to predict time to nonsurvivable cooling from initial suit-heating failure in divers. The probability of diver recovery may be less however, because consideration must also be given to the known effects of increased ambient pressure on human physiology. In this context the respiratory and cardiovascular effects of raised pressure, including a density-dependent tendency to carbon dioxide retention in some divers and gas and density-dependent alterations in cardiac neurophysiology, particularly in the presence of high circulating catecholamine levels, may increase the

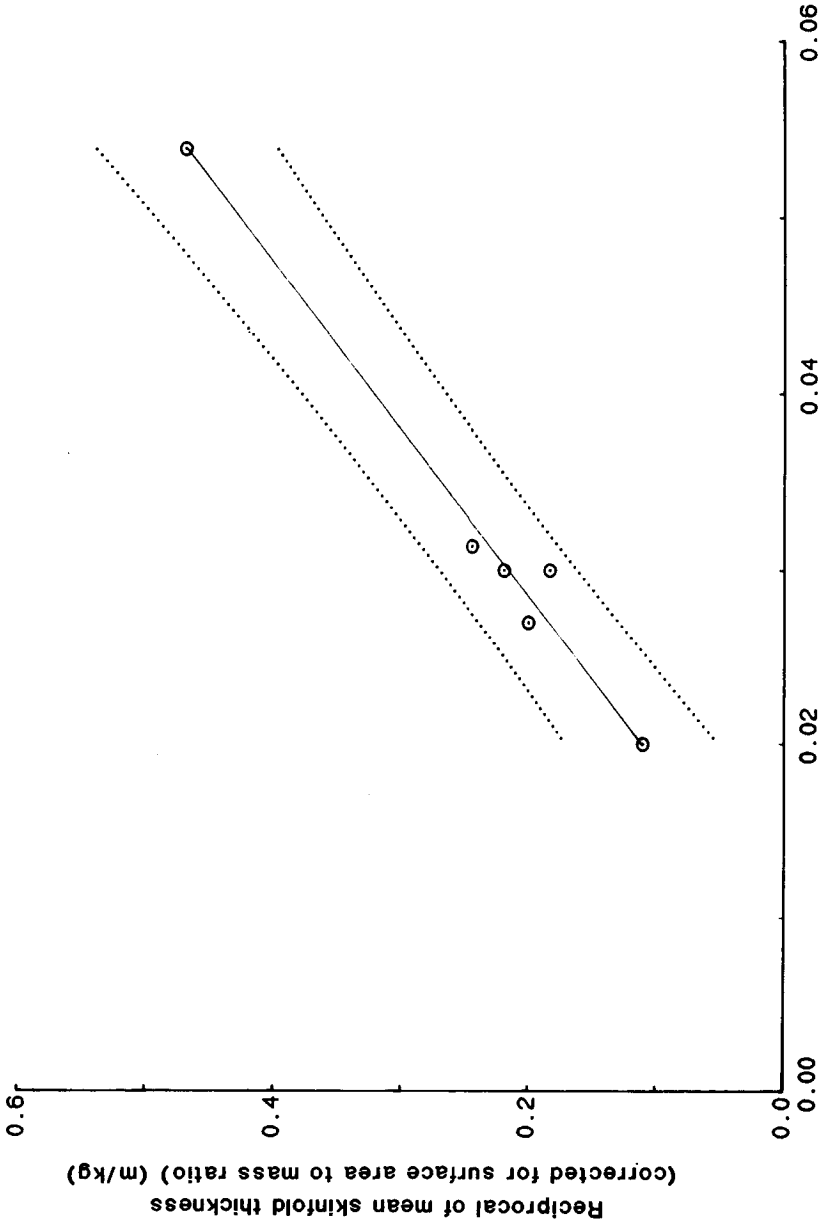

Fig. 2. The relationship between the reciprocal of subcutaneous adipose tissue (corrected for surface area-to-mass ratio) and overall rate of cooling, i.e., ΔT_{re} measured as the difference between core temperature at the beginning and end of immersion.

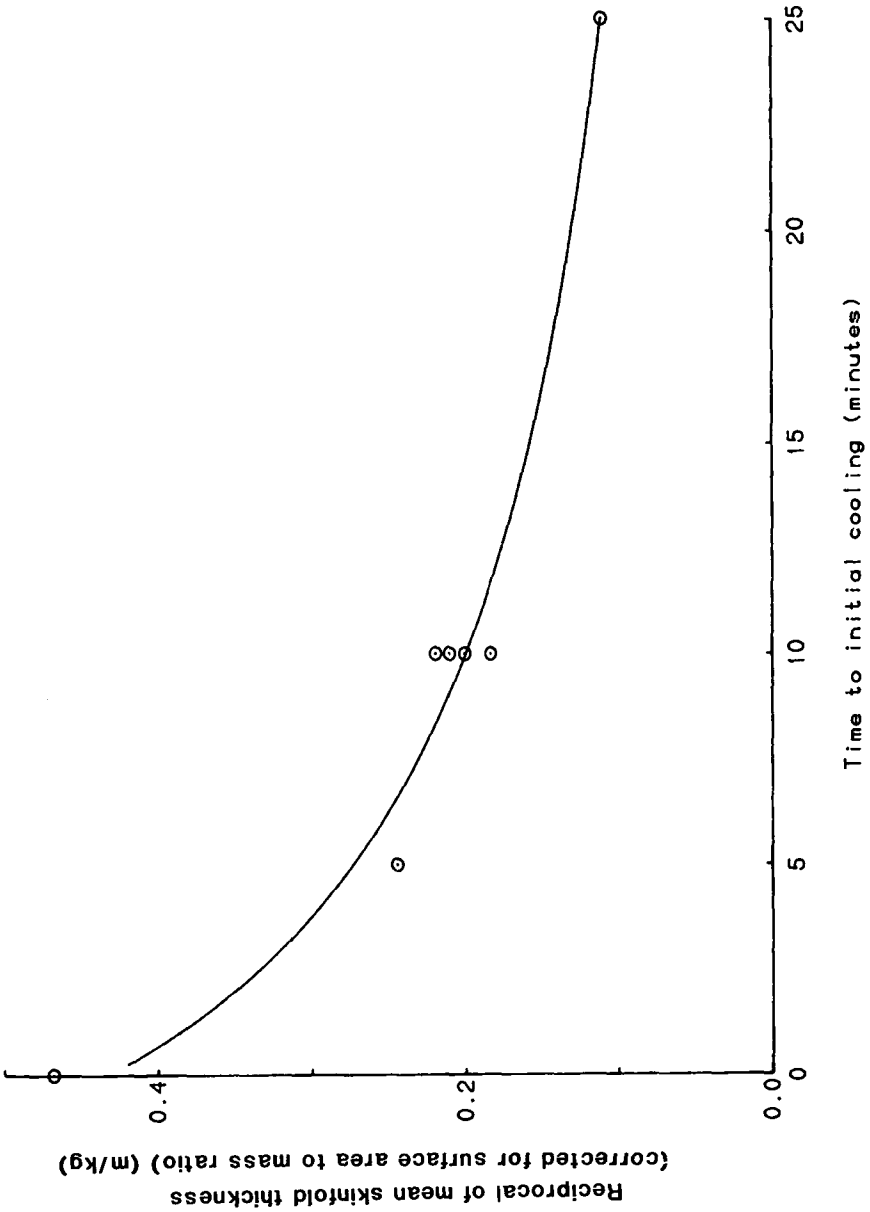

Fig. 3. Relationship between the reciprocal of mean skinfold thickness, corrected for surface-area-to-mass ratio, and the time to the beginning of cooling.

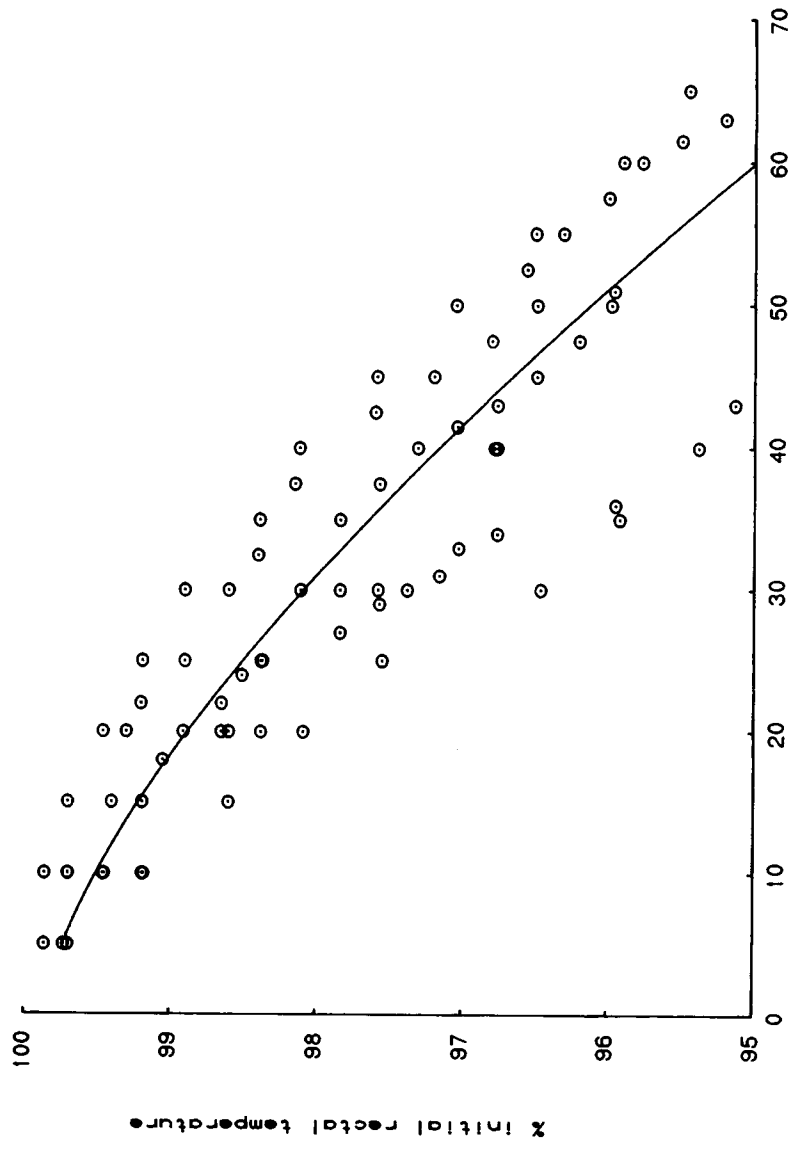

Fig. 4. The relationship between T_{re} and time in last phase cooling. T_{re} is plotted as percent of the T_{re} measured at the beginning of the fast-cooling phase against immersion time in minutes. T_{re} was plotted in this way to eliminate between-subject variation in basal T_{re}. Ninety-five percent of initial T_{re} represents a fall of 1.5 °C from basal T_{re}.

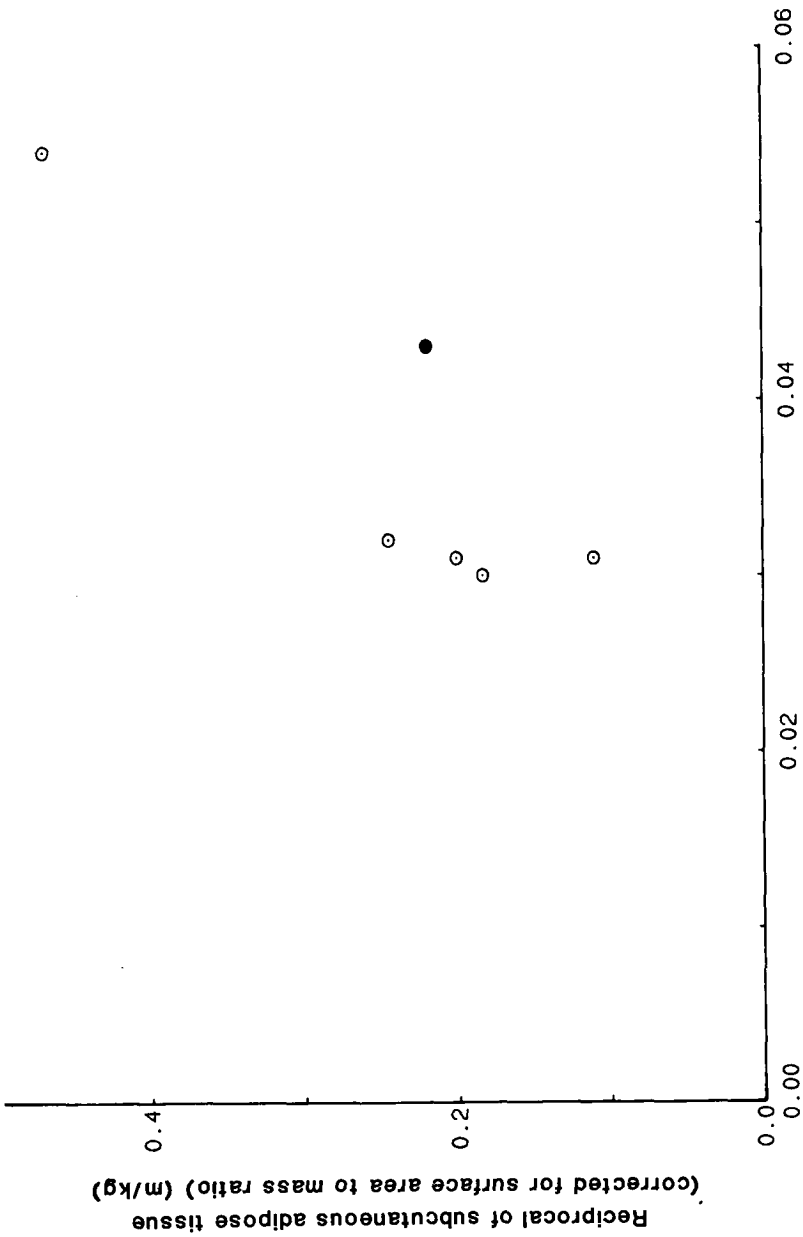

Fig. 5. Body composition related to cooling in the fast phase. The subject represented by the solid circle (subject 4) behaved differently from the rest of his group (Table 1) at median body mass and SAT.

tendency to arrhythmia (10). If the effects of increased ambient pressure and cold have synergistic effects on the cardiovascular response of the cold diver, then for the same cardiac fitness level and cold exposure the diver is at greater risk than the cold-immersion accident victim at the water surface. Cooling has also been reported in North Sea divers when suit heating had not failed but was less than optimal, with no sensation of distress reported by the diver (11). Keatinge et al. (11), reproduced this condition in the laboratory by circulating water at 29°C around his subjects and produced steady cooling without any reported sensation of cold. Of particular importance in this context may be the repression of the shivering response by the addition of heat to particularly sensitive peripheral areas, such as the upper respiratory tract. The thermal response of a diver already at risk from inadequate suit heating may be critically affected by the warmed breathing gas necessary for other purposes. The diver not exercising, i.e., stationary on the bottom for whatever reason, could be expected to cool in a manner similar to that described here, with the added insulation of the wet suit extending the time to initial cooling as in Fig. 3. Thereafter, the suit may act as an additional fixed resistance to cooling (Fig. 5). Further work needs to be done with both the metabolic response to cold of the section of the population represented by subject 4 (Fig. 5) and the precise relationship between cooling and external insulation.

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SESSION 3: THERMAL PROBLEMS IN DIVING

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Undersea and Hyperbaric Medical Society, Bethesda, MD, 1987

HUMAN THERMAL BALANCE AT REST AND EXERCISE IN WATER: A REVIEW

D. W. Rennie

This review is heavily biased toward my and my colleagues' studies inspired by the performance of the divers (Ama) of Korea and Japan. It makes no claim to be comprehensive but does allow certain basic observations made in the laboratory to be related to practice in the field by means of a simple, graphic analysis of heat exchange at rest and during exercise. Predictions can be made about the coldest operational water temperatures if overall body (and suit) insulation and metabolic heat production are known.

The response of persons immersed in water ranges from the involuntary gasp and violent burst of shivering accompanying dunking at 5°C to an insidious hypothermia developed over hours in water only 3 to 6°C colder than core temperature, water that seems luke warm to the finger or hand. It is with the latter that I will be mainly concerned, because our objectives have focused primarily on factors determining the maximal physical insulation (or minimal conductance) humans are capable of developing, and these are studied best in the absence of skeletal muscle activity. Before 1977, the women divers of Korea were clad in only a thin, cotton garment (with special attention to swathing the neck and head) in water ranging from 10°C in winter to 27°C in summer, allowing themselves to cool to a rectal temperature (T_{re}) of 35°C in all seasons before returning to shore to rewarm. A steady state of heat balance was never achieved, and net loss of stored heat was estimated to range from 293 to 466 kcal in summer and winter, respectively.

To examine how thermal balance of the Ama was controlled relative to that of nondivers, we took inspiration from an early study by Burton and Bazett (1), the principal results of which are illustrated in Fig. 1. This study used a bath calorimeter to measure both the vascular and metabolic responses to head-out water immersion at water temperatures generally ranging from 30

to 36°C. Overall thermal conductance, an index of vascular perfusion of the shell, was observed to range sevenfold (Fig 1*A*) from 45 $kcal \cdot h^{-1} \cdot m^{-2}$ at a T_{re}-T_{bath} gradient of less than 1°C to 7 $kcal \cdot h^{-1} \cdot m^{-2}$ at a T_{re}-T_{bath} gradient of 4°C. The *broken line* is theoretical hyperbola such that the product $X \cdot Y = 30$ $kcal \cdot m^{-2} \cdot h^{-1}$, as would be expected for steadystate heat balance in a thermoneutral environment at rest. The upward deviation of conductances observed at T_{re}-T_{bath} gradients in excess of 4°C coincided with an increase in Vo_2, presumably due to shivering, and was attributed to increased heat production and perfusion of peripherally located muscle.

The onset of increased metabolic heat production is seen more clearly in Fig. 1*B*. It was interpreted to signal the lowermost limit of water temperature in which physical regulation (insulative regulation) of core temperature would occur. We subsequently called this the "critical water temperature" (T_{cw}) (2) consistent with the terminology introduced by Scholander et al. (3) for critical air temperatures.

CRITICAL WATER TEMPERATURE: THE SHIVERING THRESHOLD

The simple model on which we based our early water bath studies (4) is illustrated in Fig. 2. Overall thermal conductance was visualized as having two components, one due to physical conductance of heat through tissue between the core and the skin surface and one due to convective transfer of heat by blood. Our initial objective was to minimize the convective component by long-term immersion in T_{cw} and measure the residual minimal conductance (K_{min}). We expressed this as maximal body insulation ($I_{max} = 1/K_{min}$), so we could determine the relative importance of the series' resistances to heat flux imposed by the measured thickness of subcutaneous fat (I_{fat}) and the underlying muscle (I_{muscle}).

Critical water temperature is a time-dependent variable which is higher the longer the period of immersion. We defined it in terms of the threshold metabolic response, as did Burton and Bazett (1). Because of the discomfort of immersion diuresis plus other practical considerations we compromised on 3 h of immersion as our test time. This is not long enough in most subjects to establish a new steady state of heat balance; however most subjects at the end of 3 h in T_{cw} will be undergoing a $\Delta T_{re}/\Delta t$ less than $0.1°C \cdot h^{-1}$ and hence a negligible rate of stored heat loss.

Critical water temperature was determined at the onset of our studies by trial and error, not knowing what factor (A_D, weight, $A_D \cdot wt^{-1}$, fat thickness, etc.) would be of paramount importance. As it turned out, subcutaneous fat thickness (SFT) was by far the most important single variable determining T_{cw} in nonacclimatized adult men and women (2). This is illustrated in Fig. 3 for divers and nondivers in recent studies (4, 5). A different and much more elegant approach for estimation of T_{cw} has been published by Smith and Hanna (6), who also concluded that SFT was the most important single variable determining T_{cw} in adults. Figure 3 illustrated (*broken line*) an example of

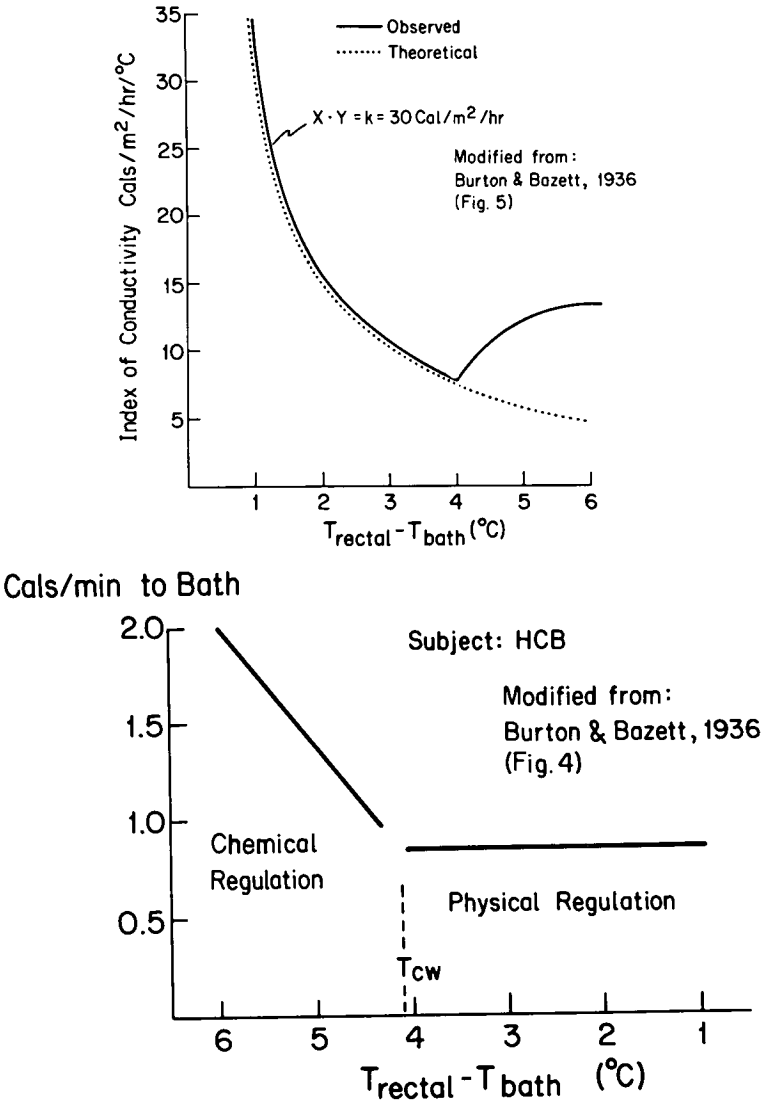

Fig. 1. Top, overall thermal conductance (expressed as $Cal/m^2/hr/^\circ C$) as a function of the rectal-to-bath water temperature gradient. Solid line depicts observed relationship. Broken line is theoretical hyperbola: $X \cdot Y = 30\ Cal \cdot m^2 \cdot h^{-1}$. Note: upward deviation of observed conductance to $T_{rectal}-T_{bath} = 4^\circ C$, marking the onset of shivering. [Modified from Burton and Bazett, 1936 (1)]. Bottom, heat loss from subject to bath (expressed as Cal/min) as a function of the rectal-to-bath water temperature gradient. The point of inclination at $T_{rectal}-T_{bath} = 4^\circ C$ marks the lowermost limit of bath water temperature where physical regulation of heat flow was sufficient to control body temperature. [Modified from Burton and Bazett, 1936 (1)].

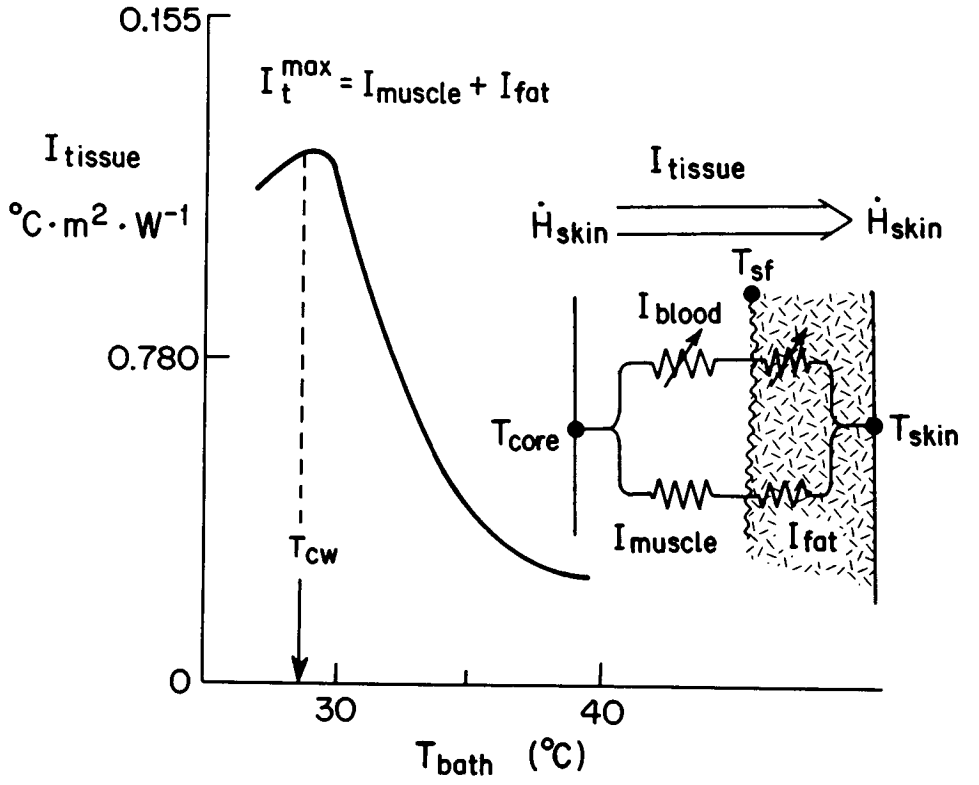

Fig. 2. Simplified model (inset) of heat flow (H_{skin}) from body core to skin surface through the thermal resistance afforded by shell tissue (I_{tissue}). The overall transfer of heat traverses two parallel paths: a) a variable convective heat transfer pathway whose resistance (I_{blood}) is an inverse function of blood flow to muscle, subcutaneous fat, and skin, and b) a conductive heat transfer pathway whose series resistances (I_{muscle} and I_{fat}) are functions of the physical properties of the tissue. Overall I increases progressively as bath temperature is lowered, until a maximal insulation, I_{max}, is achieved at a critical water temperature, T_{cw}.

T_{cw} determined from T_{core}, T_{max}, and metabolic heat productivity (M), as described below.

From first principles, $I_{max} = (T_{core}-T_{cw})/0.92$ M, where 0.92 M is skin heat-flux adjusted for respiratory heat loss and $T_{cw} = T_{sk}$ (2). The rate of stored heat loss has been ignored. Rearranging this enables T_{cw} to be calculated as: $T_{cw} = T_{core}-I_{max}\cdot 0.92$ M. T_{cw} can thus be predicted from an assigned lower limit of T_{core} and the knowledge of I_{max}. The example in Fig. 3 is for a T_{core} of 36.5°C. The practical utility of this simple graphic relationship will be discussed at the end of this review.

Any factor affecting the rate of core cooling over a 3-h period will influence the threshold for shivering, and hence T_{cw}. The most obvious is the

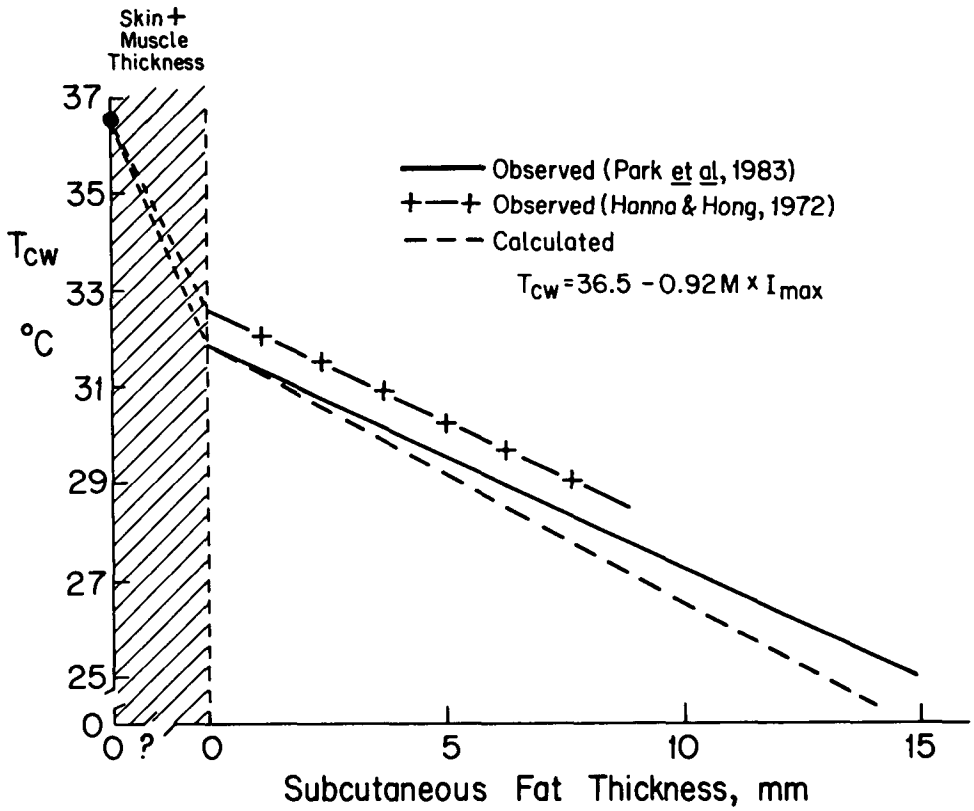

Fig. 3. Critical water temperature as a function of subcutaneous fat thickness, millimeter. Observed data are summarized from Park et al. (4). (—) and Hanna and Hong (5) (x-x-). Theoretical values for T_{cw} (---) are plotted for the formula: T_{cw} (°C) = 36.5 - 0.92 × M × I_{max} (see text for discussion).

vigor of water stirring. For this reason we have kept the bath stirred at a mean V = 0.25m•s^{-1}. Children with a significantly higher A_D•wt^{-1} would be expected to have a greatly increased T_{cw}, per millimeter thickness subcutaneous fat, than an adult and have been shown to cool faster (7). Women, who have a higher A_D•wt^{-1} than men of equal body fat, might also have a higher T_{cw} were it not for their thicker layer of subcutaneous fat (8, 9). Localized cooling of the skin, by evoking more intense vasoconstriction and shivering (10), might be expected to increase T_{cw}. These and other factors have never been systematically studied in one population of subjects nor do I expect they will be because the establishment of T_{cw} is primarily a means to an end, i.e., the measurement of I_{max}, not an end in itself.

I should point out that T_{cw} served as a useful, reproducible index of shivering threshold under conditions of prolonged, slow cooling. Comparison of T_{cw} between Ama and nondiving controls, when plotted as a contour plot of T_{cw} vs. T_{re}, provided convincing evidence of a downward shift of the T_{core}

shivering threshold in the Ama before deacclimatization occurred when wet suits were introduced into their technology (4).

MAXIMAL BODY INSULATION

Under the conditions imposed by T_{cw} as described above we assumed that I_{max} could be calculated with reasonable accuracy according to the relation: $I_{max} = (T_{core}-T_{cw})/H_{skin}$ where H_{skin} was set equal to 0.92 metabolic heat production and adjusted for rate of stored heat loss (2). The relationship observed between I_{max} and SFT for 30 acclimatized divers (Ama), 89 nondiving Koreans from early studies 1961-1973, and divers and nondivers reported by Park et al. (4) is summarized in Fig. 4. Except for the 1960 study the combined regressions were not significantly different for divers, $I_{max}(°C \cdot m^2 \cdot W^{-1}) = 0.0095$ SFT + 0.094; $r = 0.88$; for nondivers, $I_{max} = 0.010$ SFT + 0.081, $r = 0.85$) indicating that I_{max} of divers since 1980 does not differ from that of

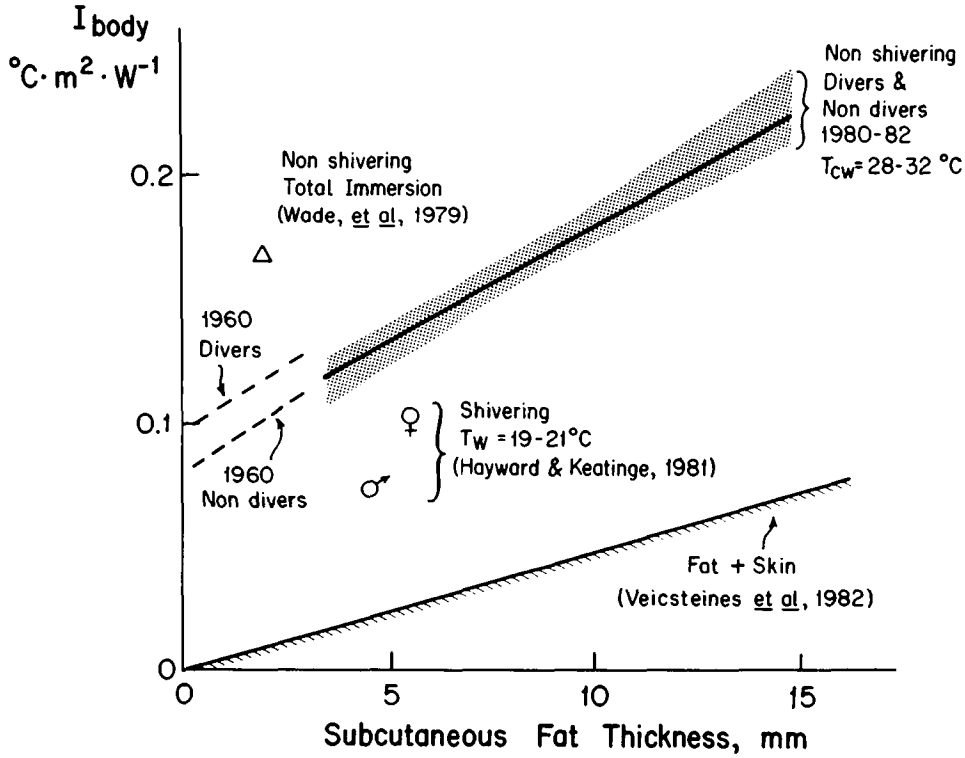

Fig. 4. Maximal tissue insulation (I_{max}, $°C \cdot m^2 \cdot W^{-1}$) as a function of subcutaneous fat thickness. Heavy line with stippled 95% confidence limits from Park et al. (4). Light line and broken line from review of Hong et al. (11). Total immersion in 25.2 °C from Wade et al. (13). Average values for 7 males and 7 females from Hayward and Keatinge (14).

nondivers for a given SFT. This deacclimatization has recently been reviewed (11) and will not be discussed here.

Figure 4 also includes (*lower line*) the relationship directly measured by Veicsteinas et al. (12) between I_{fat} and SFT in thigh skin of subjects in T_{cw}. Wade et al. (13) totally immersed 5 male subjects in 25.2°C water and measured skin-to-water heat flow with heat-flow discs from 11 regions. After 30 min, total skin heat loss was reported to be 62.7 $W \cdot m^{-2}$. I have divided this by an assumed deep body-to-water temperature gradient of 11°C to estimate the overall conductance of 5.7 $W \cdot °C^{-1} \cdot m^{-2}$ or an $I = 1/K = 0.175°C \cdot m^2 \cdot W^{-1}$ (1.13 clo). This is plotted in Fig. 5 as a function of the mean weighted subcutaneous fat thickness estimated from their skinfold data [SFT = (mean weighted skinfold thickness - 3 mm)/2]. Hayward and Keatinge (14) also have reported data for men and women that have been summarized in Fig. 4. Their objective was to determine the coldest possible water in which body temperature could be stabilized, stability being defined as a $\Delta T_r/\Delta t$ less than -0.025°C/15 min. Hence, the water temperatures were considerably colder than T_{cw} and evoked shivering that was inversely proportional to mean-weighted SFT. Calculations of I_{max} based on metabolic heat production and the

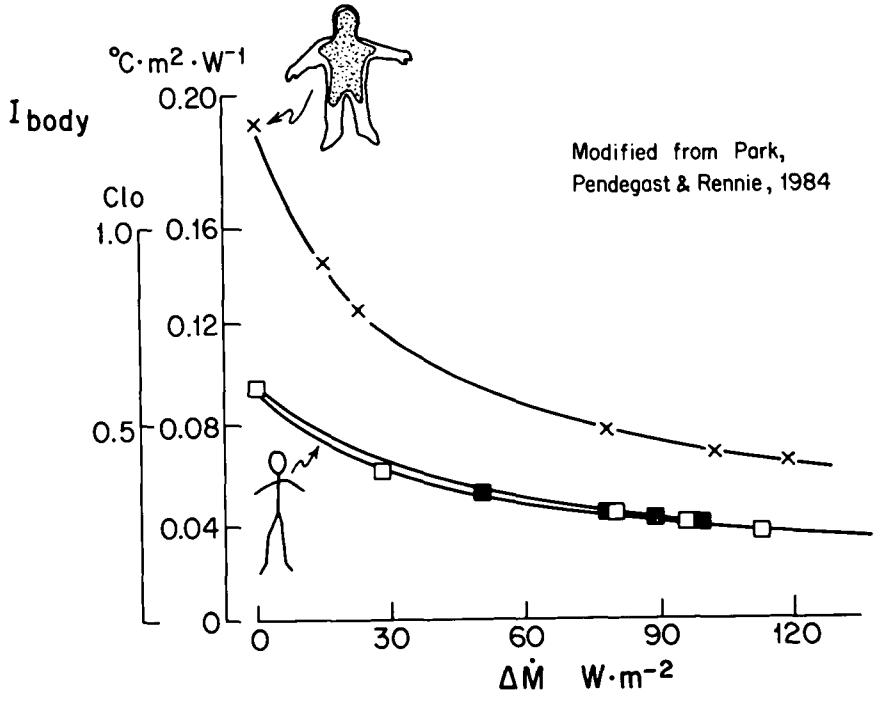

Fig. 5. Overall body insulation (I_{body}) as a function of arm and leg exercise intensity (M, $W \cdot m^{-2}$) during the 3rd h of exercise in T_{cw} (T_{cw} = 28-31 °C). Data are summarized for an obese and a thin subject. Modified from Park et al. (4).

maximal T_r-T_w gradient achieved in cold water are: I_{males} = 0.073°C•m^2•W^{-1} and $I_{females}$ = 0.111°C•m^2•W^{-1} for SFT of 4.70 and 5.7 mm, respectively.

Several attempts have been made to quantitate the relative insulative importance of subcutaneous fat and deeper tissue in resting subjects. Hayward and Keatinge (14) did so by comparing measurements of regional insulation with the theoretical insulation that could have been provided by unperfused fat of thickness equal to the measured values of SFT. The ratio "fat insulation:total insulation" so calculated averaged 0.30 for males and 0.26 for females. Veicsteinas et al. (12) measured subcutaneous fat and skin insulation directly in T_{cw} and compared calculated overall body fat insulation with measurements of I_{max}. The ratio fat insulation:total insulation for male subjects with an SFT of 5 mm averaged 0.21, in close agreement with Hayward and Keatinge (14).

Figure 4 enables the relative insulative importance of fat and deeper tissue to be visualized graphically. The vertical distance between I_{max} and the lowermost line (I_{fat}) for any given SFT is equivalent to the insulation provided by deep tissue. It is clear from this that the ratio fat insulation:total insulation varies as a function of SFT. Of great interest is the increase in the absolute value of insulative resistance of deeper tissues as SFT increases. For the nonshivering, totally immersed subject (13) deep shell insulation for a given SFT seems to be greater than during head-out immersion. Cooling of the head may induce more intense vasoconstriction in the deeper tissue, thus increasing deep shell insulation. Shivering subjects immersed in the coldest water allowing stability of body temperature (14) develop less deep shell insulation for a given SFT, probably because shivering has increased peripheral muscle blood flow. One would predict from Fig. 4 that persons with a large muscle mass might have a larger overall insulation due to the deep shell component than persons with a small muscle mass. The work of Toner et al. (15) bears out this prediction.

EFFECTS OF EXERCISE ON I_{max} AND T_{cw}

Measurements of I_{max} and T_{cw} or in the coldest water allowing stability of body temperature do not reflect heat balance during realistic operations in open water. Overall body conductance of working Ama was reported by Kang et al. (16) to be approximately 16 W•°C^{-1}•m^{-2}(1/K = 0.06°C•m^2•W^{-1} or 0.4 clo), much less than when studied at rest in T_{cw}. These values for body conductance are considerably less than the value of 29 W•°C^{-1}•m^{-2} reported by Pugh and Edholm (17) for thin subject G.P. swimming in 25.3°C water, although almost identical to the conductance of 15 W•°C^{-1}•m^{-2} reported for obese channel swimmer J.Z. swimming in 16°C water.

The combined effects of arm and leg exercise and SFT on I_{max} and T_{cw} were reported by Park et al. (18) who exercised male subjects for 3 h in T_{cw} at each of several levels of Vo_2. Figure 5 illustrates how I_{body}, measured during the steady-state heat balance of the 3rd h, declined as an exponential

function of metabolic rate. The most obese subject underwent the greatest decrease in I_{body} but still retained a higher I_{body} at the highest level of exercise investigated.

Steady-state I_{body}, measured after 3 h of exercise, is shown in Fig. 6 as a function of SFT. The linear relation between I_{body} and SFT is shifted downward and the slope is decreased as exercise intensity is increased. At M = 175 $W \cdot m^{-2}$, the I vs. SFT relationship is approaching the theoretical relationship for the measured SFT, suggesting that the deep shell has been largely replaced by core, leaving the poorly perfused superficial shell of subcutaneous fat and skin as the principle insulative barrier. Figure 7 is a conceptualization of this. I_{body} has been plotted as a percent of I_{max} for each subject studied. The dominant role of muscle as deep shell insulation at rest is seen to become progressively less as intensity of exercise increases, until at M = 180$W \cdot m^{-2}$ it has practically vanished. Recent work by Toner et al. (19) indicates that the site of muscular work, i.e., arms vs. legs, is as important as

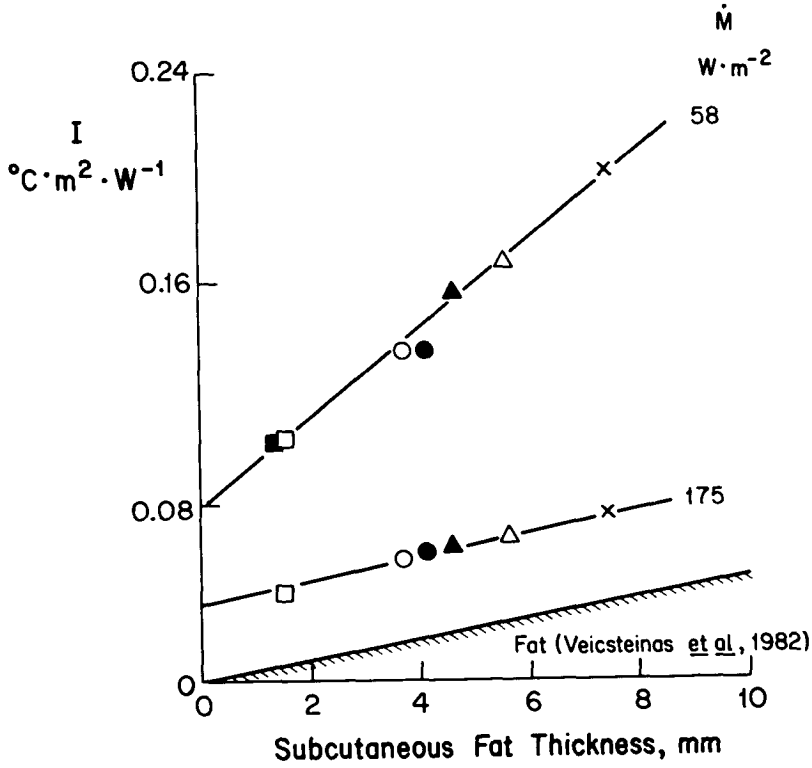

Fig. 6. Steady-state body insulation in T_{cw} (28-31 ° C) as a function of subcutaneous fat thickness. Values are for I at rest (M = 58 $W \cdot m^{-2}$) and while performing arm and leg work (M = 175 $W \cdot m^{-2}$). The theoretical relationship for unperfused fat is depicted at bottom from Veicsteinas et al. (12). Modified from Park et al. (4).

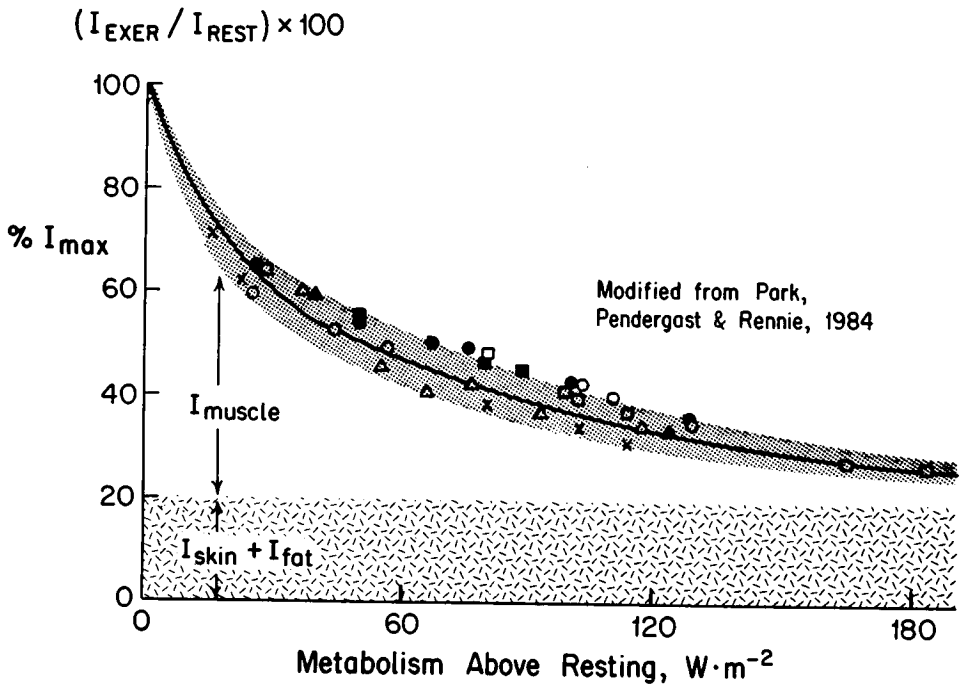

Fig. 7. Normalized body insulation, expressed as percent of I_{max}, as a function of arm plus leg exercise intensity for male subjects. The decrement in I is due to a decrease of I_{muscle} (see Fig. 2 for model) since $I_{skin} + I_{fat}$ were observed not to change (12). Modified from Park et al. (4).

the total mass of muscle exercised in determining rate of decrease of body temperature.

The results obtained in the laboratory on resting and exercising subjects have been placed in the context of field observations in Fig. 8 by substituting the observed inverse relationship between I_{body} and M summarized in Fig. 7 into the theoretical formula for T_{cw} alluded to earlier: $T_{cw} = T_{core} - I \times 0.92$ M (11).

Figure 8 depicts minimal operative water temperature (which is conceptually the same as T_{cw}) as a function of total body (plus suit) insulation. The isopleths intercepting the ordinate at 36.5°C depict the relationship for each of several levels of exercise where 36.5°C is taken to be the T_{core} for effective, long-term operations in water, I is total body insulation expressed as $°C \cdot m^2 \cdot W^{-1}$ and in clo units, and M is metabolic heat production in $W \cdot m^{-2}$. For this purpose, respiratory heat loss is assumed to equal 8% of M, useful external work has been ignored, and S is assumed to be zero.

Example U.N. depicts an unacclimatized nude male at rest and while performing two levels of exercise in water (17). As exercise increases, I_{body} decreases with very little net change in T_{cw}. The *vertical bar* on the ordinate

labeled U.N. represents the range of operational water temperature for this subject up to an exercise level of 3 times resting metabolism.

By contrast, D.N. depicts values for the current deacclimatized, thermally nude Ama (4). Their acquired SFT (averaging 9 vs. 2 mm in 1960) provides an operational water temperature of 27 to 28°C over a threefold increase in metabolism. This is sufficient for summer work with minimal wetsuit protection.

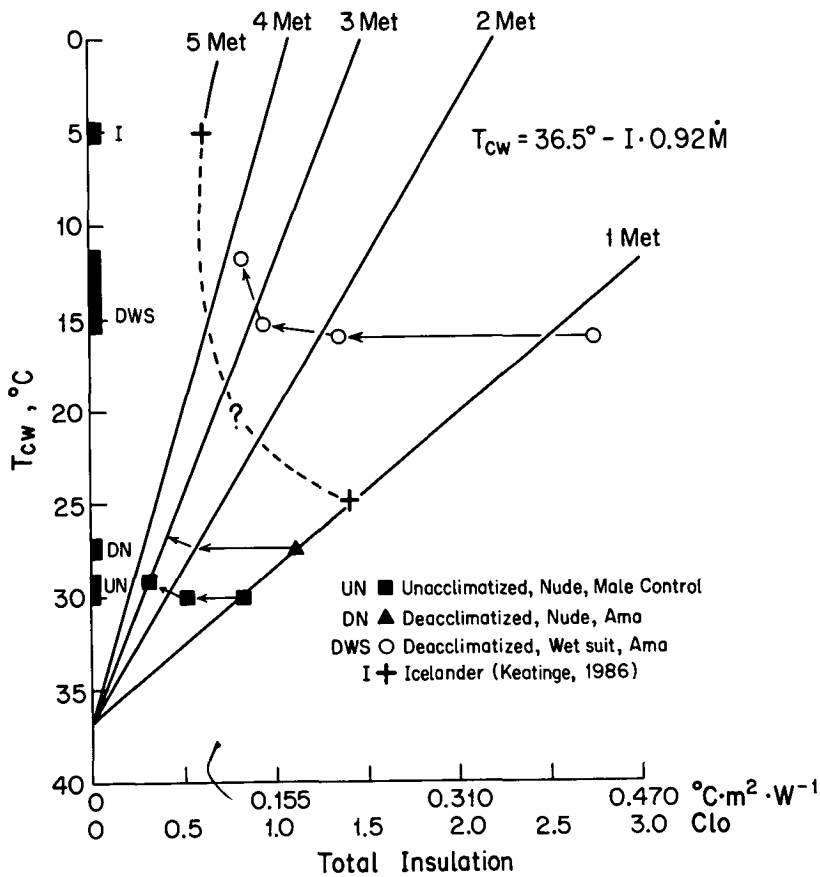

Fig. 8. Minimal operative water temperature expressed as T_{cw} as a function of total body insulation. Linear isopleths are theoretical values calculated from the formula: $T_{cw}(°C) = 36.5 °C - I \cdot 0.92$ M. Observed data are for unacclimatized males (■), deacclimatized nude Ama (▲), deacclimatized Ama (O), and an Icelandic fisherman (+). See text for details. Modified from Hong et al. (11).

Subject D.W.S. illustrates the case for the modern deacclimatized wetsuited Ama, whose operational water temperatures are seen to range from 16°C at rest to 12°C during 3.5 Met exercise. This is sufficient for winter work

with minimal loss of body heat stores.

Perhaps the most dramatic example of resistance to cold water has recently been published by Keatinge et al. (20). They recount the exploit of an obese young Icelandic fisherman who swam in 5°C water for 5 to 6 h from his swamped boat to shore. He was later studied in Keatinge's laboratory to determine his I during exercise in 5 to 6°C water. The results are illustrated as + in Fig. 8. During exercise at 5 Met his observed insulation of 0.7 clo would afford an operational water temperature of 5°C—by actual observation and by this simple graphic analysis. His insulation at rest (M = 1 Met) was not measured but his SFT was 14 mm, and I have determined from Fig. 4 that his I_{max} would be approximately $0.22°C \cdot m^2 \cdot W^{-1}$ (1.4 clo units). T_{cw} would therefore be approximately 25°C (Fig 3). Clearly, he would enjoy the greatest span of operational water temperature of any of the examples cited, purely on the basis of his thick subcutaneous fat layer and his muscular endurance.

In summary, fat remains the single most readily measurable variable determining overall body insulation at rest and during exercise. However, the insulative resistance of deeper shell tissue, presumably skeletal muscle, is far more important quantitatively than fat in resting subjects, whether shivering or nonshivering. This insulative resistance is progressively reduced as voluntary exercise intensity increases up to 3 Mets, almost exactly offsetting the increased heat production, and leaving the physical resistance of subcutaneous fat alone as the dominant barrier to heat loss in the heavily exercising subject.

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COLD ACCLIMATION CAN BE INDUCED IN HUMANS BY REPEATED COLD WATER IMMERSION

A. J. Young, S. R. Muza, M. N. Sawka, and K. B. Pandolf

Studies of individuals experiencing a lifetime of repeated exposure to cold stress have clearly demonstrated that the process of cold acclimatization does occur in humans. For example, Australian aborigines, South African bushmen, and professional breath-hold divers of the South Korean and Japanese coasts all show different thermoregulatory responses to cold stress than do subjects from populations native to warm or temperate climates (1-3). However, consensus is lacking as to whether cold acclimation can be induced in humans who lack a lifetime experience of cold stress. Chronic exposure to cold stress (both continuous and repeated intermittent) has been reported to produce adaptations in the responses to cold (4-8). Several of those investigations, however, were "field" studies in which the effects of altered environmental conditions, clothing, or physical activity were not adequately measured, controlled for, or reported (6, 7). In addition, it has been suggested that adaptations in physiologic responses to cold are really indicative of the development of habituation (decreased responsiveness) and not acclimation (enhanced responsiveness) to cold (8).

As a basis for the present investigation, it was hypothesized that the requisite physiologic stimulus to induce development of a measurable degree of cold acclimation in humans is a large and repeated lowering of body temperature, as opposed to cold exposure per se. Previous studies investigating the effects of cold air exposure may have failed to provide sufficient stimulus to induce cold acclimation in their subjects. A greater and more rapid lowering of body temperature can be achieved during cold water immersion than with cold air exposure. Thus cold water immersion would provide a greater thermoregulatory challenge than exposure to cold air. The purpose of this report is to review the results of our recent investigation (9) which was undertaken to

determine the effects of repeated cold water immersion on human thermoregulatory, cardiorespiratory, and body fluid responses to acute cold stress.

METHODS

Seven male Caucasians, native to the continental United States, volunteered as test subjects. None had experienced any significant cold exposure during the 6 mo. before beginning the study. Descriptive characteristics (mean ± SE) of the subjects were: age, 24 ± 2 yr; total body mass, 70 ± 4 kg; body surface area, 1.98 m^2; body fat, 17 ± 2%; skinfold thickness (14 sites), 11 ± 2 mm; maximal oxygen uptake, 45 ± 2 $ml \cdot kg^{-1} \cdot min^{-1}$.

The subjects performed a standardized cold air exposure 2 d before and 2 d after completion of the cold acclimation program. The standardized cold air exposure consisted of a 30-min baseline period and a 90-min cold period. During the baseline period, the subject was wrapped in blankets and reclined on a nylon-mesh lounge chair in a comfortable environment (T_a = 24°C, rh = 30%). After the baseline period, the subject stood, entered the environmental chamber (T_a = 5°C, rh = 30%), and then reclined for 90-min wearing only swim trunks.

The cold acclimation program consisted of a daily immersion in cold (18°C, stirred) water, repeated 5 d (generally consecutive) each week for 5 consecutive wk. Each immersion was 90-min unless the subject's rectal temperature fell below 35°C, in which case that session was terminated early for safety reasons. A total of 24 acclimation sessions were scheduled. During immersion, the subject reclined quietly with the water level adjusted to the bottom of the neck. Only swim trunks were worn during immersion. The first and last cold water immersions were carried out using the same protocol and data collection procedures as in standardized cold air exposures. During the remaining cold water immersions, only limited data collection was performed and no baseline period was employed.

All cold exposures were completed at the same time of day to avoid the influence of circadian rhythms. Cardiorespiratory measurements were made once during the baseline period and at regular intervals during the cold period of both cold air exposures and the first and last cold water immersion. Rectal (T_{re}) and mean weighted skin ($\overline{T}_{sk}$) temperatures were measured at 2-min intervals during both baseline and cold periods. Venous blood samples were obtained during the last 5 min of baseline and cold periods for determination of hematocrit and hemoglobin concentration, as well as plasma osmolality and concentration of proteins, epinephrine, and norepinephrine. Urine production rate was measured during each period and urine aliquots from each period were saved for later analysis of specific gravity, and Na^+ and K^+ concentration.

The data were analyzed using multifactor analysis of variance (ANOVA). When ANOVA indicated significant main or interaction effects for the factors "acclimation" (pre vs. post), or "cold exposure" (baseline vs. final for blood parameters or the periodically repeated physiologic measurements), Tukey's

critical difference was calculated and used to determine if differences between means were significant.

RESULTS AND DISCUSSION

As previously stated, it was hypothesized that to induce development of a measurable degree of cold acclimation, an individual's core temperature must be repeatedly reduced by a substantial amount for an extended time. This was accomplished with the acclimation program used; the subjects sustained a reduction in T_{re} of about 1°C for 90 min on 24 d during a 35-d period. None of the physiologic responses to cold water immersion (e.g., thermoregulatory, cardiorespiratory, body fluid responses) were changed by the acclimation program to indicate development of any particular pattern of cold adaptation. Thus, while cold water immersion provided what was thought to be a sufficient stimulus to induce cold acclimation, adaptations in cold responses were apparently inadequate to offset the severity of the stress imposed by this aqueous environment. The possibility that evidence for cold acclimation would be masked during water immersion by the magnitude of the cold stress had been anticipated and was the reason for inclusion of the cold air exposures in the experimental design.

In contrast to the responses to the cold water, the physiologic responses to cold air did show significant adaptations after the acclimation program. Aerobic metabolic rate before and during the two cold air exposures is shown in Fig. 1. During the postacclimation cold air exposure, aerobic metabolism was lower ($P < 0.05$) during the first 10 min, but by the 30th min and thereafter there was no difference in pre- and postacclimation metabolic rate. These observations may indicate that the onset of shivering was delayed by acclimation, as has been suggested by others (5).

Figure 2 shows the T_{re} measured before and after 30, 60, and 90 min of the pre- and postacclimation cold air exposure. Before and at all times during the cold air exposure after acclimation, T_{re} was reduced ($P < 0.003$) compared to before acclimation (Fig. 2 *A*). Even after correcting for the effect of a lower initial T_{re} in the postacclimation test by calculating individual changes relative to initial T_{re} (Fig. 2 *B*, there was a greater and more rapid fall ($P < 0.01$) in T_{re} during the cold air exposure compared to preacclimation. A greater and more rapid fall in T_{re} during cold air exposure after acclimation has also been observed in other studies of human cold acclimation. This adaptation has been referred to as hypothermic cold acclimation (6) or habituation (8). A reduction in baseline temperature is not usually observed with seasonal cold acclimatization; in fact, others have observed an increase in basal metabolic rate during the winter months (2). However, consistent with a lower baseline temperature, the subjects in the present investigation had slightly lower baseline aerobic metabolic rates (Fig. 1) after acclimation than before, but the difference was not statistically significant. These observations might suggest that cold acclimation results in a resetting of the normally regulated body

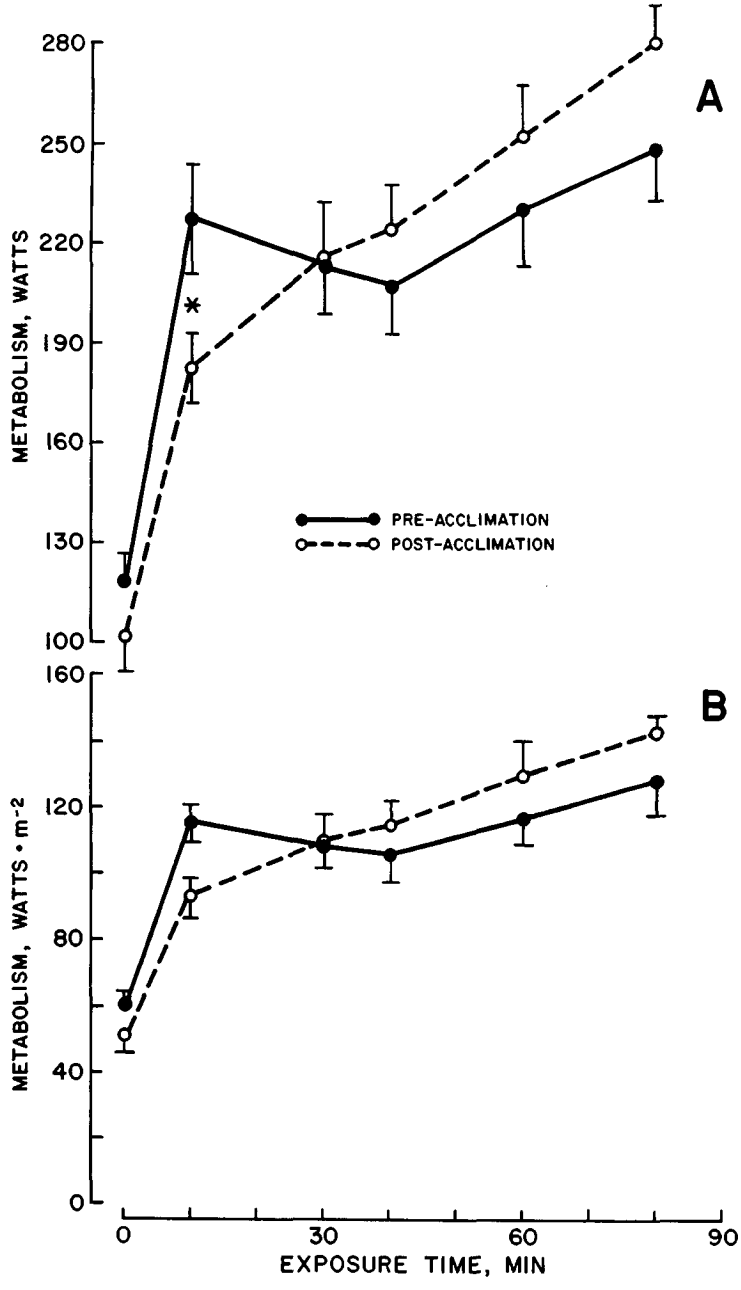

Fig. 1. Aerobic metabolism before (0 min) and during 90-min exposure to cold (5 ° C) air shown as absolute value (A) and relative to body surface area (B). Values are means ± SE; $n = 7$. a * Significant ($P < 0.05$) difference between pre- and postacclimation.

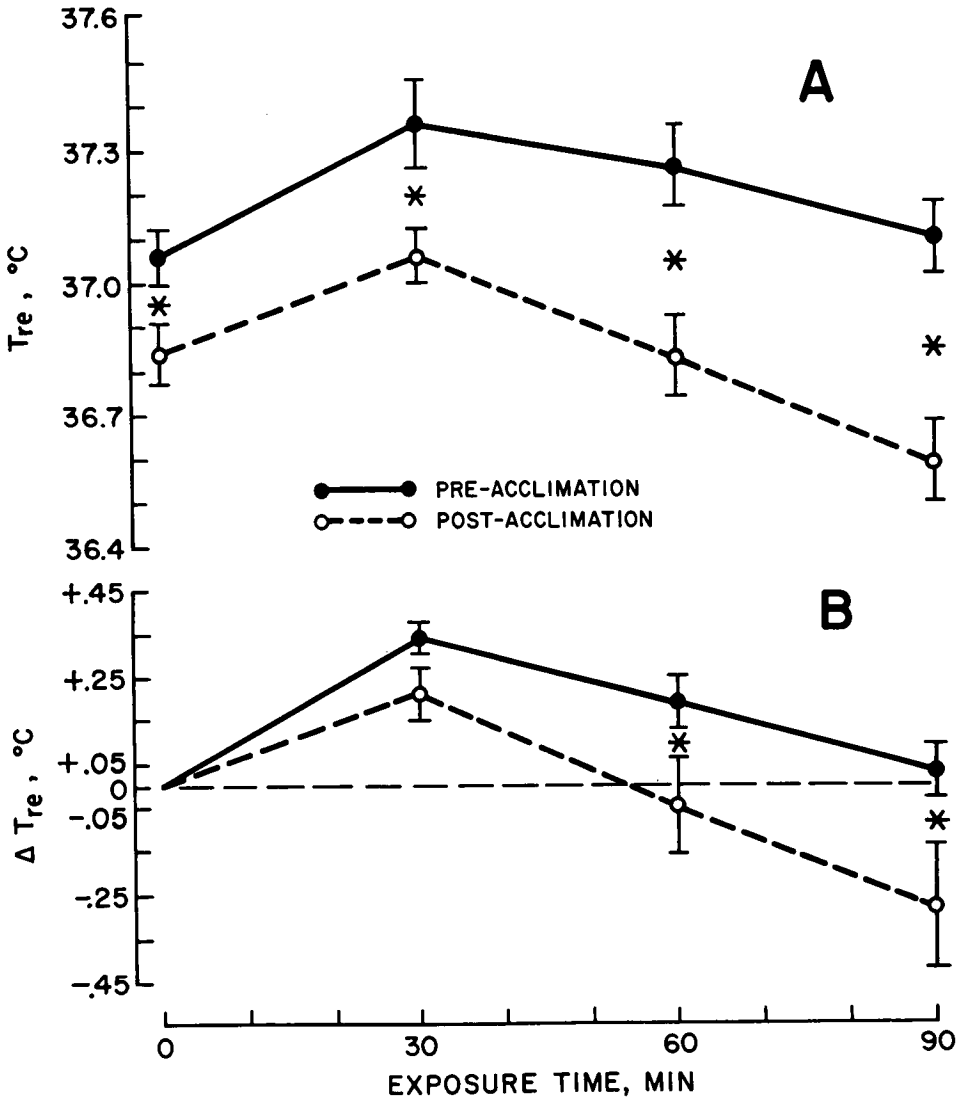

Fig. 2. Rectal temperature (T_{re}) before (0 min) and during 90-min exposure to cold (5 ° C) air (A), and the change in T_{re} during cold exposure relative to the initial value (B). Values shown are means ± SE; $n = 7$. * Significant ($P < 0.01$) difference between pre- and postacclimation.

temperature, but this speculation must be confirmed.

Figure 3 shows the effect of cold air exposure on $\overline{T}_{sk}$. During the first 30-min of both pre- and postacclimation cold air exposures, $\overline{T}_{sk}$ fell but thereafter remained plateaued. Figure 3 shows that $\overline{T}_{sk}$ plateaued at about 4°C lower ($P < 0.01$) during the postacclimation cold air exposure compared to preacclimation. This observation is consistent with the observations that

persons acclimatized to cold over a lifetime maintain lower skin temperatures during cold exposure than nonacclimatized subjects (2). On the other hand, others have reported that repeated cold water immersion programs had no effect on $\bar{T}_{sk}$ during subsequent cold air exposure (8). However, the failure to observe lower $\bar{T}_{sk}$ may have been due to a lesser degree of cold acclimation in their subjects (8). The reduction in $\bar{T}_{sk}$ may reflect reduced skin blood flow due to greater cutaneous vasoconstriction. The increase in plasma norepinephrine (NE) concentration during cold air exposure (Table 1) after acclimation was 2 times greater ($P < 0.004$) than the increment in NE before acclimation. Acclimation had no significant effect on plasma epinephrine (E) concentration. Therefore, the increment in plasma NE response is due to enhanced sympathetic nervous activity, not adrenal medullary activity. Enhanced sympathetic responsiveness to cold air could cause more pronounced cutaneous vasoconstriction thereby resulting in lower $\bar{T}_{sk}$ during the postacclimation cold air exposure. Furthermore, an increased sympathetic responsiveness to cold air stress is clear evidence that repeated cold water immersion induced a true cold acclimation, not habituation.

Table 1
Changes in Plasma Catecholamine Concentration
During 90-min Exposure to Cold Air

	Norepinephrine, $ng \cdot l^{-1}$		Epinephrine, $ng \cdot l^{-1}$	
	Preacclimation	Postacclimation	Preacclimation	Postacclimation
Initial	485 ± 90	293 ± 33	62 ± 12	34 ± 5
Final	969 ± 206*	1257 ± 166*	73 ± 17	96 ± 30
Change	484	694**	11	62

Values are means ± SE. * Significant ($P < 0.002$) difference between initial and final. ** Significant ($P < 0.004$) difference between pre- and postacclimation.

The two components that comprise the total insulative shell of the body are the superficial shell (skin and subcutaneous fat) and the subcutaneous muscle shell. The insulation provided by subcutaneous fat is proportional to its thickness and therefore relatively constant, at least for short periods such as the duration of the present study. However, the insulation provided by skin and muscle is in large part determined by blood flows to these regions and thus can be regulated. Total insulation of the body's shell increases during cold exposure due to a sympathetically mediated cutaneous vasoconstriction and a reduction in skeletal muscle perfusion. The reduction in $\bar{T}_{sk}$ with acclimation enhances insulation in two ways. First, insulation provided by the skin is increased due to a smaller temperature gradient for heat transfer between the

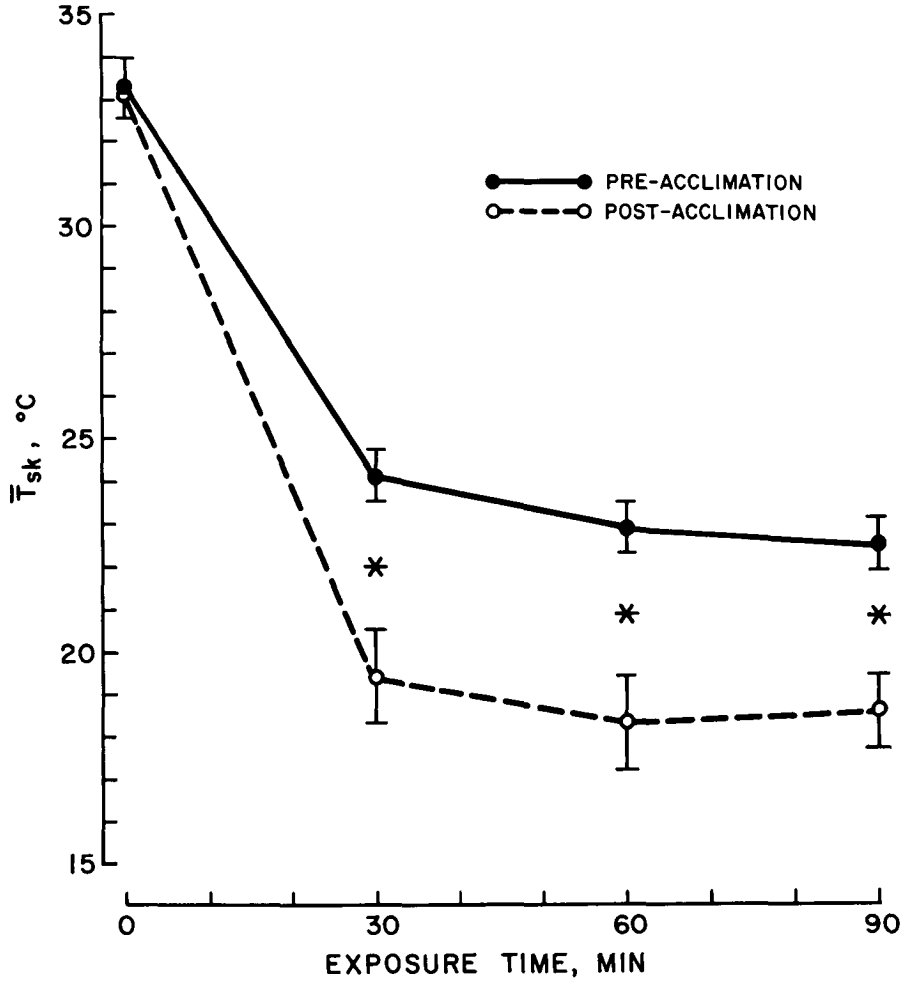

Fig. 3. Mean weighted skin temperature ($\bar{T}_{sk}$) before (0 min) and during 90-min exposure to cold (5 °C) air. Values shown are means ± SE; $n = 7$. * Significant ($P < 0.01$) difference between pre- and postacclimation.

skin and the environment. Second, the enlarged T_{re}-$\bar{T}_{sk}$ difference (Fig. 4) after acclimation ($P < 0.02$) facilitates the transfer of heat from the body core to the muscle shell, whereas improved skin vasoconstriction would limit the loss of heat from the muscle shell. Thus, acclimation may allow maintenance of a warmer, better-perfused muscle shell. The lower T_{re} during cold exposure after acclimation may reflect redistribution of body heat stores from the core to the muscle shell. The development of this type of insulative acclimation would allow better maintenance of muscle function.

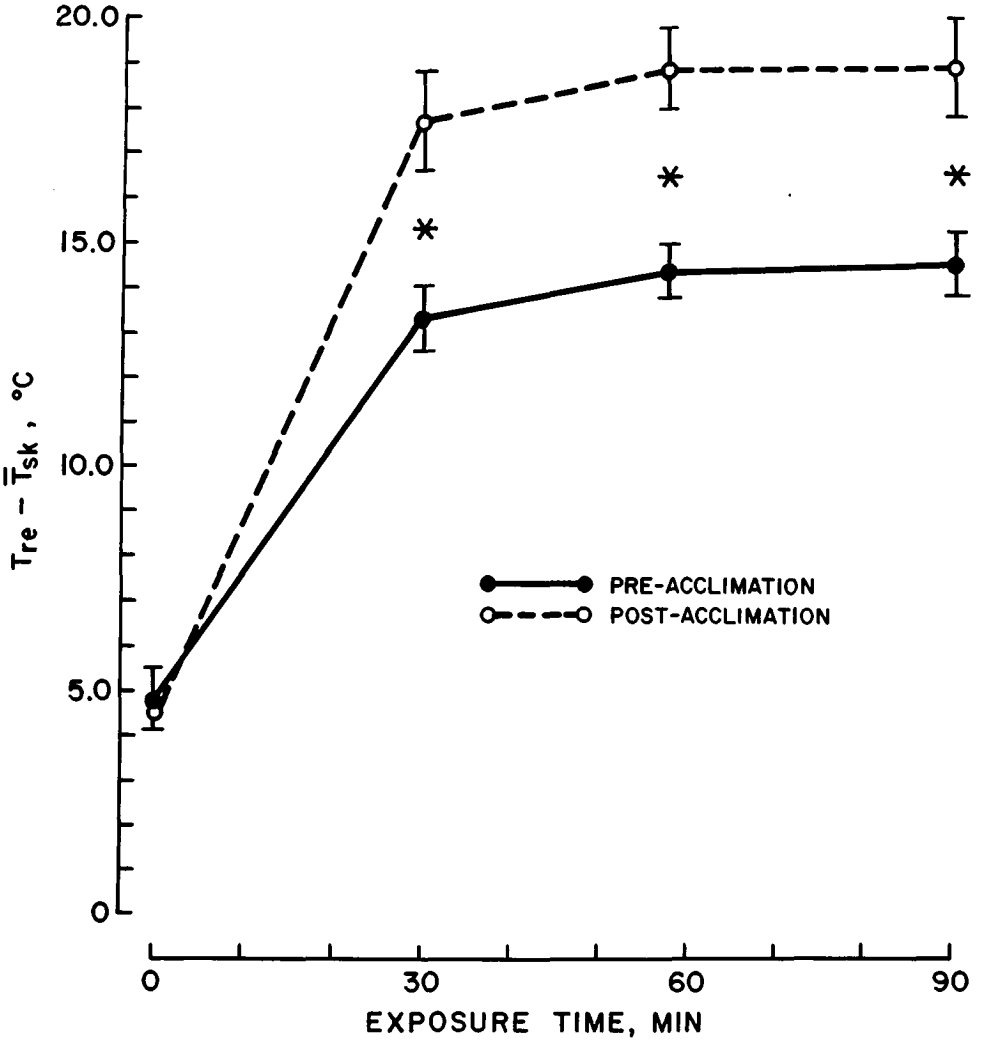

Fig. 4. Gradient between rectal and mean weighted skin temperature (T_{re}-$\bar{T}_{sk}$) before (0 min) and during 90-min exposure to cold (5 ° C) air. Values shown are means ± SE; $n = 7$. * Significant difference between pre- and postacclimation.

Cardiorespiratory responses to cold air exposure were not influenced by the acclimation program to any significant degree. For example, cardiac output (Fig. 5) and pulmonary ventilation (Fig. 6) increase during cold air exposure. However, the increments in cardiac output and ventilation during cold air exposure were not different between pre- and postacclimation. Likewise, body fluid regulation does not seem to be altered by the acclimation program. Plasma volume decreased ($P < 0.05$) and urine flow rate increased ($P < 0.05$) by

the same amount (12 and 52%, respectively) during cold air exposure before and after the acclimation program.

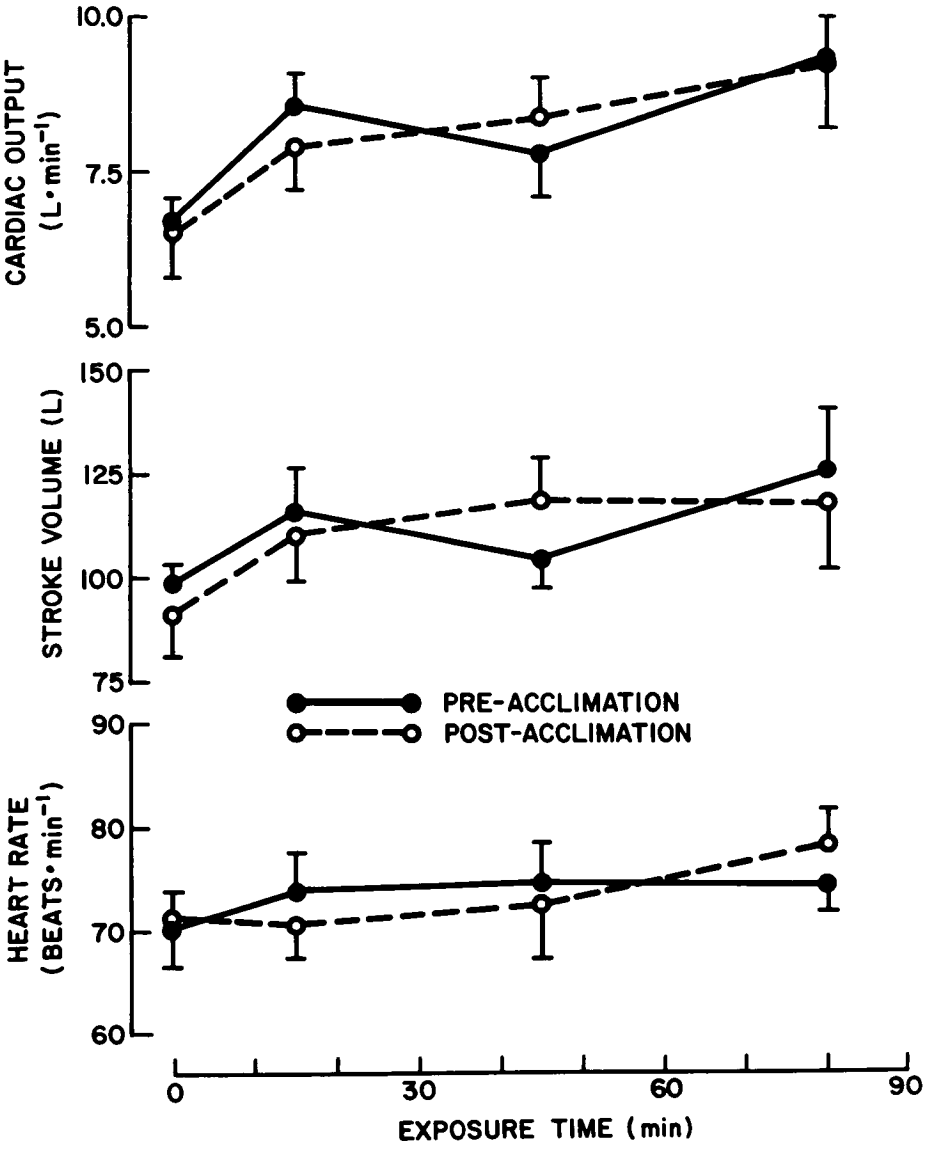

Fig. 5. Heart rate, stroke volume, and cardiac output before (0 min) and during 90-min exposure to cold (5 ° C) air. Values shown are mean ± SE; $n = 7$.

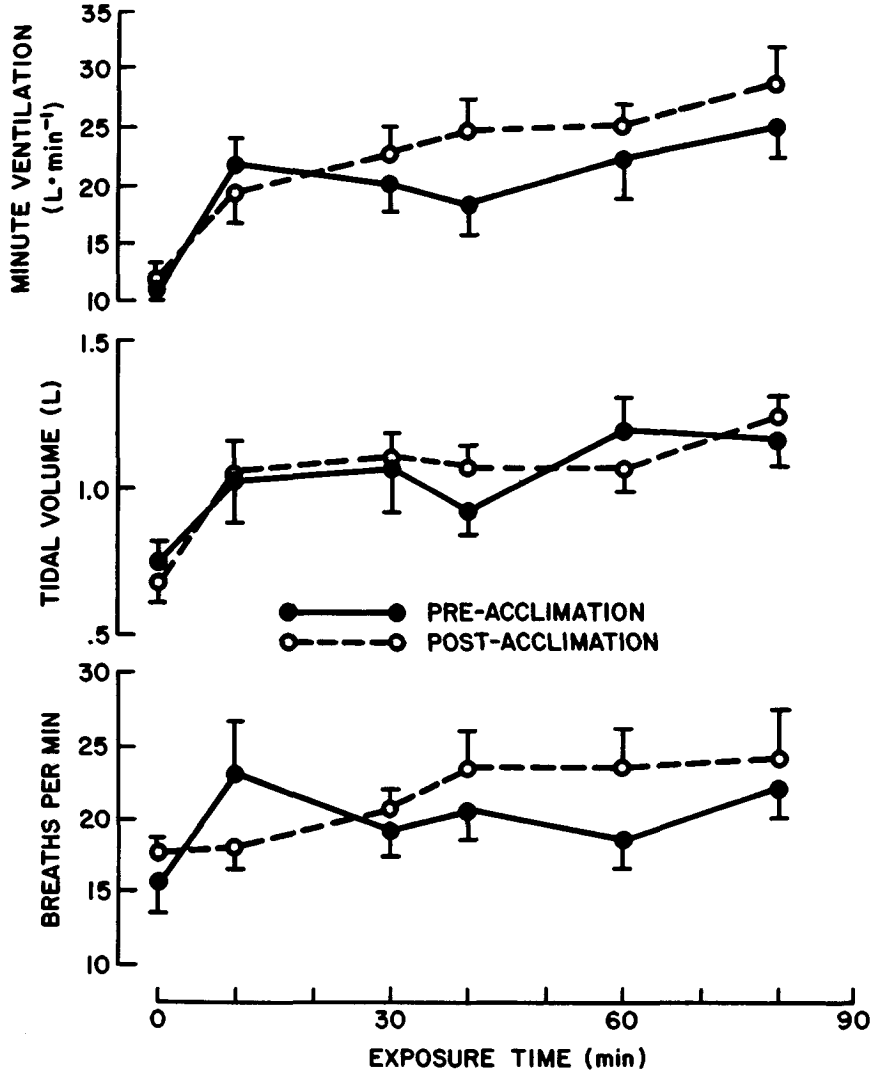

Fig. 6. Tidal volume, respiratory frequency, and minute ventilation before (0 min) and during 90-min exposure to cold (5 ° C) air. Values shown are means ± SE; $n = 7$.

In summary, the program of repeated immersion in cold water induced a true acclimation to cold air in nonadapted men. The type of acclimation produced seems to be insulative, resulting in lower skin temperature during cold exposure. The physiologic mechanism for enhanced insulation is probably an increased sympathetic responsiveness to cold stress, which in turn mediates more pronounced cutaneous vasoconstriction. Frequency, magnitude, and duration of core temperature reduction as opposed to exposure conditions seem

to be key determinants of whether cold acclimation or habituation are induced.

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THERMAL BALANCE OF WET-SUIT DIVERS DURING EXERCISE IN COLD WATER AT 1, 2, AND 3 ATA

D. J. Suh, D. S. Yeon, H. J. Kim, J. K. Choi, Y. S. Park, Y. H. Park, and S. K. Hong

The major thermal problem in divers working in cold water is direct heat loss through the skin (1, 2). It has been documented that insulation of the human body undergoes a dramatic reduction during exercise in water (3). Moreover, physical insulation of wet suits decreases as pressure increases due to compression of trapped air (4, 5). Thus, the overall insulation (and hence the skin heat loss) of wetsuited subjects will change as a function of both pressure and workload. We therefore investigated the combined effect of exercise and pressure on the thermal balance of wet-suit divers in cold water.

METHODS

In 3 male wet-suit divers (average age 32 yr, height 168 cm, weight 67 kg, body surface area 1.76 m^2, mean skinfold thickness 12.5 mm) changes in body temperature, heat exchange, and thermal insulation were determined during immersion in 15 to 16°C water at 1, 2, and 3 ATA. The subjects were clad in usual neoprene wet suits consisting of jacket, pants, boots, and mittens of 5-mm thickness and immersed up to the neck in the wet pot of a hyperbaric chamber. The chamber pressure was maintained at 1, 2, or 3 ATA air. The subjects either rested or exercised at a constant intensity for 2 h. Exercise was performed using a bicycle ergometer submersed in water. The external workload was empirically established for each subject by modifying the frequency of pedaling. The rectal (T_r) and skin (T_s) temperatures were measured at 10-min intervals with appropriate thermistor probes (Yellow Springs Instrument Co., Yellow Springs, OH). The skin probes were attached to the skin surface using stomaseal (no. 1500, Minnesota Mining and Manufac-

turing Co.) and secured with a surgical adhesive plaster. The skin temperature was measured at chest (T_c), back (subscapular, T_{sc}), upper arm (T_{ua}), thigh (T_{ul}), and calf (T_{ll}), and the mean temperature of skin exposed to water ($\bar{T}_s$) was calculated as: $\bar{T}_s = 0.15\ (T_c + T_{sc}) + 0.3\ T_{ua} + 0.2\ (T_{ul} + T_{ll})$.

Oxygen consumption ($\dot{V}O_2$) was measured directly at 30-min intervals using a closed-circuit, 9-liter Collins spirometer (Warren E. Collins, Inc., Baltimore, MD), and metabolic heat production ($\dot{M}$, kcal·m^{-2}·h^{-1}) was calculated as 4.83 $\dot{V}O_2$/A, where A is the DuBois body surface area (m^2).

Thermal insulation of the subject (I, °C·kcal^{-1}·m^{-2}·h^{-1}) was determined at rest and during exercise using the following formula (6): $I_{body} = (T_r - \bar{T}_s)/\dot{H}_S$, $I_{total} = (T_r - T_w)/\dot{H}_S$, where T_r is the rectal temperature, T_w the water temperature, T_s the mean skin temperature, and $\dot{H}_S$ the skin heat loss (kcal·m^{-2}·h^{-1}). $\dot{H}_S$ was assumed to be equal to $\dot{M}$ minus respiratory heat loss (0.08 $\dot{M}$). In cases where mean body temperature ($T_B = 0.6\ T_r + 0.4\ \bar{T}_s$) was decreasing during the last hour of immersion, the change in body heat store ($S = \Delta T_B \times 0.83 \times$ body weight/A) was added to 0.92 $\dot{M}$ in estimating $\dot{H}_S$. All calculations were made when the change in heat exchange was minimal during the last hour of immersion.

RESULTS

Figure 1 illustrates average time courses of T_r, $\bar{T}_s$, and $\dot{M}$ of 3 wet-suited subjects at rest and during light exercise in 15-16°C water at 1, 2, and 3 ATA. At rest in water, T_r declined continuously during the 2-h immersion at all pressures tested. The total reduction in T_r for 2 h was 1.1°C (from 37.5 to 36.4) at 1 ATA, 0.9°C (from 37.4 to 36.5) at 2 ATA, and 1.8°C (from 37.6 to 35.8) at 3 ATA. The $\bar{T}_s$ dropped rapidly by about 5°C (from 33.6 to 28.5) at 1 ATA and 7°C (from about 33 to 26) at 2 and 3 ATA over the initial 10 to 20 min and then it decreased very slowly or remained unchanged during the rest of the immersion period.

Table 1 summarizes the average value for T_r, $\bar{T}_s$, and metabolic rate ($\dot{M}$) along with calculated skin heat loss ($\dot{H}_S$) and thermal insulation (I) at rest in the 2nd h of immersion at 1, 2, and 3 ATA. although the T_r did not seem different between 1 (36.4°C), 2 (36.4°C), and 3 (35.9°C) ATA, the $\bar{T}_s$ seemed significantly lower at 2 (24.5°C), and 3 (24.4°C) ATA than at 1 ATA (26.9°C). On the other hand, $\dot{M}$ at 2 and 3 ATA (85 and 95 kcal·m^{-2}·h^{-1}, respectively) were significantly higher than the corresponding value at 1 ATA (72 kcal·m^{-2}·h^{-1}), probably due to shivering induced by lower skin temperature at depth than at the surface (1 ATA). Likewise, the $\dot{H}_S$ (74, 88, and 94 kcal·m^{-2}·h^{-1} at 1, 2, and 3 ATA, respectively) increased as pressure increased, indicating that the overall thermal insulation of subjects decreased with pressure. In fact, the calculated total insulation with wet suits (I_{total}) reduced significantly from 0.298°C·kcal^{-1}·m^{-2}·h^{-1} at 1 ATA to 0.253 at 2 ATA and to 0.224 at 3 ATA. Body tissue insulation (I_{body}), however, did not apparently change with pressure.

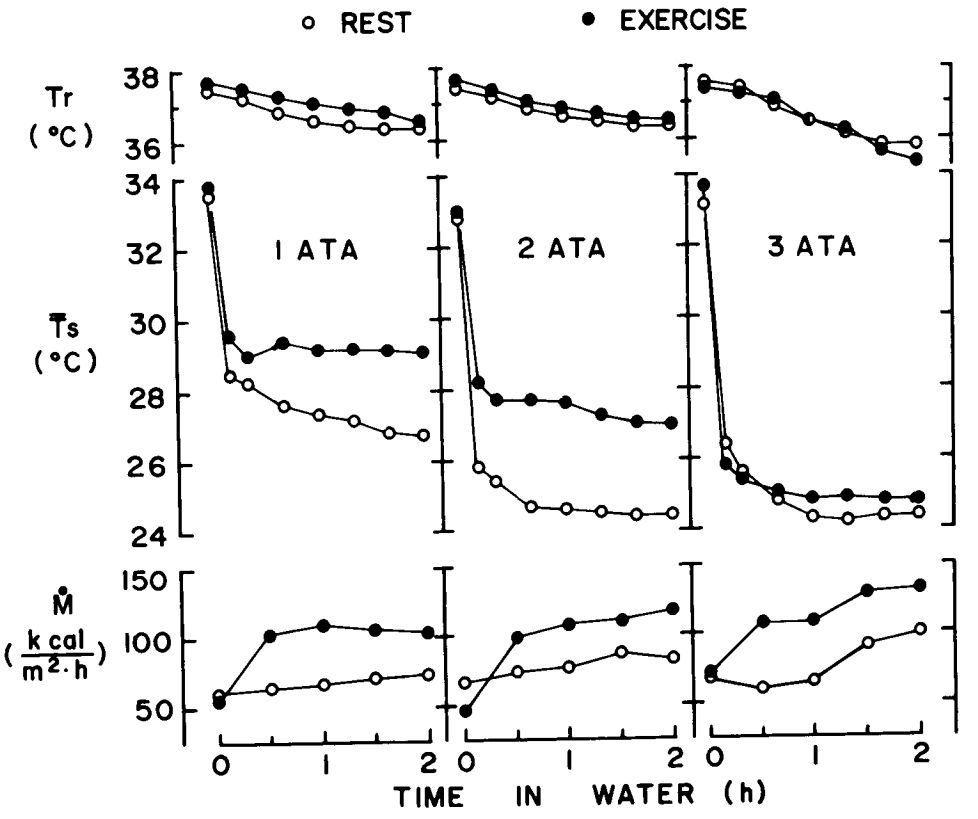

Fig. 1. The average time course of rectal temperature (T_r), mean skin temperature ($\bar{T}_s$), and metabolic rate ($\dot{M}$) of 3 wet-suit divers while resting (open symbol) or performing light exercise (solid symbol) in water of 15-16 ° C at 1, 2, and 3 ATA. Values are mean ± SE.

During exercise in water ($\Delta \dot{M}$ = 29 - 36 kcal•m^{-2}•h^{-1}, the change in T_r was similar, but that in $\bar{T}_s$ was much smaller as compared with those observed in resting subjects at the corresponding pressure (Fig. 1). Consequently, as depicted in Fig. 2, the final T_r at the end of 2-h immersion was not apparently different between the resting and exercising subjects, but the final $\bar{T}_s$ seemed significantly higher in the latter than the former, although the difference was small at 3 ATA. At all levels of workload the $\bar{T}_s$ decreased as the pressure increased.

Figure 3 presents the average skin heat loss ($\dot{H}_S$) in the 2nd h of immersion as a function of exercise intensity at 1, 2, and 3 ATA. The $\dot{H}_S$ either at rest or during exercise was higher at depths than at the surface. At all pressures, $\dot{H}_S$ increased linearly with the exercise intensity, implying that

Table 1
Effect of Pressure on the Body Temperature, Metabolism, Skin Heat Loss, and Thermal Insulation of Wet-Suit Divers While Resting in Water at 15-16 °C

	1 ATA	2 ATA	3 ATA
T_r, °C	36.4 ± 0.2	36.4 ± 0.1	35.9 ± 0.3
$\bar{T}_s$, °C	26.9 ± 0.5	24.5 ± 1.1**	24.4 ± 0.6**
$\dot{M}$ (kcal·m^{-2}·h^{-1})	72.2 ± 8.1	85.0 ±10.1**	95.3 ± 23.4*
$\dot{H}_S$ (kcal·m^{-2}·h^{-1})	74.0 ± 8.4	88.1 ± 5.5**	93.9 ± 14.7*
I_{total} (°C·kcal^{-1}·m^{-2}·h^{-1})	0.298 ± 0.032	0.253 ± 0.023*	0.224 ± 0.034**+
I_{body} (°C·kcal^{-1}·m^{-2}·h^{-1})	0.136 ± 0.020	0.145 ± 0.025	0.136 ± 0.027

Data represent average values of 3 subjects during the 2nd h of immersion; ** and * difference from the corresponding values at 1 ATA is significant at $P < 0.05$ and $P < 0.10$, respectively, in 1-tailed paired t test; + significantly different from the corresponding value at 2 ATA.

thermal insulation of subjects decreased with exercise. Indeed, as depicted in Fig. 4, the calculated overall insulation (I_{total}) declined gradually as the exercise intensity increased at all pressures tested (1, 2, and 3 ATA). This decrease in I_{total} appeared to be due in part to the reduction in body insulation (I_{body}) and in part to the decrease in external insulation provided by wet suits. The I_{body} was 0.136 to 0.145°C·kcal^{-1}·m^{-2}·h^{-1} at rest but it decreased gradually as the exercise intensity increased. Likewise, the apparent suit insulation estimated from the difference between the total and body insulation ($I_{suit} = I_{total} - I_{body}$) at each pressure declined with exercise intensity (Fig. 4, *inset*). The value of I_{suit} either at rest (0.16, 0.11, and 0.09°C·kcal^{-1}·m^{-2}·h^{-1} at 1, 2, and 3 ATA, respectively) or during exercise decreased as pressure increased. The actual thickness of wet suits reduced from 5 mm at 1 ATA to 3.5 at 2 ATA and 2.6 at 3 ATA.

DISCUSSION

Although it has been widely recognized that the insulative value of foam neoprene wet suits reduces with pressure (1, 4, 5) few studies on the thermal balance of wet-suited subjects at high hydrostatic pressure have been carried out.

The result of the present study indicate that the protective effect of wet suits on the thermal balance of subjects immersed in cold water reduces

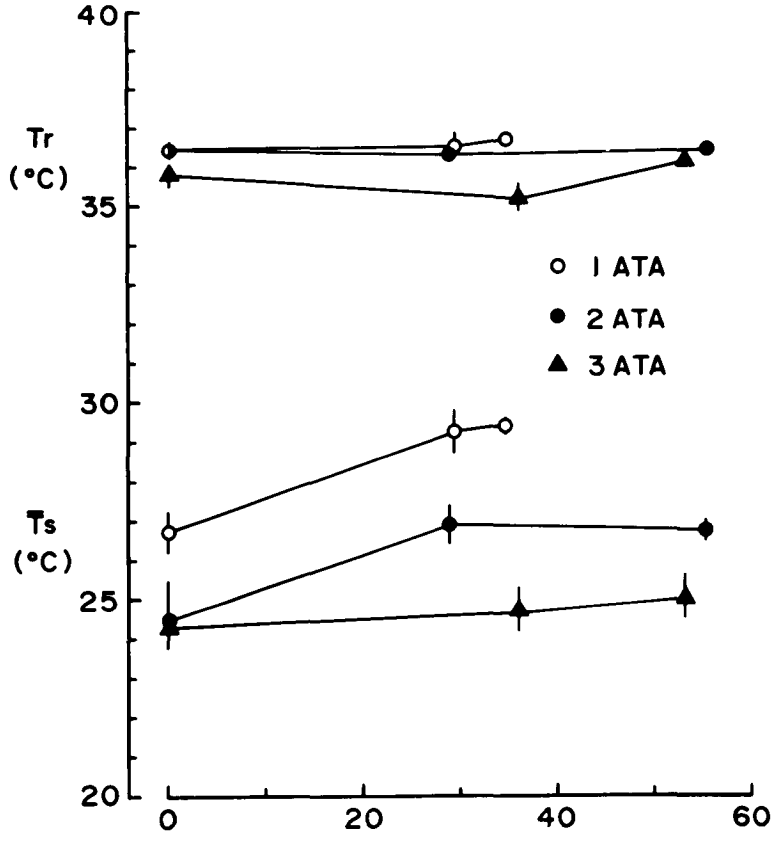

Fig. 2. Rectal (T_r) and mean skin ($\bar{T}_s$) temperatures of wet-suit divers at the end of 2 h rest or exercise in 15-16 ° C water at 1, 2, and 3 ATA. The exercise intensity is expressed as the metabolism above the resting value. Values are mean ± SE of 3 subjects

gradually as the pressure increases from 1 to 3 ATA. In both resting and exercising subjects, the fall in rectal temperature during immersion did not change extensively with pressure but that in skin temperature and the rates of metabolism and skin heat loss increased gradually with pressure, the change being particularly significant between 1 and 2 ATA (Figs. 1-3). These results may be compatible with variations in suit insulation with pressure. The apparent suit insulation (I_{suit}) estimated in resting subjects, for instance, reduced from 0.16°C•kcal^{-1}•m^{-2}•h^{-1} at 1 ATA to 0.11 at 2 ATA and to 0.09 at 3 ATA (Fig. 4, *inset*). Such a change in I_{suit} was obviously attributed to compression of wet suits at pressure. The value of suit thickness reduced from 5 mm at 1 ATA to 3.5 mm at 2 ATA and to 2.6 mm at 3 ATA, the degree of compression being exactly comparable to that for 0.25-in. neoprene wet suits observed by others (1). It thus seems that the reduction in external insulation

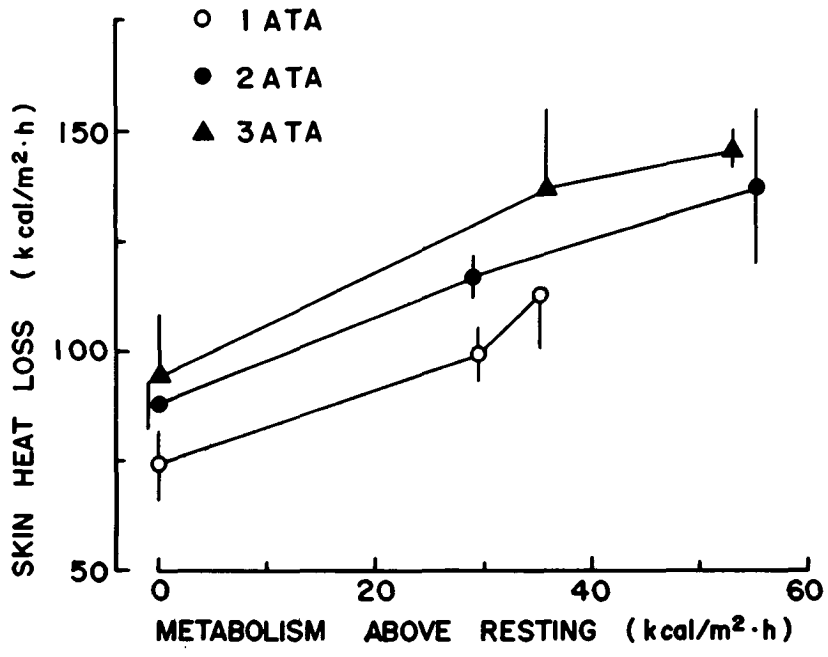

Fig. 3. Rate of skin heat loss of wet-suit divers during the 2nd h of immersion in 15-16 ° C water as a function of exercise intensity at 1, 2, and 3 ATA. The exercise intensity is expressed as the metabolism above the resting value. Values are mean ± SE of 3 subjects.

due to suit compression allowed higher skin heat flux and hence lower skin temperature at pressure than at the surface. The metabolic rate was accordingly increased at pressure, compensating for the increased heat loss. As a result, the rectal temperature was maintained at a similar level at all pressures tested (1, 2, and 3 ATA).

The total thermal insulation (I_{total}) of subjects at pressure as well as at the surface underwent drastic reduction during exercise in water (Fig. 4). Interestingly, at all pressures tested, the magnitude of reduction was far greater than can be accounted for by the change in body insulation (I_{body}), indicating that the external insulation afforded by wet suits decreased with exercise. The apparent suit insulation (I_{suit}) estimated from the difference between I_{total} and I_{body} declined progressively with exercise intensity (Fig. 4, *inset*). This finding is consistent with previous observations made on Korean women wet-suit divers at 1 ATA (7). In both present and previous studies, the 1 ATA value of I_{suit} for resting subjects ($0.16°C \cdot kcal^{-1} \cdot m^{-2} \cdot h^{-1}$ in the present study and 0.27 in the previous study) was significantly higher than the physical insulation of 5-mm neoprene wet suits ($0.12°C \cdot kcal^{-1} \cdot m^{-2} \cdot h^{-1}$) obtained using a copper manikin (8). However, the value for exercising subjects gradually approached the physical insulation as exercise intensity increased. Presumably, actual physical insulation of the suit did not vary during exercise

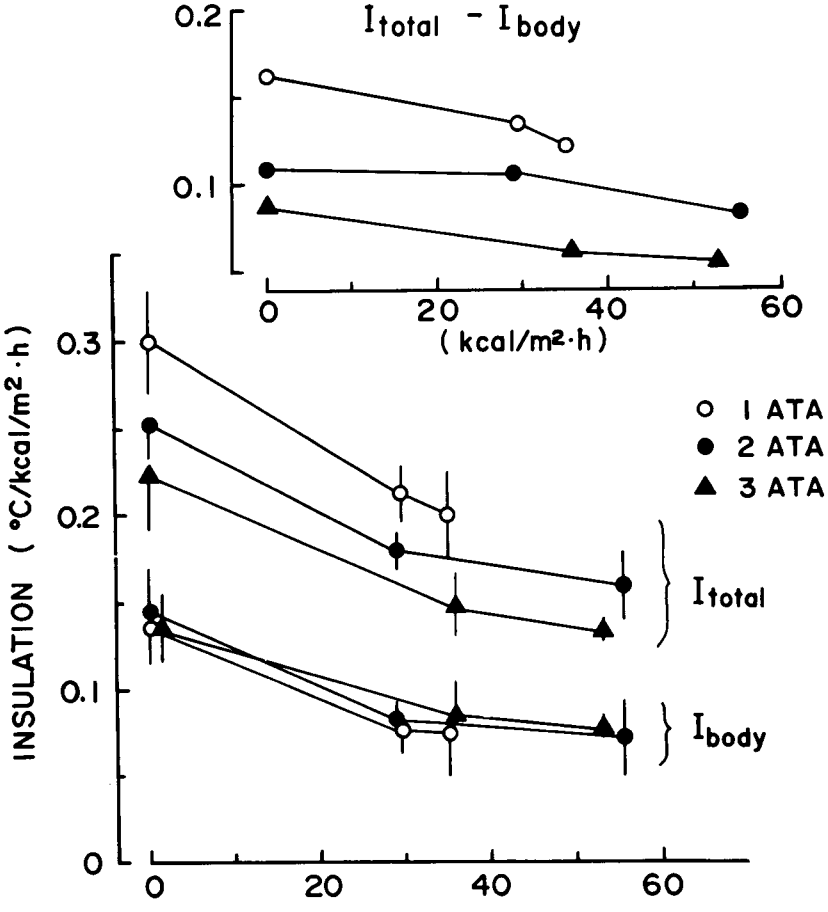

Fig. 4. Insulation (I) of wet-suit divers at rest and during exercise in 15-16 ° C water at 1, 2, and 3 ATA. Exercise intensity is expressed as the metabolism above the resting value. Values are mean ± SE of 3 subjects.

inasmuch as its thickness at a given pressure was not changed. We therefore speculate that the relatively high functional insulation in resting subjects is a consequence of physiologic regulation in cold water.

The overall insulation of the human body in cold is largely determined by the degree of limb vasoconstriction which in turn is sensitively affected by the skin temperature on the extremities (9, 10). In wet-suited subjects, external insulation provided by suits will be much lower in the limb than in the trunk, due to design of wet suits and the curvature effect. Although most of the trunk surface is covered by double sheets (jacket and pants), the limb is covered by a single sheet. Furthermore, the physical insulation of the suit material will decrease as the curvature of surface increases, as first pointed out by Van Dilla et al. (11) in connection with the insulation of clothing.

Thus, the limb surface of wet-suited subjects will be selectively cooled during immersion in cold water, and this will lead to intense vasoconstriction in the extremities. As a consequence, most of the heat exchange between body core and water in resting subjects will take place at the trunk surface where the suit insulation is relatively high. During exercise, however, hyperemia of skeletal muscles in the limbs will increase the area for heat exchange over a poorly insulated region in parallel with the trunk. Therefore, much of the heat produced in skeletal muscle is dissipated through a large surface area of the limbs rather than returning to the body core. For these reasons wet suits provide far greater physiologic insulation at rest than during exercise.

Another factor that reduced insulative protection of wet suits during exercise may be increased convective heat loss from water movement under the suit. Wolff et al (12) observed in wet-suited subjects immersed in water that a significant water exchange takes place through the neck and zipper seals, and the exchange increases during exercise. The extent of such water flow has not been assessed in the present study. However, inasmuch as the subjects complained of chest chilling during immersion and the skin temperature at chest (about 23°C in 1 ATA) was significantly lower than that in other parts of the trunk surface (32°C at subscapular) we presume that significant water convection took place, especially at the chest surface.

The 1 ATA value of I_{suit} estimated for resting subjects in the present study ($0.16°C \cdot kcal^{-1} \cdot m^{-2} \cdot h^{-1}$) was much smaller than that (0.27) obtained in a previous study on Korean women wet-suit divers (7). Such a wide difference in I_{suit} between the two studies is probably due to difference in the magnitude of convective heat loss. In the latter study, subjects wore wet suits without zippers and the suit was taped at the wrists, ankles, and waist; thus, water convection under the suit was probably not extensive.

A practical implication of these findings is that if a wet-suit diver is in a situation where escape from cold water is not possible, he is better off to move to the surface and hold still than to swim if wasting of energy is to be prevented. Even with wet suits, exercise increases heat loss as much as heat production in cold water.

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CUTANEOUS HEMODYNAMICS AND THERMOREGULATION IN A HYPERBARIC ENVIRONMENT

K. Shiraki, S. Sagawa, and N. Konda

Heat exchange at the body surface is more closely linked with ambient temperature in a hyperbaric environment than in a normal atmosphere. Some fatal accidents in hyperbaric environments are thought to be related to thermal stress. As the thermal gradient between the skin surface and the ambient temperature is the main site of heat exchange of the body, the regulating mechanism of the blood flow in the skin is a very important factor in limiting the thermal regulation of the body. In a normal atmosphere, distal parts of the body, i.e., the extremities, play an important role in the heat exchange of the body by active vasomotor response in these areas. In a normal atmosphere, the redistribution of blood flow can effectively control heat dissipation or conservation when needed; however, at abnormal barometric pressure the redistribution of blood flow during heat exposure may not be in the same direction as at sea level. For example, Sagawa et al. (1) observed at half an atmosphere (0.5 ATA) an attenuated vasodilation in the fingers during heat exposure. Little is known about the vascular response in human skin to heat exposure in hyperbaric environments. However, a different response of vasomotor function is expected in hyperbaric environments because the possibility exists that increased gas density or hyperoxia may modify the physiologic responses to thermal stress. Rather extensive observations have been made on thermal problems in cold in the past (2-4), but the necessity to study thermal responses in humans to heat exposure has increased due to those instances where laborers work in caisson chambers or pressurized or closed tunnel work where they encounter high environmental temperatures in hyperbaric environments. In this regard, it is important to know whether there is any difference in the circulatory response in maintaining the homeostasis of the body and how the acral parts of the body act in regulating thermal balance when man is

superimposed against heat exposure in a hyperbaric environment. Therefore, the aim of this study is: a) to estimate changes in the vascular tone of the whole integument by measuring the body heat transfer coefficient and total peripheral resistance during heat exposure to high pressure (4 ATA); b) to measure the rate of heat exchange in the different regions of the skin to examine the influence of hyperbaria on regional cutaneous heat dissipation during heat exposure at hyperbaric environments; c) to determine whether the vascular tone differs in various cutaneous regions in response to heat exposure in hyperbaric environments; and d) to measure blood flow in the finger and forearm to examine the influence of hyperbaria on regional cutaneous circulation during heat exposure.

METHODS

Seven healthy male volunteers, average age 30 yr, none of whom had prior experience in diving, participated in this experiment. Physical characteristics of the subjects are summarized in Table 1. All of the subjects signed a consent form after being thoroughly familiarized with the experimental procedures. Each subject dressed in shorts only, lay supine on a Potter bed scale (Potter, 23B, resolution: 0.5 *g*). At 0900 h the subjects reported to the laboratory where they were harnessed with skin thermocouples and heat flow discs, and measurements were started at 1000 h.

After 90 min resting at 1 ATA in a pressure regulation chamber (3.3 m long, 2.5 m wide, 2.0 m high), the subjects were exposed to a simulated depth of 30 m (4 ATA) by compressing the chamber air at a rate of 114 Torr/min. The chamber temperature (T_a) and relative humidity were maintained at 31 ± 0.1°C and 25%, respectively, except for an indispensable transient period for compression. Following a 40-min equilibrium period, chamber temperature was raised to 38°C at a rate of 1.0°C/min and maintained there until the termination of the experiment. The heat loading was terminated when the subject sweated markedly (about 50 min after T_a was increased).

All temperature measurements were made with copper-constantan thermocouples. Esophageal temperature (T_{es}) was measured with a thermocouple swallowed to the level of the left atrium (38 cm from the incisors). Mean skin temperature (T_{sk}) was calculated from 15 point measurements by the formula of Winslow et al. (5).

For measurement of nonevaporative heat exchange (R+C), a heat flux transducer (Thermonetics, HA-13-18-10PC) was placed on the various regional sites of the skin and secured with surgical tape (6). Total nonevaporative heat exchange on the skin was calculated as the regional heat exchange of the 15 sites times the respective regional areas. For the regional area, the same weighting factor as that for T_{sk} (5) was applied. Each heat flux transducer was calibrated and found to be identical in both 1 and 4 ATA. Body temperature, heat flux, and heart rate (HR) were recorded every 15 s throughout the entire experiment, and the data were stored on a data logger

Table 1
Characteristics of the Subjects

Subject	Age, y	Height, cm	Weight, kg	SA, m^2
A	47	173	76.1	1.9
B	34	169	61.4	1.7
C	21	163	58.6	1.6
D	28	180	75.2	2.0
E	27	165	61.9	1.7
F	30	171	64.3	1.8
G	21	170	55.7	1.7
Mean ± SE	29.7 ± 3.4	170.1 ± 2.1	64.8 ± 3.0	1.8 ± 0.1

SA = DuBois surface area.

(San-ei Sokki, 7V07 Tokyo, Japan) and analyzed by a computer (Sord, M243 Tokyo, Japan).

Cardiac output (CO) was measured by using an impedance cardiograph (7) every 10 min. The validity of this technique was confirmed in our laboratory (12). Forearm blood flow (FBF) and finger blood flow (FiBF) were measured every 2 min by venous occlusion plethysmography. A Whitney mercury-in-silastic circumference gauge (9) was placed at the left midforearm for FBF and at the phalanx media of the left middle finger for FiBF, and a cuff was secured at the upperarm and the base of the finger. Venous congestive pressure was 50 Torr.

Blood pressure was determined with a pressure transducer (Validyne, DP15) connected to a conventional pressure cuff applied on the left arm of the subject. Systolic and diastolic pressures were read from the pressure wave recorded on a pen recorder. Total peripheral resistance (TPR) was calculated by dividing mean arterial pressure (MAP) by CO, where MAP = 1/3 pulse pressure + diastolic pressure. Forearm vascular conductance (FVC) and finger vascular conductance (FiVC) were calculated as FBF/MAP and FiBF/ MAP.

The mean heat transfer coefficient (h_k) from the core to the skin was calculated according to the following equation:

$$h_k = [(R+C)_{sk} + E_{sk}]/(T_{core} - \bar{T}_{sk}) \ (W\ m^{-2}\ °C^{-1}) \qquad (1)$$

In the above equation, T_{core} represents the core body temperature (°C) which was measured as T_{es}; $\bar{T}_{sk}$ is the mean skin temperature (°C); $(R+C)_{sk}$ represents the nonevaporative heat exchange from the skin (W m^{-2}); E_{sk} is the evaporative heat loss from the skin (W m^{-2}), which was calculated from the cutaneous water loss, measured by subtracting respiratory water loss from whole body water loss. The whole body water loss of the subject was measured as the body weight change measured on the Potter bed scale before sweat began dripping, and the respiratory water loss (m_{res}) was estimated according to the following equation (10):

$$m_{res} = 0.019 \times Vo_2 (44-Pa) \quad (g\ min^{-1}) \qquad (2)$$

In Eq (2), Vo_2 is oxygen consumption (liter/min STDP) measured by an open-circuit method. The expiratory gas was collected into a Douglas bag through a mouthpiece in the chamber, and then its volume and O_2 content were determined at 1 ATA by leading the gas through a pipe. The 0.019 is a constant ($g \cdot liter^{-1} \cdot Torr^{-1}$) and Pa represents water vapor pressure (Torr) at ambient temperature which is practically independent of the atmospheric pressure under the present experimental conditions.

To provide baseline data, 2 control studies were performed separately; 1 was the pressure-control study performed at 1 ATA using the identical time schedule including heat loading, and the other was the time-control study. In the time-control study, each subject followed the identical time schedule at thermoneutrality (31°C) at both 1 and 4 ATA. Since the measured physiologic parameters did not change in the time-control study, we then considered that all functional changes during the experiments were induced by the heat loading at both atmospheres.

Statistical Analysis

Steady state measurements during the equilibrium period were compared with values measured during the course of heat exposure by using repeated measurement analysis of variance. Significant differences in the analysis of variance were further tested using the Bonferroni method of simultaneous multiple comparisons (11). The 4 ATA studies were compared with those of time-matched 1 ATA by using a paired t test. Data are expressed as means ± SE, with a value of $P < 0.05$ considered significant.

RESULTS

Body Temperature

Changes in body temperature during heat exposure at high pressure and at sea level are shown in Fig. 1. The average T_{es} during the equilibrium period was identical at 1 and 4 ATA (36.6 ± 0.2°C). At 4 ATA, a pronounced fall in T_{es} was initially observed after heat exposure, and then T_{es} began to increase after 8 min, exceeding its preheat value after the 20th min of heat exposure

(Fig. 2). However, the fall was slight at 1 ATA in comparison with that at 4 ATA, and T_{es} began to increase 6 min after heat exposure, exceeding its preheat value after 10 min. At 4 ATA, T_{es} value during the period between the 4th and 21st min after exposure was significantly lower ($P < 0.05$) than those at 1 ATA. The difference became insignificant during the remaining period of heat exposure. Therefore, at 4 ATA T_{es} increased at a higher rate than at sea level.

The average T_{sk} at 4 ATA was slightly but significantly lower ($P < 0.05$) during the equilibrium period, but upon heat exposure it increased at a higher rate than at sea level, and the difference of $\bar{T}_{sk}$ between 1 and 4 ATA increased throughout course duration (Fig. 2). The average forearm T_{sk} at 4 ATA (32.9 ± 0.02°C) was significantly lower ($P < 0.05$) than that at 1 ATA (33.4 ± 0.02°C) at the equilibrium period, but the latter caught up with the former by the 10th min of heat exposure. The average finger T_{sk} at the equilibrium period (34.5 ± 0.04) was slightly but significantly lower ($P < 0.05$) at 4 ATA (34.3 ± 0.03); however, a quicker rising rate at 4 ATA resulted in identical finger T_{sk} with that at 1 ATA a few minutes after heat exposure.

Relationship Between Skin Temperature and Blood Flow

In Fig. 3, differential responses of blood flow of the forearm and finger during heat exposure at 2 different atmospheric pressures are depicted. Changes in blood flow from the preheat equilibrium value (Δblood flow) are plotted against the corresponding changes in regional skin temperature (ΔT_{sk}). A curvilinear relationship between the forearm ΔT_{sk} and ΔFBF was observed at both 1 and 4 ATA. However, the response of FBF to the increase in forearm T_{sk} was attenuated significantly ($P < 0.05$) at 4 ATA, when the forearm ΔT_{sk} exceeded 2°C, suggesting a retardation of the vasodilation reflex to heat exposure at 4 ATA. This attenuation may prevent excess heat gain from the forearm, resulting in an identical core temperature of 1 ATA in spite of a higher T_{sk} (Figs. 1 and 2). On the other hand, the response of FiBF to the increase in finger T_{sk} was negligible at 1 ATA; but at 4 ATA FiBF increased as finger ΔT_{sk} increased and reached a plateau at a significantly higher level ($P < 0.05$) when the finger ΔT_{sk} exceeded 2°C. The poor response of FiBF at 1 ATA means the vasodilation of the finger was at near maximum level at this T_a (31°C) and did not show further increase in FiBF during heat exposure. On the other hand, a further increase in FiBF occurred at 4 ATA because base line FiBF was at submaximum level. Accordingly, FiBF of both atmospheres reached the same level at heat exposure termination (Table 2).

Responses of the Vascular State

Effects of high pressure and heat exposure on vascular states are summarized in Table 2. Averages of the last 10 min during the preheating equilibrium period (*left two columns*) and averages of the 20th to 30th, and 40th to 50th min of heat loading (*right four columns*) are presented. At 31°C,

a significant decrease in h_k ($P < 0.05$) was observed at 4 ATA indicating cutaneous vasoconstriction. Also, a significant decrease in the FBF, FiBF, and

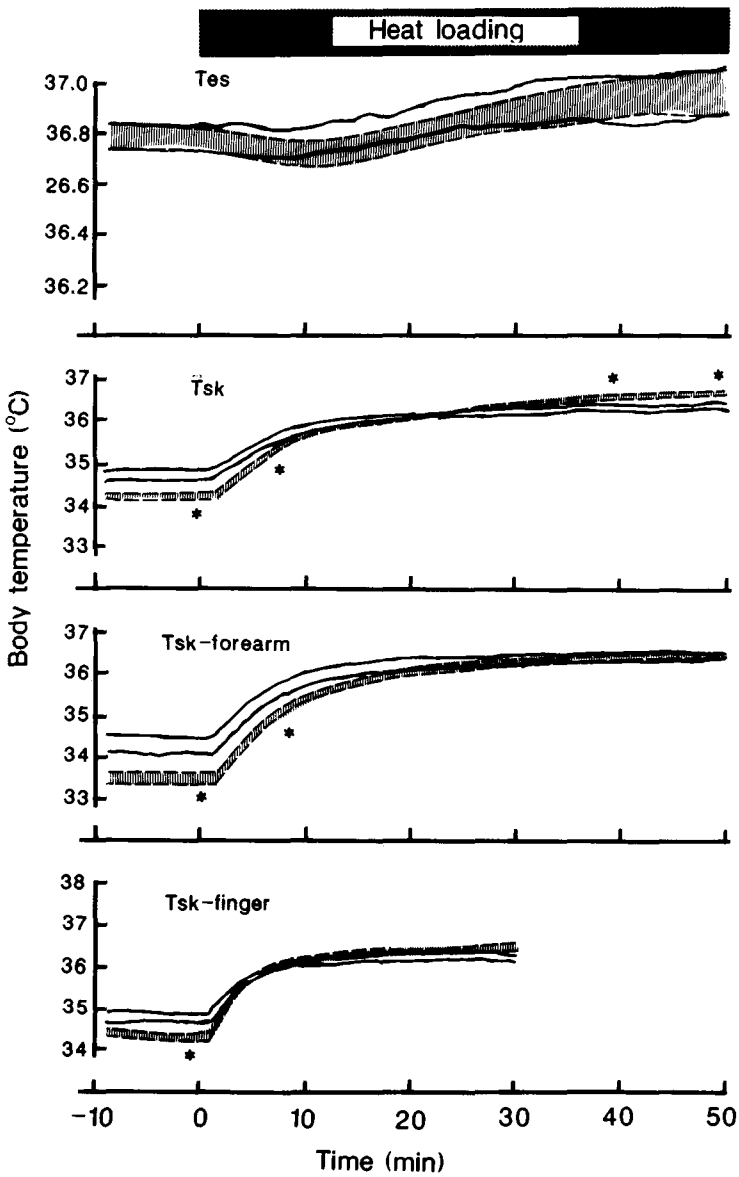

Fig. 1. Changes in esophageal (T_{es}), mean skin ($\bar{T}_{sk}$), forearm (T_{sk}-forearm), and finger (T_{sk}-finger) skin temperatures during heat exposure. Open area with solid lines represents the mean ± SE of 7 subjects at 1 ATA, and shaded area with broken lines represents the mean ± SE of 7 subjects at 4 ATA. * = Significant difference ($P < 0.05$) between the average values of every 10 min at 4 ATA and the corresponding average at 1 ATA.

Table 2
Effect of High Pressure and Heat Exposure on General Hemodynamics and Regional Vascular States

	Thermoneutral, 31 ° C		Heat Loading, 38 ° C			
	1 ATA	4 ATA	1 ATA		4 ATA	
Variables			30 min	50 min	30 min	50 min
h_k, W ·m^{-2}·°C^{-1}	15.0 ± 1.1	13.2 ± 0.5*	21.5 ± 4.8	32.4 ± 2.6**	16.1 ± 5.0*	39.8 ± 4.5*,**
MAP, Torr	82.0 ± 5.0	84.0 ± 4.0	82.0 ± 5.0	89.0 ± 7.0	87.0 ± 4.0	90.0 ± 6.0
CO, liter/min	5.4 ± 0.3	5.3 ± 0.2	6.0 ± 0.6		5.6 ± 0.3	
TPR, Torr·$liter^{-1}$·min^{-1}	15.9 ± 1.2	15.9 ± 0.8	15.0 ± 1.6		16.0 ± 1.1	
FBF, ml·min^{-1}·100ml^{-1}	7.6 ± 1.0	4.6 ± 0.7*	12.2 ± 1.5**	13.7 ± 1.6**	9.7 ± 1.4**	10.0 ± 2.1**
FVC, ml·min^{-1}·100 ml^{-1}·100·$Torr^{-1}$	10.6 ± 0.8	6.1 ± 1.3*	16.4 ± 1.9**	15.7 ± 2.2**	9.5 ± 1.3**	11.7 ± 3.0**
FiBF, ml·min^{-1}·100 ml^{-1}	74.3 ± 12.1	48.3 ± 5.7	70.0 ±14.9		88.1 ±18.6	
FiVC, ml·min^{-1}·100 ml^{-1}·Torr	0.93± 0.20	0.59± 0.10	0.85± 0.26		1.00± 0.24**	

Values are means ± SE during last 10-min preheat equilibrium period (thermoneutral) and the 20-30th min and 40-50th min during heat loading. * $P < 0.05$ comparing 1 with 4 ATA; ** $P < 0.05$ comparing thermoneutrality and heat exposure.

FiVC ($P < 0.05$) at 4 ATA confirmed vasoconstriction in these sites. The unchanged MAP, CO, and TPR suggested that whole body vasoconstriction was not evident.

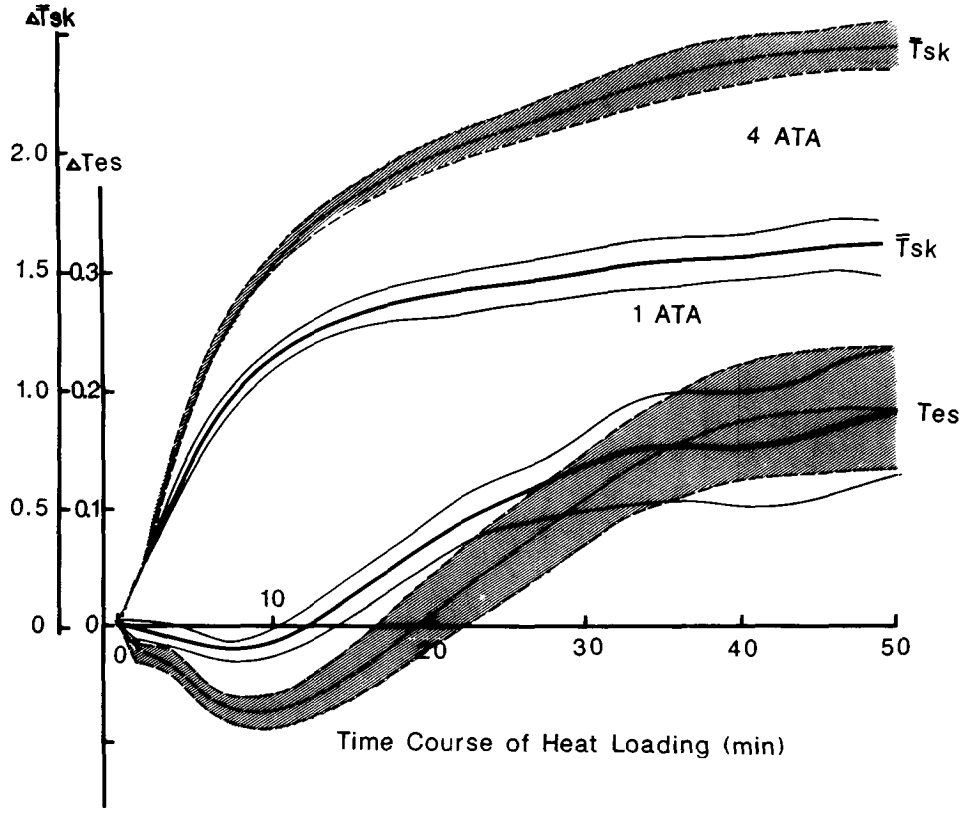

Fig. 2. Changes of esophageal (ΔT_{es}), and mean skin ($\Delta \bar{T}_{sk}$) temperatures during heat exposure. ΔT_{es} and $\Delta \bar{T}_{sk}$ were calculated as difference of T_{es} and $\bar{T}_{sk}$ from preheat equilibrium values. Open area with solid lines represents the mean + SE at 1 ATA and shaded area with broken lines represents the mean + SE at 4 ATA. * = Significant difference ($P < 0.05$) between the average values of every 10 min at 4 ATA and the corresponding average at 1 ATA.

During the last 10 min (50-60 min) of heat exposure, where body temperatures and blood flow were in a relatively steady state, the h_k value at both atmospheres increased significantly ($P < 0.05$) from the preheat value. The increase in h_k was slower at 4 ATA at the beginning, but the h_k was extreme during the last 10 min. This may be explained by a higher cutaneous vasodilation at 4 ATA due to a higher T_{sk} during this period (Fig. 2). No significant changes in MAP, CO, and TPR were observed during this period. These results indicated that the observed changes in cutaneous vasoconstriction during

exposure to 4 ATA at thermoneutrality disappeared during heat loading and, moreover, enhanced vasodilation was expected.

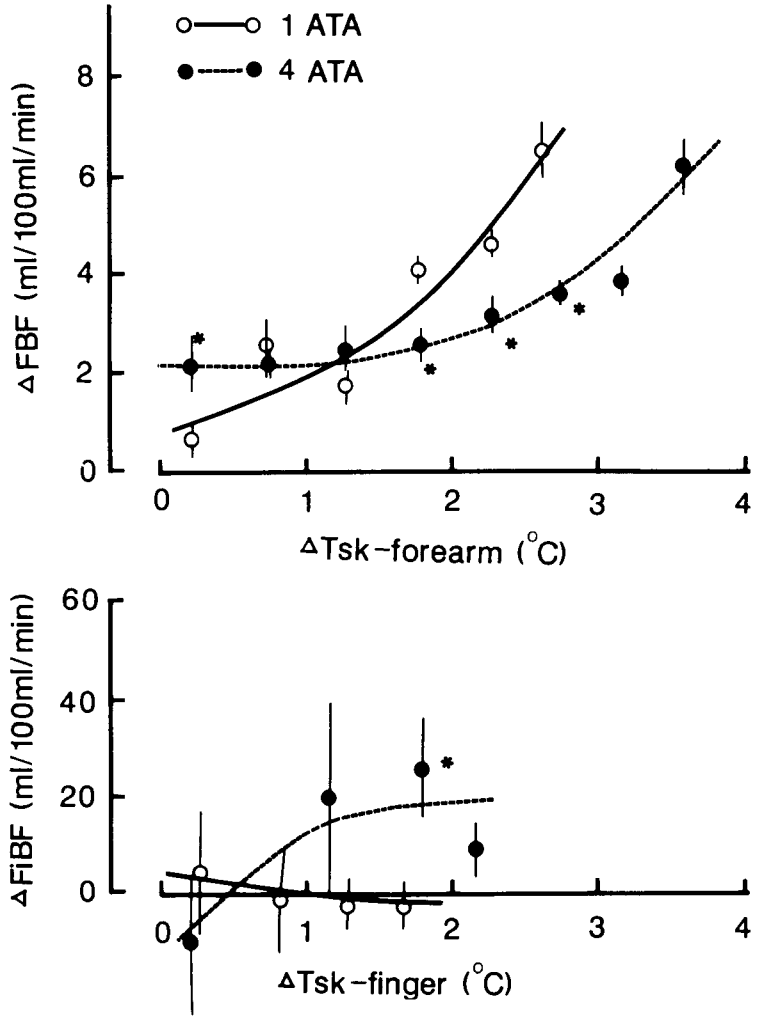

Fig. 3. Relationship between changes in forearm skin temperature (ΔT_{sk}-forearm) and changes in forearm blood flow (ΔFBF) (top), and between changes in finger skin temperature (ΔT_{sk}-finger) and changes in finger blood flow (ΔFiBF) (bottom). Open circles, 1 ATA; closed circles 4 ATA; bars ± SE. Absolute baseline value of blood flow ($\Delta T_{sk} = 0$) of the forearm and finger are shown in Table 2 as the data of thermoneutrality (31 ° C) in each atmospheric pressure. Solid line (1 ATA) and broken line (4 ATA) curves were drawn by inspection. The ΔT_{sk}-forearm and ΔT_{sk}-finger were calculated as the differences of forearm T_{sk} and finger T_{sk} from the preheated equilibrium values. ΔFBF and ΔFiBF represent the changes of FBF and FiBF from the preheat equilibrium values. * = Significant difference ($P < 0.05$) at 4 ATA compared with corresponding value at 1 ATA.

The FBF and FVC increased significantly during heat exposure ($P < 0.05$) and this change was similar at both atmospheres. No increase in FiBF and FiVC was observed at 1 ATA, indicating near maximum vasodilation at T_a of 31°C. Approximately the same magnitude (45%) of increase in FiBF and FiVC was observed at 4 ATA during heat exposure as at 1 ATA. These observations, together with data on $\bar{T}_{sk}$ (Fig. 1), suggested that the vascular responses during exposure to heat in a hyperbaric environment were not homogeneous in every part of the body.

DISCUSSION

As our previous work (12) has confirmed that a T_a of 31°C is within the zone of thermoneutrality at both 4 and 1 ATA, we compared the vascular responses during exposure to hyperbaric environment with those of normal atmosphere at T_a of 31°C in the present experiment. At 31°C, a significant decrease in FBF was observed in simulated high pressure, whereas T_{es} was identical to that of 1 ATA conditions. The decrease in FBF coincided with a mean cutaneous vasoconstriction as indicated by the decreased $\bar{T}_{sk}$ and h_k. These observations agreed basically with the report of Moore et al. (2), who estimated a decrease in peripheral circulation by observing a decreased skin heat flux. Since TPR and CO were maintained constant at 4 ATA (Table 2), the observed cutaneous vasoconstriction may represent vasodilation in some organs or tissues other than the skin. Additionally, vasoconstriction may be caused by an increase in cutaneous arteriolar resistance.

The decreased cutaneous blood flow observed at the equilibrium period of 4 ATA supports indirect evidence that has been estimated by the changes of T_{sk} and heat flux in the skin. For example, decrease in T_{sk} and hence in vasoconstriction was reported at 16.1 ATA by Moore et al. (2), and at 7 ATA by Matsuda et al. (13). In our experiment we compared the vascular tone during the equilibrium period at thermoneutrality (T_a = 31°C) in which a decreased evaporation at 4 ATA is comparable to an increased nonevaporative heat loss as reported previously (12). In other words, the total heat dissipation from the skin was equal at both 4 and 1 ATA. The response of FBF to the increase in forearm T_{sk} was attenuated at high pressure when local skin temperature exceeded 35°C. The FiBF, on the other hand, responded more to the increase in the local skin temperature. We may consider that the finger and the hand have comparable vascular responses (1), thus at least qualitative changes of the blood flow in the hand can be interpreted from the blood flow change in the fingers.

The mechanism of the differential vascular response, i.e., attenuation of vasodilation and a further vasodilation in the acral parts of the body, is not clear and deserves further investigation. A reduction of evaporative cooling of the skin surface and an increase in the convective heat inflow to the skin surface accord with other reports (4, 14, 15). These two facts evidently explain the characteristic changes in the skin temperature of an increased rising rate

and elevated level during heat exposure at hyperbaric environments. Considering the above fact, an attenuated skin blood flow in the forearm during heat exposure at hyperbaria will be advantageous to prevent the core temperature from hyperthermia through large heat inflow from the skin and thus homeothermy maintenance. On the other hand, an increase in blood flow in the hand and fingers from a rise in local skin temperature during heat exposure in hyperbaria will be disadvantageous. Thus, in hyperbaric environments fine control of thermal balance may be difficult since acral parts of the body contribute to the effective heat exchange by means of an increase in the counter-current heat flow by regulating the skin blood flow.

At 31°C T_{es} was the same in hyperbaria and normal environments, although T_{sk} decreased during hyperbaria. The low T_{sk} does not result in decreased convective heat loss since the coefficient of convective heat loss is increased at hyperbaric environment (3, 16, 17). A significant fall in T_{es} was observed in all subjects during the first 20 min of heat exposure at 4 ATA. The same phenomenon was much smaller in magnitude and shorter in duration at 1 ATA. The fall in the core temperature was first described and named as "initial fall" of core temperature by Ogata and his colleagues (18, 19) and later confirmed by others (1, 20). This phenomenon may be interpreted as a redistribution of heat from the core to the shell of the body during rapid exposure to heat. The fall of core temperature corresponds to relaxation of the vascular tone of the skin, which in turn increases outflow of core heat to the skin and increases the inflow of cooled blood from the peripheral skin layers. Enhancement of this phenomenon at 4 ATA suggests a typical change in heat flow and redistribution of blood flow during heat exposure. We interpret the enhancement of the initial fall of core temperature as a result of enlargement of vasodilator reserve due to baseline vasoconstriction. This baseline vasoconstriction is supported by a diminished $\bar{T}_{sk}$, lower h_k value and decreased blood flow during the equilibrium period at 4 ATA. The CO did not significantly increase by heat loading. This finding is in disagreement with Rowell et al. (21) who reported increased CO during heat loading (T_{sk} = 39°C). The relatively short heat load used in the present experiment may account for the disagreement.

We speculate that thermal homeostasis is maintained at neutral temperature by the reduction of blood flow to protect heat dissipation in an environment that has a high convective heat transfer coefficient (hyperbaria), but will be impaired by imposed heat loading because of the high heat flow from the environment to the acral parts of the body. Further studies to understand the above relationship during chronic exposure at high pressure are needed. Investigation of heat dissipation from the respiratory tract is also needed for further understanding of the homeothermy of the human body. In conclusion, results of the present experiment suggest strongly that cutaneous vasodilation caused by heat exposure is not induced uniformly over the whole body in a hyperbaric environment.

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INFLUENCE OF BODY MASS, MORPHOLOGY, AND GENDER ON THERMAL RESPONSES DURING IMMERSION IN COLD WATER

K. B. Pandolf, M. M. Toner, W. D. McArdle, J. R. Magel, and M. N. Sawka

Subcutaneous adipose tissue is known to provide insulatory benefits to humans during immersion in cold water (1-3). Individuals with greater subcutaneous fat demonstrate smaller physiologic and thermal adjustments during cold-water immersion and greater exercise tolerance when contrasted to more lean individuals (4-6). However, far less is known about the impact of morphologic and dimensional differences between individuals, such as body mass and surface area-to-mass ratio ($A_D \cdot wt^{-1}$), on the thermoregulatory adjustments to cold-water exposure.

The thermal and metabolic responses between individuals who differ in body mass and morphology may be further altered by movement of the limbs as in exercise compared to rest. When compared to rest, water-immersion exercise in cold water has been shown to either increase (7, 8) or decrease (2, 9) the rate of decline in deep body temperature. Finally, comparative data for cold-water exposures during rest and exercise between genders who generally display morphologic and dimensional differences are relatively meager.

The purpose of this presentation is to summarize the recent experimental observations from our laboratory and from the government contracted collaborative research with our laboratory that directly addresses these topics. Therefore, this paper will primarily focus on the influence of body mass, morphology, and gender on thermal and metabolic responses during both rest and exercise after cold-water exposure.

METHODS

Series 1

A series of experiments were conducted to describe the metabolic and thermoregulatory responses between genders during both rest and exercise (36 W) for 1 h in air (25-28°C) in water at 20, 24, and 28°C. The physical

characteristics of the subjects, test protocol, and associated methodology have been described in detail previously (1, 2); nevertheless, a brief summary of pertinent information necessary for this presentation is provided. Ten Caucasian, college-aged male volunteers were categorized as low (< 12%, $n = 4$), average (15-18%, $n = 4$), and high (>22%, $n = 2$) in percent body fat while 8 female volunteers of similar age were classified as low (<22%, $n = 4$) and average (24-27%, $n = 4$). However, 2 subgroups between genders ($n = 4$ each) were evaluated that had similar ($P < 0.05$) percent body fat and total skinfolds, but differed ($P < 0.05$) in weight, lean body weight, limb divided by trunk skinfold, A_D, and A_D/wt.

All testing in air and water at each of the three water temperatures (T_w) during both rest and exercise was assigned randomly. During all tests in both air and water the subjects wore nylon swimsuits. Experiments in water were conducted with the subjects immersed to the level of the first thoracic vertebra. During the resting experiments, the subjects sat quietly for 1 h during each exposure, and for the exercise experiments subjects pedaled an arm-leg ergometer at 30 rpm for a similar time period. All water experiments were performed in stirred water which was maintained within ± 0.5°C of the target T_w.

Percent body fat was determined by hydrostatic weighing, and mean skinfold thickness was evaluated from the average of 10 skinfold sites. Metabolic measurements were evaluated using standard techniques for open-circuit spirometry. Rectal temperature (T_{re}) and mean-weighted skin temperature ($\bar{T}_{sk}$) which was determined from three sites (forearm, chest, and calf) were continuously monitored during both rest and exercise.

Series 2

Another series of experiments was completed to describe the metabolic and thermoregulatory responses of male volunteers differing in body morphology and mass during both rest and exercise in water at 26°C for 1 h. The physical and morphologic characteristics of these subjects (10), test protocol, and associated methodology (10-12) have been presented in detail elsewhere; therefore, only a brief description will be reported. Ten male subjects were divided into large mass (LM) and small mass (SM) groups ($n = 5$ each) in an attempt to maximize differences in body mass and A_D/wt, but match groups for both subcutaneous and total body fat. The two groups were found to differ ($P < 0.05$) in total body weight, lean body weight, A_D/wt, arm and leg volumes but were similar ($P > 0.05$) in percent body fat and total skinfolds (10).

Before all experiments, subjects sat quietly in a room with a T_a 22°C while wearing nylon swimsuits. Then, subjects either rested on a chair or performed leg exercise on a modified water ergometer at a moderate exercise intensity ($\dot{V}O_2 \approx 1.5$ liter/min) for 1 h while immersed to the neck in stirred water at 26°C (± 0.5°C). These experiments were conducted in a systematically varied fashion.

Similar measurements and techniques as described above for series 1 were

also employed for these experiments. However, esophageal temperature (T_{es}) was measured and tissue insulation (I) calculated. In comparison to series 1, total skinfold thickness was evaluated from 11 skinfold sites whereas $\bar{T}_{sk}$ was determined from 5 sites (calf, thigh, chest, triceps, and forearm).

Statistical Analysis

For series 1 experiments, independent or paired *t* tests, analysis of variance for repeated measures and Duncan's multiple range tests, and in some instances Pearson product-moment correlation coefficients (*r*) were employed (1, 2). For series 2 experiments, analysis of variance for repeated measures with the Tukey multiple range and interaction post hoc test, and Pearson product-moment correlations coefficients were utilized (10). In all instances, the 0.05 level of significance was chosen for these analyses.

RESULTS AND DISCUSSION

Table 1 presents selected observations from series 1 experiments between genders with comparable levels of body fat (16.8% men; 18.5 women). During rest at all three T_w, the decline in T_{re} over the 1-h immersion was greater for the women as compared to the men, being statistically significant at 20 and 24°C. Although not statistically significant, the T_{re} to $\bar{T}_{sk}$ thermal gradient was always greater for the men while final $\bar{T}_{sk}$ was consistently higher for the women. Although the women demonstrated a greater decline in T_{re}, their resting metabolic rate (M) was not seen to differ significantly from the men at any of these T_w after 1 h of immersion. During exercise at all three T_w, the differences between genders after the 1-h immersion period for ΔT_{re}, final T_{re}, final $\bar{T}_{sk}$, final T_{re}-$\bar{T}_{sk}$, and absolute M(W) were not statistically significant ($P > 0.05$). The M in relation to surface area was also not different between genders during exercise at all three T_w for these same experiments (2).

Table 2 displays selected findings from series 2 experiments comparing men divided into 2 groups to maximize differences in body mass and A_D/wt while minimizing differences in subcutaneous and total body fat between groups. During rest, the M, T_{re}, and $\bar{T}_{sk}$ did not differ ($P > 0.05$) between groups after 60 min of exposure at 26°C. However, T_{es} was significantly lower ($P < 0.05$) for the LM group during the same resting experiments at 60 min, but the change in T_{es} from preimmersion to final values did not differ (SM, -0.2°C; LM, -0.4°C) between groups (10). Tissue insulation (I) during rest was seen to be higher ($P < 0.05$) for the LM compared to the SM group at 60 min. During exercise, M, T_{es}, T_{re}, $\bar{T}_{sk}$, and I did not differ significantly ($P > 0.05$) at 60 min between groups. In general, experimental findings from additional rest and exercise tests on these same subjects, but performed in 18 and 30°C water, provided support for the above observations during immersion in 26°C water (13).

Table 1
Metabolic and Thermoregulatory Responses Between Genders of Similar Percent Body Fat for Rest and Exercise During Immersion (≈ 1 h) in Water at 20, 24, and 28 °C

	20 °C			24 °C			28 °C		
	Male	Female	Diff	Male	Female	Diff	Male	Female	Diff
Rest									
ΔT_{re} (°C)	-1.1	-1.6	-0.5*	-0.5	-1.2	-0.7*	-0.5	-0.9	-0.4
Final T_{re} (°C)	36.1	35.7	-0.4	36.6	36.2	-0.4	36.5	36.6	0.1
Final $\bar{T}_{sk}$ (°C)	21.4	22.3	0.9	24.7	25.2	0.5	28.3	28.8	0.5
Final T_{re}-$\bar{T}_{sk}$ (°C)	14.7	13.6	-1.1	11.7	11.1	-0.6	8.2	7.8	-0.4
Final M (W)	212	205	-7	142	163	21	118	135	17
Exercise									
ΔT_{re} (°C)	-0.7	-0.5	0.2	-0.1	-0.1	0.0	0.2	0.2	0.0
Final T_{re} (°C)	36.6	37.2	0.6	37.4	37.4	0.0	37.2	37.7	0.5
Final $\bar{T}_{sk}$ (°C)	21.3	21.2	0.1	24.8	24.9	0.1	28.3	28.8	0.5
Final T_{re}-$\bar{T}_{sk}$ (°C)	15.4	16.1	0.7	12.6	12.5	0.1	8.9	9.0	0.1
Final M (W)	340	330	-10	291	357	66	305	343	38

* Statistically significant at the $P < 0.05$ level.

During rest in cold water, metabolic rate might be expected to differ between genders and/or groups differing in body mass, making the interpretation of findings difficult. Thus, a constant intensity of leg exercise was also employed in these experiments to help eliminate this interindividual variability in metabolism (10). Further, exercise with the legs only has been recently shown to be more effective than rest in preventing the decline in core temperature over time in both cool (30°C) and cold (18-20°C) water (11). Figure 1 illustrates that 1 h of leg exercise resulted in higher internal

Table 2
Metabolic and Thermoregulatory Responses Between LM and SM Groups for Rest and Exercise During Immersion After 60 Min in Water at 26 °C

	Rest			Exercise		
	Large	Small	Diff	Large	Small	Diff
Final M	152	198	46	550	527	-23
Final T_{es} (°C)	36.3	36.7	0.4*	37.2	37.3	0.1
Final T_{re} (°C)	36.5	36.5	0.0	37.4	37.3	-0.1
Final $\bar{T}_{sk}$ (°C)	26.2	26.4	0.2	26.2	26.2	0.1
Final I ($°C \cdot m^{-2} \cdot W^{-1}$)	0.116	0.106	-0.06*	0.039	0.036	-0.003

*Statistically significant at the $P < 0.05$ level.

temperature responses (either T_{es} or T_{re}) when compared to rest (11). In contrast, arm and combined arm-leg exercises were not as effective as strict leg exercise in preventing the drop of core temperature in cold water as shown in Fig. 2 (12). Figure 3 indicates greater conductive and convective heat loss after 45 min of exercise in cold water while employing the arms as compared to strict leg exercise (12). Mean weighted heat flow was area weighted from 5 sites (back, forearm, triceps, calf, and thigh) in these experiments. In series 1 or series 2 experiments, rest (1, 2, 10), arm-leg exercise (1, 2), and strict leg exercise (10) were evaluated.

During our rest experiments in cold water, the women with the same percent body fat as the men were found to have greater difficulty in maintaining T_{re} when compared to these men (1). It should be noted that these women had a smaller lean body mass and a relatively larger A_D/wt than the men, indicating the importance of a larger inactive muscle mass in providing added insulation for the men during resting exposures in cold water. However, these same authors suggest the possibility that the sensitivity of the thermogenic responses may be enhanced in men when compared to women during rest in cold water (1). The thermal response contrasts between the LM and SM groups of men during rest in cold water further support the concept of added insulatory potential being associated with a larger muscle mass but for asubject population of the same gender (10). Although these two groups were similar in terms of percent body fat but differed in total body mass, lean body mass, and A_D/wt, the large mass group most likely defended deep body temperature by employing a greater volume of muscle tissue to provide insulation without

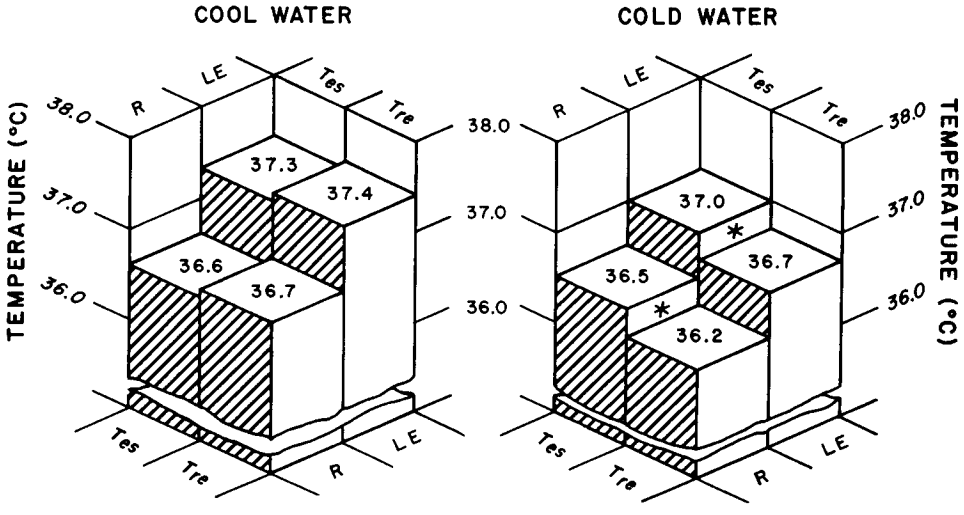

Fig. 1. Comparison between rest and strict leg exercise of esophageal (T_{es}) and rectal (T_{re}) temperatures after 60 min in cool (30 ° C) and cold (18-20 ° C) water.

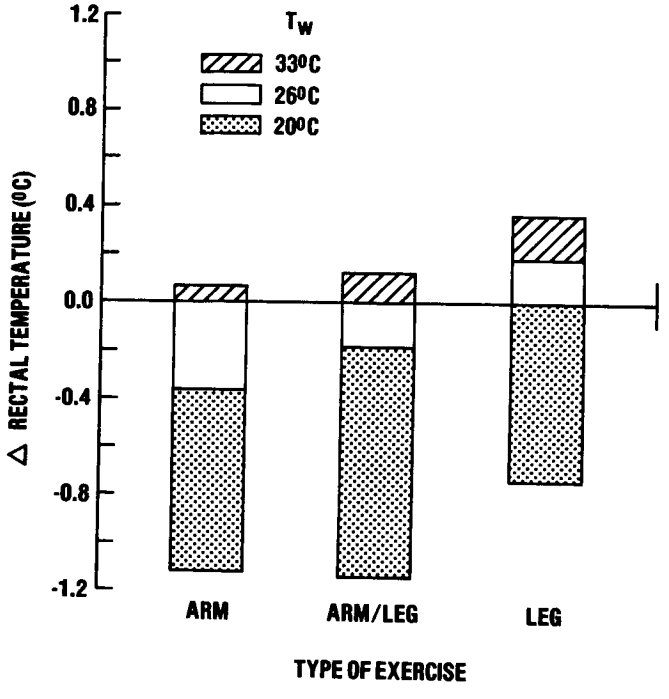

Fig. 2. Change (Δ) in rectal temperature responses after 45 min of exposure for arm, combined arm-leg, and leg exercise at water temperatures (T_w) of 20, 26, and 33 ° C.

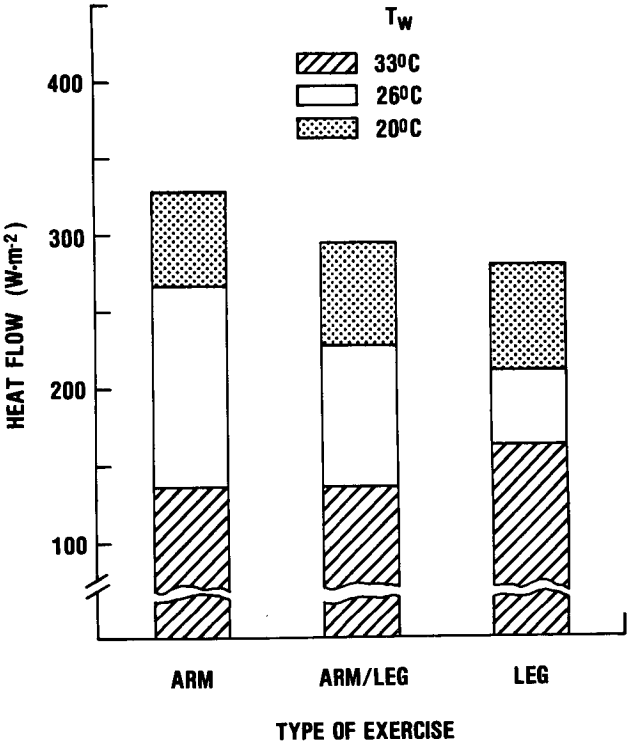

Fig. 3. Final mean weighted heat flows after 45 min of exposure for arm, combined arm-leg, and leg exercise at water temperatures (T_w) of 20, 26, and 33 ° C.

an increase in muscle metabolism. In contrast, the SM group appeared to increase metabolism twofold between preimmersion and final immersion values to defend deep body temperature in cold water (10).

During exercise in cold water, a different picture emerges as the active skeletal muscle mass becomes perfused with warm blood and looses its resistance for heat transfer from the body core to water. When a major portion of the muscle mass is perfused by warm blood during exercise, all metabolic and thermal responses are similar between either men and women (2) or LM and SM groups of men (10), despite significant differences in A_D/wt and body mass between groups. However, further data from our laboratory indicate that distribution of the active muscle mass during exercise is an important consideration (12). Arm exercise results in greater conductive and convective heat loss than strict leg exercise in cold water. This probably results from a) less fat insulation on the arms, b) perfusion of an equal cardiac output to a smaller muscle mass causing greater blood flow per unit limb volume, and/or c) a shorter axial conductive pathway from core to surface in arms than in legs (14). Further experimentation is necessary to fully understand the impact of

exercise type on thermal responses during cold-water immersion.

In conclusion, when these findings are taken collectively, it appears that the water temperature, body mass, and exercise type and intensity are more critical factors to be considered in preventing a decline in deep body temperature during cold-water immersion than surface area-to-mass ratio and gender. We believe, however, that this conclusion is more applicable to individuals at exercise than at rest during cold-water immersion.

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UNDETECTED HYPOTHERMIA: FURTHER INDICATIONS

E. H. Padbury, I. Rønnestad, A. Hope, G. Knudsen, E. Myrseth, and R. Værnes

Undetected hypothermia occurs when a body core temperature falls and causes little or no physiologic or psychologic response on the part of the person. It seems to be the result of reduced response to stimuli for thermoregulation, as during cooling in lukewarm water (28-32°C) and/or when there is a conflict between peripheral and central receptor input, as in the case of a subject with warm skin but slowly cooling core. The situation is dangerous because even mild degrees of hypothermia produce striking impairments of cognitive function. Coleshaw et al. (1) found that memory registration, for example, was progressively impaired when core temperature fell below about 36.7°C, with a 70% loss of data retention at core temperatures of 34 to 35°C. Webb (2) has also reported degradations in reaction time of 16% when falls in core temperature of between 0.2 and 0.9°C have been produced by slow cooling in a suit calorimeter.

However, the diving industry is skeptical whether undetected hypothermia is a real enough danger to divers to warrant monitoring, let alone development of equipment or procedures to prevent it. Laboratory evidence of undetected hypothermia at 1 atm comes from Hayward and Keatinge (3), van Someren et al. (4), and Padbury (5). Hyperbaric occurrences have been presented by Keatinge and coworkers (6) at depths of 130 to 145 m, by Piantadosi et al. (7) at 190 to 570 m, and in *New Scientist* (8) at unstated, presumably shallow depth. We now document further development of undetected hypothermia under hyperbaric conditions.

MATERIALS AND METHODS

In 1985, NUTEC performed an onshore chamber dive to 450 m. During the bottom phase the thermal status of the 6 male divers was monitored on most

occasions when a diver was "locked out" (i.e., was in the water in the wet chamber). While locked out the divers had to perform manual work on a construction rig, swim against a trapeze and an arm ergometer, and carry out performance tests. In addition, the divers were periodically asked to respond to a status questionnaire, which included their subjective thermal evaluation of hot water and breathing gas temperature and water distribution in the suit. Where appropriate, the response "none," "some," "much," or "severe" was requested. The physical characteristics of the divers are given in Table 1.

Table 1
Description of Divers

Diver	A1	A2	A3	B1	B2	B3
Height, cm	178	191	181	179	185	170
Predive weight, kg	73	90	81	88	76	73

Temperatures measured were those of the skin at 4 sites (chest, arm, thigh, and calf); rectum (T_{re}); inspired-expired breathing gas (T_i/T_e); and hot water (T-HW) to diver at the inlet to the suit and cold water (T_w cold) in the wet chamber. A Fenwall thermistor (response time approximately 200 ms at 360 m) was placed in the oronasal mask of the divers' helmet to measure breathing gas temperature. All other temperatures were measured with Yellow Springs Instruments (YSI) series 700 thermistors. Mean skin temperature (MST) was calculated as the average of the available measurements. The "start" MST value was taken within 2 min of the diver entering the water.

Minute ventilation (VE), oxygen consumption (Vo_2), and carbon dioxide production Vco_2) were measured using the method of Myrseth and colleagues (9). Total respiratory heat loss (convective plus evaporative; RHL) was calculated from the equation given in Piantadosi et al. (7). Oxygen consumption was converted to metabolic rate (Hm) by multiplying its value by 4.9 kcal/min and converting to watts.

The complete performance measurement system (PMS) is described by Værnes and coworkers (10). During the predive training period the last 5 to 10 repetitions of each test are used to calculate control average values and SD for each diver. The following PMS tests were administered while the diver was in the water:

Motor Tests: a) Finger Dexterity Test for evaluation of finger arthralgia, b) Manual Dexterity Test for evaluation of gross manual performance, and c) Arm Wrist Speed for testing the ability to make repeated, rapid arm movements.

Cognitive Tests: a) Operational Test for testing the ability to select rapidly the correct arithmetic operation and b) Visual Reaction Time for testing the ability to respond rapidly to a discrete stimulus.

The divers wore open-circuit hot water suits when diving and were supplied with water in the temperature range of 37 to 42°C, at a flow rate of between 20 and 43 liter/min. Diving Unlimited International open-circuit suits were used for all dives except 66 and 68 (Table 2) when a Finn Christian Olsen suit was used. Two types of breathing equipment were used: a Draeger closed circuit and a Rexnord system. Both were capable of providing sufficiently heated breathing gas for comfort and safety.

RESULTS

The changes in rectal and mean skin temperatures of the divers during lockouts are shown in Table 2. When a lockout was interrupted, i.e., the diver left the water for a short time, the durations and data for each in-water period have been given separately. It can be seen that rectal temperature fell to below 37°C on only four occasions: A3/54, A3/60, B3/55, and B3/59.

Diver B3 is particularly interesting with regard to the development of an undetected hypothermia. This diver's rectal temperature decreased from 37.8 to 36.3°C in 66 min (lockout 55), as shown in Table 3, during which time he did not voluntarily complain of cold or shivering.

Skin temperatures are given in Table 4. It can be seen that at times, especially in the beginning of the lockout, thigh and calf temperatures were somewhat lower than chest and arm temperatures.

During the status questionnaire, diver B3 rated hot water temperature as "some" too cold, and distribution as "some" uneven, however when asked directly how he felt (thermally), he said "fine" except that his expired breathing gas temperature was uncomfortably warm. In fact he had turned off his gas heating for his own comfort, and did not want it on although his inspired gas temperature was between 15 and 19°C for most of the 66 min.

Table 5 presents the limited amount of data that we have available on VE, RHL, $\dot{V}o_2$, and Hm. Ventilation rate varied between 20 and 25 liter/min at time 2040, and between 25 and 30 liter/min at time 2110.

Calculations of RHL have therefore been made for all VE. It can be seen that RHL was between 59 and 74% of heat production at 2040 and between 80 and 96% at time 2110. (Due to computer failure no data were collected after 2110.)

Due to technical problems the lockout was then interrupted for an interval of 11 min before it was continued. During the time the diver was in the dry part of the chamber—its temperature approximately 28°C—his rectal temperature rose by 0.3 to 36.6°C. The diver's changes in T_r during the second part of the lockout are also shown in Table 2. The decrease, stabilization, and small increase may be attributable to changes in breathing gas temperature combined with the diver's work program. During the status questionnaire he stated that breathing gas and hot water temperature were "OK."

The results of the PMS indicate that no significant changes occurred in

Table 2
Changes in T_{re} and MST During Lockouts at 450 m

Diver and Dive No.	Approx. T_w Cold,* °C	Approx. Lockout Duration, min **	Approx. HW Temp., °C*	MST, °C Start	MST, °C End	No. of Skin Thermistors Start	No. of Skin Thermistors End	T_{re}, °C Start	T_{re}, °C End
A1/50	10	22	40	35.5	33.8	3	3	37.8	37.4
	10	4	36	34.5	34.3	3	3	37.0	37.0
B1/51	9	12	41	36.9	36.9	4	4	37.9	37.7
	9	61	40-42	33.6	35.2	4	3	37.7	37.2
B2/53	9	94	37	34.1	35.3	4	4	37.5	37.3
A3/54	10.5	80	38	34.3	32.5	4	2	37.8	37.2
	11	80	38	33.4	33.5	2	2	37.5	36.8
A1/54	11	53	38	34.6	32.6	4	3	37.9	37.8
B3/55	8	66	38.5	29.3	34.8	4	4	37.8	
36.3									
		120	39	30.5	34.8	4	4	36.6	36.4
B1/55	11	117	40	36.2	37.5	4	2	37.3	37.5
A2/56	11-17	173	40	34.4	—	4	1	37.4	37.4
B1/57	10	173	40	36.8	36.6	4	4	37.4	37.2
B2/57	11	72	41-39	36.4	37.7	4	4	37.5	37.7
	11.5	72	39	33.3	37.4	4	4	37.6	37.6
A2/58	12	116	41	36.6	37.7	2	2	36.3	36.4
A1/58	12	160	41	33.3	—	4	1	38.0	37.3
B2/59	12	152	40.5	35.9	35.3	4	4	37.4	37.4
B3/59	9-12	180	40.5	32.7	31.7	4	4	37.5	36.6
A1/60	12	130	41	34.7	33.2	3	2	—	—
A3/60	10	161	41	36.5	34.9	4	3	37.5	36.9
A1/64	3.5	26	40	33.6	35.3	3	3	36.3	36.7
A2/64	8	49	40	34.9	34.8	3	2	39.5	39.0

Table 2 (Cont.)
Changes in T_{re} and MST During Lockouts at 450 m

Diver and Dive No.	Approx. T_w Cold,* °C	Approx. Lockout Duration, min **	Approx. HW Temp., °C*	MST, °C Start	MST, °C End	No. of Skin Thermistors Start	No. of Skin Thermistors End	T_{re}, °C Start	T_{re}, °C End
B3/65	6.5	11	41.5	36.8	38.0	4	4	37.4	37.5
A1/66	7	67	41	33.2	34.4	3	3	38.2	38.0
	9	58	42	36.9	34.4	3	3	38.0	38.1
A1/68	10	13	41	35.7	38.1	3	3	—	—

* Both cold (T_w cold) and hot water (HW) temperatures varied somewhat during the lockouts, the values given here are therefore subjective representative values. The HW value is that of the water in the splitter block at the diver; where this was not measured directly, an estimated value has been stated here.

**These values attempt to indicate the time that the diver was totally immersed in the water, do not include hot water cut periods, and may differ slightly from the "lockout durations" quoted elsewhere.

peripheral motor performance, i.e., gross manual performance, arm wrist speed, or finger dexterity, throughout the lockout. However, vigilance, i.e., operational test and visual reaction time, were worse in the first PMS session (time 2048 to 2108) than in later sessions of lockout 55. Visual reaction time improved from 325 ms in the first PMS session to 256 and 238 ms in the second and third. The first value was more than 3 SD (1 SD = 10%) outside the predive, in-water average value of 242 ms whereas the later reaction times were within 1 SD. The operational test scores improved from 32 to 35 and 36 correct replies, whereas incorrect answers decreased from 11 to 7 and 7. These scores were within 1 SD (7%) of the predive, in-water average score of 34.5 correct answers.

In lockout B3/59 the breathing gas temperature was somewhat higher (varying approximately between 19 and 27.5°C). It is noticeable that the rate and magnitude of fall in T_{re} of diver B3 were correspondingly less, 1.0°C in 180 min (Table 2). Only one PMS was performed during the lockout, when visual reaction time for B3 was 203 ms, and operational test scores were 36 correct and 5 wrong replies.

DISCUSSION

Even though a high hot water temperature was measured at the inlet to the divers suit, there will be a decrease in T-HW as the water flows through

Table 3
Thermal Status of Diver B3 During Lockout 55 at 450 m

Time	T_w Cold, °C	T-HW, °C	MST, °C	T_{re}, °C	T_i/T_e, °C	Comments
2015	—	—	35.0	37.9	—	prelockout
2030	9.6	39.2	34.7	37.8	—	20.30 into water
2040	9.8	38.7	31.5	37.4	18/28	20.33 diver immersed
2050	10.0	38.5	32.8	37.0	16/28	20.48 PMS started
2100	10.3	38.9	33.7	36.7	17/31	21.10 status questionnaire
2110	10.1	38.6	34.9	36.4	19/34	21.13 arm ergometer started
2120	10.3	38.6	35.2	36.4	17/28	21.20 status questionnaire
2130	10.3	38.1	34.1	36.3	18/28	21.30 construction rig started
2139	10.4	38.7	34.8	36.3	22/30	21.39 out of water
2150						21.50 into water again
2153	10.6	38.8	30.5	36.6	20/29	21.55 construction rig cont.
2155	10.4	38.9	31.3	36.6	20/30	
2205	10.5	39.5	34.0	36.5	17/28	
2215	10.6	40.1	34.6	36.3	17/28	
2225	10.9	40.1	35.6	36.2	17/29	
2235	10.9	40.1	35.9	36.2	16/29	22.40 status questionnaire
2245	10.9	40.6	35.2	36.2	18/26	22.43 arm ergometer
2255	11.8	40.3	35.7	36.2	19/29	22.53 PMS started
2305	11.7	40.1	36.2	36.2	19/28	23.13 status questionnaire
2315	11.5	40.0	35.0	36.4	18/28	23.15 trapeze started
2325	12.1	40.5	34.8	36.4	19/30	23.30 PMS started
2335	12.1	40.5	35.4	36.3	19/29	
2345	11.8	40.2	34.8	36.4	19/31	23.50 arm ergometer started
2350	—	—	—	36.6	—	23.51 out of water

the suit. Neoprene is highly compressed at 450 m, and its insulative value correspondingly reduced. The temperature of the skin in the thigh and calf indicate that the hot water was considerably cooler in those areas. This could have led to a small heat loss through the skin. The diver was in fact aware of the uneven temperature distribution and "some" coolness of the hot water. In addition, whenever the main route of heat loss is through the respiratory tract, there will be a reversal of temperature gradients within the center of the body. This would also contribute to the general core cooling observed. However, the diver was unaware of the respiratory heat loss, as indicated by his reluctance to increase the temperature of his breathing gas.

The close association between core cooling and inspired breathing gas

Table 4
Skin Temperature of Diver B3 During Lockout 55 at 450 m

	Temperature °C				
Time	Chest	Arm	Thigh	Calf	Comments
2030	35.8	35.8	33.8	33.4	20.30 diver into water
2040	34.3	39.6	26.2	26.0	20.33 fully immersed
2050	33.0	36.7	29.4	31.9	
2100	35.8	35.8	31.5	31.6	
2110	37.4	37.7	32.8	31.5	
2120	37.4	37.0	33.4	32.9	
2130	34.8	35.6	32.9	33.0	
2139	34.4	36.8	32.2	35.8	21.39 out of water
2151					21.50 into water again
2153	31.6	37.6	28.2	24.6	
2155	31.6	36.2	28.6	28.8	
2205	35.4	36.7	32.0	32.9	
2115	35.4	37.2	33.0	32.8	
2225	35.9	37.5	33.6	35.3	
2235	35.4	37.5	34.3	36.3	
2245	36.2	34.7	32.9	36.8	
2255	36.1	37.9	33.2	35.6	
2305	37.2	39.5	33.9	34.0	
2315	35.5	40.5	31.0	33.0	
2225	36.2	39.0	33.2	30.8	
2335	35.3	38.9	33.2	34.3	
2345	36.0	35.9	33.7	33.4	
2351					23.51 out of water

temperature was investigated by Piantadosi et al. (7) and has also been observed during a 350-m dive at NUTEC (11). We believe that diver B3 exhibited a classic example of the development of undetected hypothermia during lockout 55, and to a lesser extent in lockout 59 due to his preference for a cool breathing gas temperature. Had his breathing gas temperature been higher, any heat losses through the skin could have been compensated for by the metabolic heat production. It should be noted that he did not respond to and was not aware of his falling body temperature.

While a dangerously low level of T_{re} per se cannot be considered to have been reached, in the study by Coleshaw et al. (1) subjects with core temperatures in the same range as diver B3 have been described as having impaired cognitive performance. The results of the cognitive performance tests were significantly worse at the time when diver B3's body core temperature was

Table 5
Ventilation Rate, Respiratory Heat Loss, Oxygen Consumption, and Metabolic Heat Production of Diver B3 During the First Part of Lockout 55

Time	VE, liter/min	T_i, °C	T_e, °C	RHL, W	V_{O_2}, liter/min	Approx, Hm, W
20.40	20	18	28	162	0.8	274
	25	18	28	203		
21.10	25	19	34	301	1.1	376
	30	19	34	361		

falling most rapidly in lockout 55, improving when body temperature stabilized. Stabilization of core temperature may have been the result of his active work on the arm ergometer and construction rig, but it is noticeable that the heat production was not sufficient to institute a rise in core temperature. Peripheral motor performance was not affected by the central core cooling, being more likely to be influenced by a peripheral cold stress which did not occur in this case.

CONCLUSION

There is growing (albeit slowly) evidence that the occurrence of undetected hypothermia can be a real possibility in certain divers, particularly where breathing gas heating is inadequate or the diver himself prefers a cool inspired gas temperature.

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SESSION 4: DECOMPRESSION

9th International Symposium on Underwater and Hyperbaric Physiology
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LIKELIHOOD ANALYSIS OF DECOMPRESSION DATA USING HALDANE AND BUBBLE GROWTH MODELS

R. D. Vann

Haldane introduced the first mathematical model of decompression in 1908 (1). Since that time, mathematical models have been used extensively in the development of decompression procedures, but because the mechanisms of decompression are not well understood, empirical testing has been necessary for model validation. Validation has been hindered, however, by the inability to measure a model's success in predicting an empirical outcome. As a result, there has been no quantitative means for separating models that are useful from those that are unsatisfactory.

The calculation of decompression procedures usually assumes that decompression sickness (DCS) occurs only when a critical threshold is exceeded. This assumption is in conflict with observations that DCS is often unpredictable and behaves as a statistical rather than a threshold phenomenon. The principle of maximum likelihood introduced by Weathersby et al. (2) was an important step toward resolving this conflict.

Maximum likelihood has been used for many years in genetics, engineering, and pharmacology but is new to environmental physiology (3). It is analogous to least squares in that it finds the best fit of a mathematical model to experimental data. Hence, it furnishes a quantitative measure by which models may be compared. This eliminates the subjectivity that has plagued decompression research for so many years.

LIKELIHOOD DEFINED

The link between theory and experiment is provided by a dose-response curve such as that shown in Fig. 1. This curve relates the DCS probability to a decompression dose which is predicted by a decompression model. The dose-

response curve shown in Fig. 1 is the Hill equation

$$P(\text{DCS}) = (\text{Dose})^a/(\text{Dose})^a + b \qquad (1)$$

whose shape is determined by the parameters a and b. As the decompression dose increases, the DCS probability rises sigmoidally from zero to one. The Hill equation is one of many dose-response models that are available.

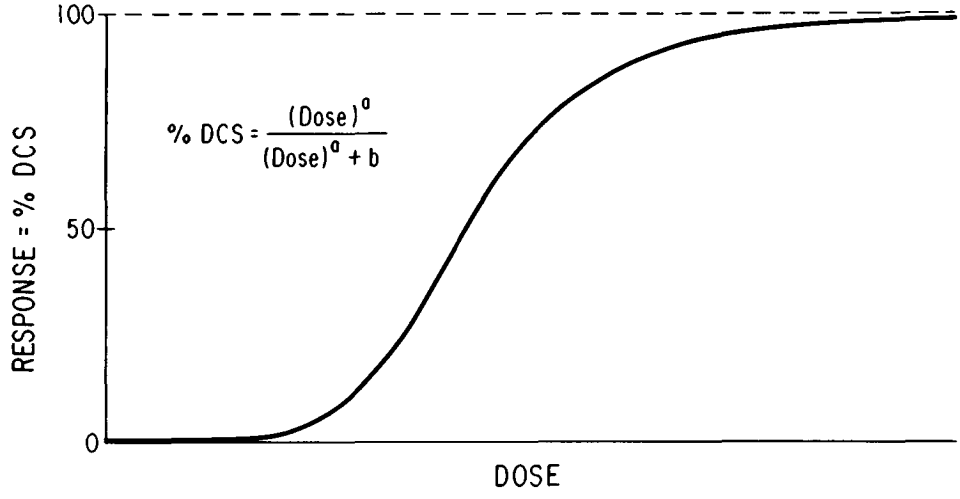

Fig. 1. A dose-response curve defined by the Hill equation.

This link between theory and experiment contains a disparity, however. The dose-response curve predicts that the DCS probability, P(DCS), will be between 0 and 1 and that the probability of no DCS will be 1 minus P(DCS). The experimental outcome, which shall be called X, on the other hand, is 1 if DCS occurs and 0 if DCS does not occur.

Theory	Experiment
$0 < P(\text{DCS}) < 1$	X = 1 if DCS
$P(\text{no DCS}) = 1 - P(\text{DCS})$	X = 0 if no DCS.

Likelihood resolves this disparity. Likelihood is defined as the theoretical probability of an experimental outcome. Thus, when DCS occurs and the outcome X is 1, the likelihood is equal to P(DCS). When DCS does not occur and the outcome X is 0, the likelihood is equal to P(no DCS).

If X = 1, likelihood = P(DCS)
If X = 0, likelihood = P(no DCS)

Expressed quantitatively,

$$\text{Likelihood} = P(\text{DCS})^{X} * P(\text{no DCS})^{1-X}.$$

This formulation combines information from both theory and experiment. Indeed, likelihood is a measure of the agreement between theory and experiment, as the following examples illustrate.

Consider a theory that is always correct. When DCS occurs, this theory predicts that P(DCS) is 1, and calculation indicates that the likelihood also is 1.

$$X = 1 \text{ and } P(\text{DCS}) = 1$$
$$\text{Likelihood} = P(\text{DCS})^{X} * P(\text{no DCS})^{1-X} = 1^{1}*0^{0} = 1.$$

When DCS does not occur, the correct theory predicts that P(no DCS) is 1. Again, the likelihood is 1.

$$X = 0 \text{ and } P(\text{DCS}) = 0$$
$$\text{Likelihood} = 0^{0}*1^{1} = 1$$

Now consider a theory which is always wrong. When DCS occurs, this theory predicts that P(DCS) is 0. The likelihood also is found to be 0.

$$X = 1 \text{ and } P(\text{DCS}) = 0$$
$$\text{Likelihood} = 0^{1}*1^{0} = 0$$

When DCS does not occur, the incorrect theory predicts that P(no DCS) is 1, and the likelihood is again 0.

$$X = 0 \text{ and } P(\text{DCS}) = 1$$
$$\text{Likelihood} = 1^{0}*0^{1} = 0$$

The concept of likelihood may be extended to a series of observations made on different decompression procedures by multiplying the likelihoods of the individual observations. This is analogous to finding the probability of a series of coin tosses by multiplying the probabilities of individual tosses. The ability to pool decompression data from many different profiles for simultaneous analysis is a great advantage over traditional statistical methods where profiles must be treated separately.

Suppose, for example, there were 3 DCS incidents in 5 trials of one decompression schedule and no incidents in 5 trials of a second schedule. A simple model, called the "null" model (4), assumes that DCS probability is the same for each trial and is equal to the observed incidence. The null model has only one parameter, DCS probability, which is 3/10 or 0.3. The probability of no DCS is 7/10 or 0.7. The likelihood for the 10 trials is found by multiplying the likelihood of each trial.

$$(0.3^1 \times 0.7^0)^3 \times (0.3^0 \times 0.7^1)^7 = (0.3)^3 \times (0.7)^7 = 0.00222.$$

Since multiplying likelihoods results in a small number, the natural logarithm of the likelihood is frequently reported and appears as a negative quantity

$$ln\ (0.00222) = -6.109.$$

A second model assumes that each schedule has a different DCS probability that is equal to the observed incidence. The data described above have two parameters, the DCS probabilities for each schedule. These are 0.6 for the first schedule and zero for the second schedule. As before, the likelihood for the 10 trials is found by multiplying individual likelihoods where different probabilities are now used for the two schedules.

$$(0.0)^0 \times (1.0)^5 \times (0.6)^3 \times (0.4)^2 = 0.03456$$
$$ln\ (0.03456) = -3.365.$$

This model fits the data perfectly and will be called the "perfect" model. It defines the greatest possible likelihood that any model can attain (2).

The ability of the null model to fit the data can be measured against the perfect model by comparing their maximum likelihoods. The likelihood ratio test and chi-square distribution are used for this purpose (2). The likelihood ratio is twice the difference of the likelihoods, and the number of degrees of freedom is the difference between the number of parameters in each model. Applying a ratio test to the null and perfect likelihoods, they are found to be significantly different at a 0.025 confidence level. Thus, the null model is not a good description of the data.

APPLICATION OF LIKELIHOOD TO HALDANE DECOMPRESSION MODELS

To illustrate the use of likelihood, several decompression models were applied to altitude exposures conducted by the National Aeronautics and Space Administration (NASA) and the U.S. Air Force during trials of 30 pressure profiles (5). These trials included 548 individual exposures in which there were 58 cases of mild DCS symptoms for an 11% incidence. Figure 2 shows examples of the profiles tested. Included were ascents to pressures of 7.8 to 10 psia without oxygen prebreathing, ascents to the Shuttle spacesuit pressure of 4.3 psia following 3.5 to 8 h of oxygen prebreathing, and stage decompression to 10.2 psia followed by ascent to 4.3 psia. During several stage decompressions, repetitive ascents to 4.3 psia were performed. Applying the perfect model to this data, the maximum log likelihood was -142.4 and there were 30 parameters.

The original Haldane model had five tissue compartments with halftimes of 5, 10, 20, 40, and 75 min and a critical decompression ratio of 2 in each compartment (1). To apply likelihood to a Haldane model, a decompression dose

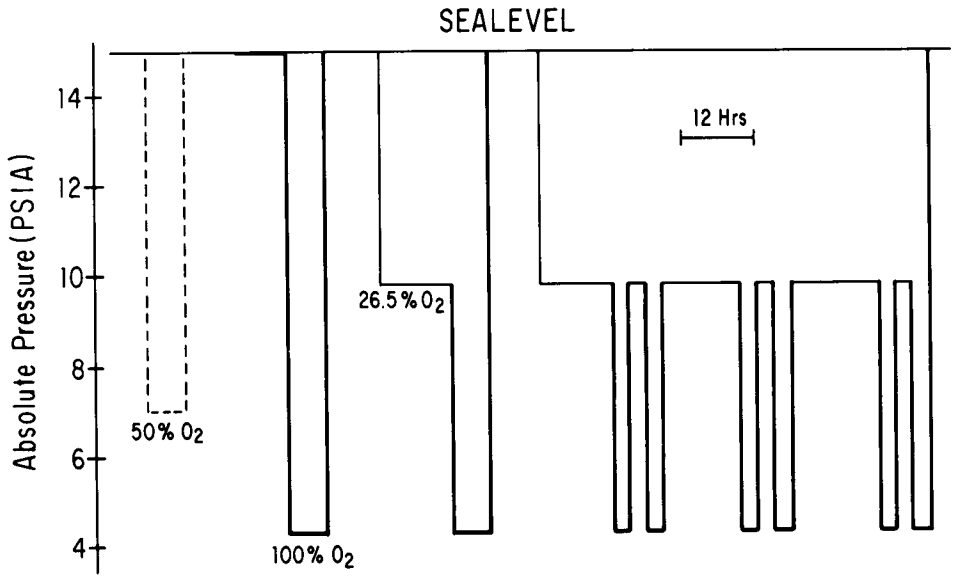

Fig. 2. Examples of the 30 pressure profiles tested by NASA and the Air Force.

is defined for each tissue as

$$\text{Dose} = \text{tissue tension/absolute pressure} - \text{critical ratio}$$

This dose is a measure of *excess* supersaturation where the critical ratio is the supersaturation at which the DCS probability becomes greater than zero and increases sigmoidally toward 1. This differs from traditional usage where the critical ratio is a threshold beyond which DCS is inevitable. The largest dose in all tissues is substituted into the dose-response equation to find the DCS probability at any point during a pressure profile.

With the Haldane model to predict the tissue tensions, the dose equation to predict the decompression dose, and the dose-response model to predict the probability, a DCS probability can be computed for every pressure profile in a dataset. By combining the probabilities with the experimental results according to the definition of likelihood, and taking the product of the individual likelihoods, the likelihood for the entire dataset may be found.

The magnitude of this likelihood depends on the values assigned to the

parameters a and b in the Hill equation (Fig. 1). The initial values chosen for such parameters, however, will rarely be those that determine the maximum likelihood, and the optimal parameter values must be found by searching. Many search procedures or optimization methods are available. The one used here was slow and unsophisticated but robust and easy to understand.

This method operates much as a man on the side of a foggy hill might proceed to reach the hilltop. By analogy, the maximum likelihood is located on the hilltop and the parameters a and b are perpendicular axes oriented before and beside him. The man takes one step forward, backward, and to each side of his initial position and measures at which of the four new positions he is highest. He moves to the highest position and repeats his search one step around him for still higher ground. This process continues until his initial position is highest of all. Should he desire, he can reduce the step size and carry on his search until he is as close to the hilltop as he chooses. This method can be generalized to multidimensional space and has been used with as many as 18 parameters. All optimization methods, however, sometimes find a local maximum near the initial parameter values, and it is often necessary to try several initial parameter groups to ensure a high probability of estimating an overall maximum.

When optimal values were found for the parameters a and b in the Hill equation, the maximum log likelihood for the altitude data and original Haldane model was -934.9, far less than for the perfect model. After a modification to allow the critical ratio to seek its optimal value during the search procedure, the maximum likelihood increased to -185.5 at a critical ratio of nearly 0. No further improvement in likelihood occurred when separate ratios were determined for each of the five tissues.

Using likelihood ratio tests, the differences in maximum likelihood between the perfect model and both Haldane models were found to be statistically significant, indicating that the Haldane models provided poor descriptions of the data.

In further modifications of the Haldane model, optimal parameter values were found for the halftimes and critical ratios in models with one to five tissues. A single-tissue model with four parameters raised the maximum log likelihood to -153.6, with a halftime of 508 min and a critical ratio of 1. Additional tissues produced no further improvement.

Figure 3 shows the lower left corner of the dose-response curve for the single-tissue model and illustrates how a statistical approach to decompression differs from the traditional threshold approach. When the supersaturation ratio exceeded the critical ratio, the DCS risk increased slowly rather than immediately to 100%. At a supersaturation ratio of 1.2, for example, the decompression risk was 1.3%. At a ratio of 1.3, the risk rose to 3.5%.

The maximum likelihood of the single-tissue Haldane model was 11 log likelihood units less than the maximum likelihood of the perfect model, indicating that the perfect model was a better description of the data. A likelihood ratio test, however, showed that the difference in likelihoods was

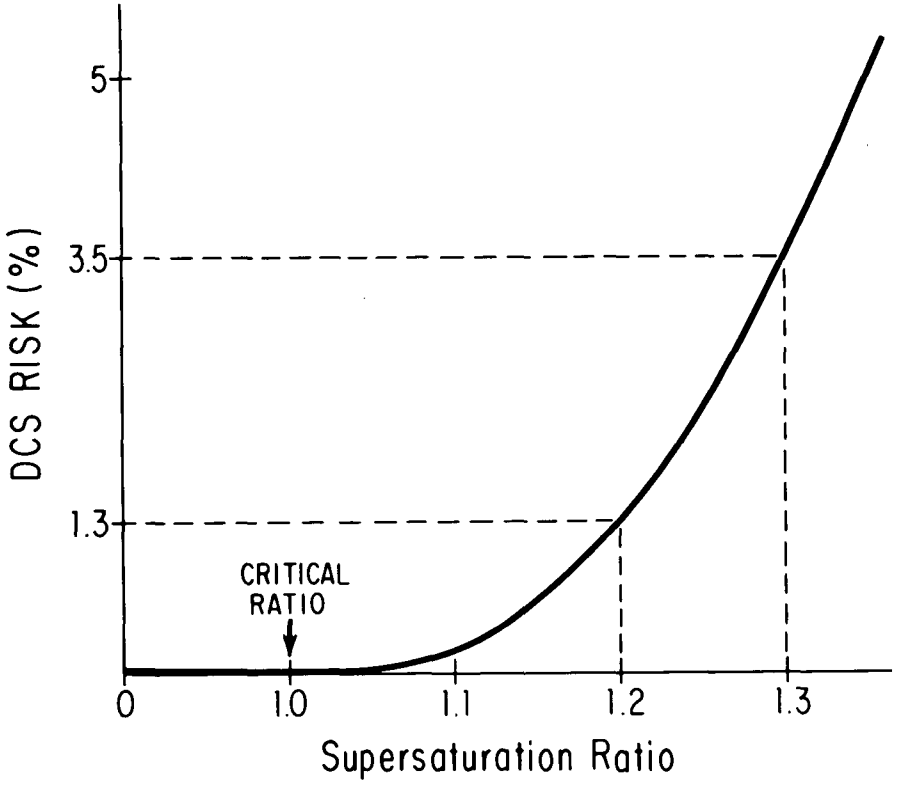

Fig. 3. The low-risk end of the dose-response curve for the single-tissue Haldane model with a tissue halftime of 508 min and a critical ratio of 1.0007.

not statistically significant because the 4-parameter Haldane model was accorded greater certainty than the 30-parameter perfect model. Thus, these models were statistically indistinguishable in their ability to describe the altitude data.

APPLICATION OF LIKELIHOOD TO BUBBLE GROWTH MODELS

One of likelihood's strongest attributes is its capability to estimate optimal parameter values directly from experimental data which, in effect, incorporates the data into the model. This applies to any model or theory that can be stated mathematically. For example, the hypothesis that stationary bubbles cause DCS pain suggests that bubble volume might be used as a decompression dose. Figure 4 illustrates an application of this hypothesis in which a bubble has formed in a Haldane tissue compartment (6). As the compartment is well stirred, the bubble and surrounding tissues are in diffusion equilibrium. During nitrogen uptake and when no bubble is present, the tissue

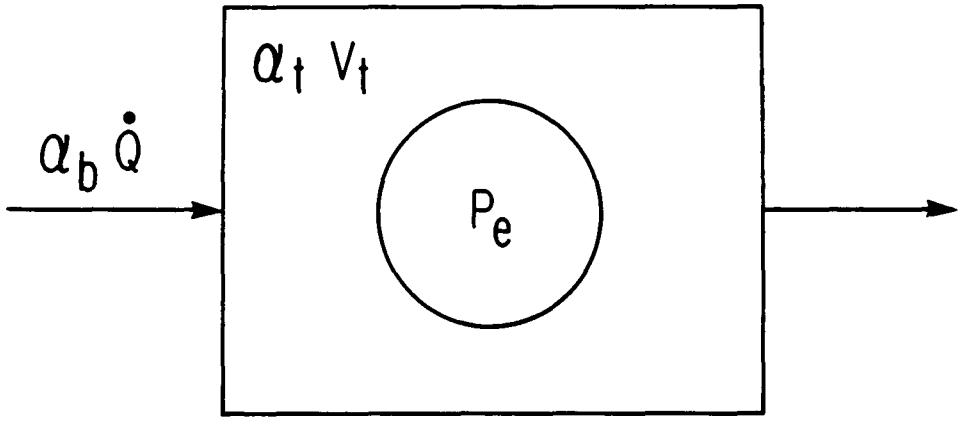

Fig. 4. Diffusion equilibrium between a bubble and a well-stirred Haldane tissue. $\alpha_b * Q$ is the nitrogen solubility in blood times the blood flow rate. $\alpha_t * V_t$ is the nitrogen solubility in tissue times the tissue volume. P_e is the pressure in the bubble due to tissue elasticity and surface tension less the tissue tensions of oxygen, carbon dioxide, and water vapor.

has the exponential gas exchange kinetics typical of the Haldane model. When a bubble is present during a decompression stop, however, the tissue tension remains constant while the bubble volume decreases as a linear function of time. The gas exchange kinetics of this model are conceptually the same as those of a single compartment of the exponential-linear model conceived by Thalmann (7).

The diffusion-equilibrium model of Fig. 4 was extended to multiple parallel tissues as in the Haldane or Thalmann model. With multiple tissues, the largest bubble volume determined the decompression dose. Each tissue compartment had three undetermined parameters (Fig. 4) in addition to the two Hill equation constants. $a_b * Q$ is the nitrogen solubility in blood times the blood flow rate. $\alpha_t * V_t$ is the nitrogen solubility in tissue times the tissue volume. P_e is the pressure in the bubble due to tissue elasticity and surface tension minus the tissue tensions of oxygen, carbon dioxide, and water vapor. When optimal values were found for these constants from the altitude data, the corresponding maximum log likelihoods were -159.6 for a single-tissue model, -156.5 for two tissues, and -155.6 for three tissues.

The assumption of bubble-tissue diffusion equilibrium in Fig. 4 is actually incorrect because the nitrogen partial pressure in the bubble is greater than the nitrogen tension in tissue as a result of the oxygen window (6). Figure 5 shows simulations of concentration gradients around a bubble in tissue for oxygen, nitrogen, and helium (8). The oxygen gradient is steep because it is metabolically consumed in tissue. The helium gradient extends further into tissue than the nitrogen gradient because helium is more diffusible than nitrogen.

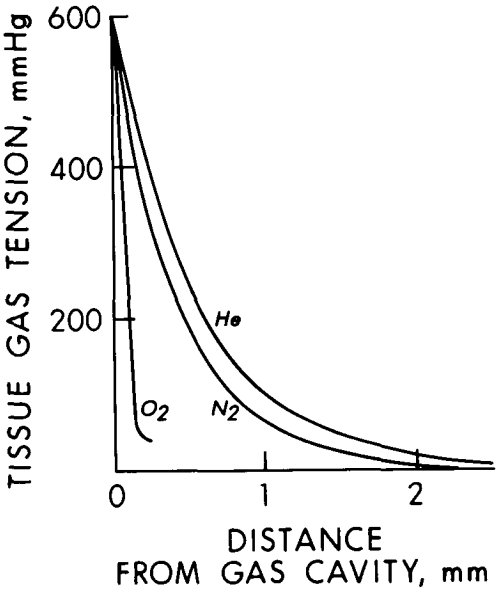

Fig. 5. Tissue tension gradients around a bubble. [Redrawn from Van Liew (8)].

Because of these concentration gradients, diffusion is a limiting factor in inert gas exchange when a bubble is present, and tissue is not well stirred. A barrier placed between the bubble and tissue can provide a first order simulation of this diffusion limitation. This simulation is shown in Fig. 6 where the permeability of the diffusion barrier is defined by the parameter k. When a single tissue bubble model with a diffusion barrier was applied to the altitude data, the maximum log of likelihood increased to -151.9 (Table 1). Additional tissues produced no further improvement.

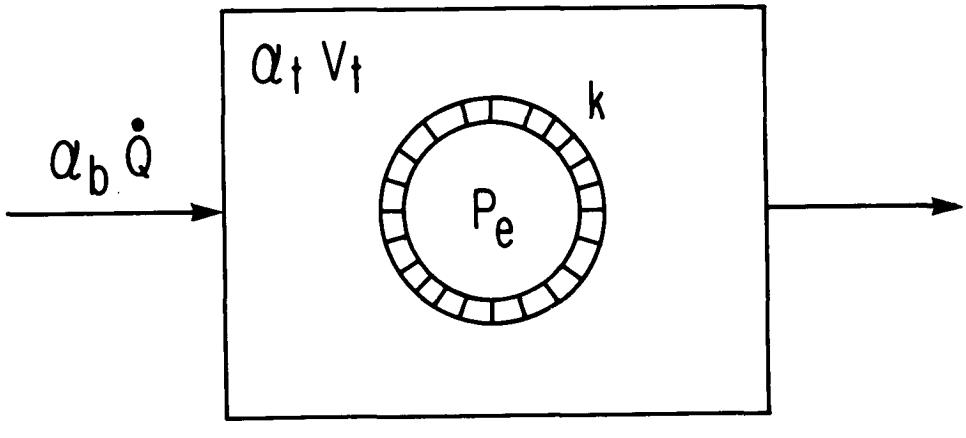

Fig. 6. A diffusion barrier between a bubble and a well-stirred tissue. k is the permeability of the barrier.

Table 1
Maximum Log Likelihoods for Various Decompression Models and Datasets*

Model	No. Parameters	Dataset A	AC	D	E	F	G
Null	1	-377.12	-382.94	-185.07	-108.60	-24.27	-31.71
Perfect	-	-191.54	-191.54	-142.39	-50.55	0.0	-17.98
No. of per mod param.		117	121	30	109	5	10
Haldane (5 tissue)							
Original	2	-931.25	-932.03	-934.85	-272.55	-112.82	-338.48
1 ratio	3	-378.35	-384.45	-185.52	-109.24	-24.40	-31.72
5 ratio	7	-378.35	-383.90	-185.39	-91.93	-24.38	-31.71
Modified Haldane							
1 tissue	4	-312.18	-314.81	-153.55	-93.63	-24.37	-21.15
2 tissue	6	-305.01	-307.45	-153.43	-93.11	-24.37	<u>-20.68</u>
3 tissue	8	-303.94	-307.33	-153.43	-91.95	-24.37	-20.68
Bubble tissue diffusion equilibrium							
1 tissue	5	-313.23	-339.17	-159.62	-89.42	-13.95	-21.53
2 tissue	7	-300.83	-326.34	-156.49	<u>-87.77</u>	<u>-12.72</u>	-21.53
3 tissue	9	-300.79	-326.34	-155.47	-87.77	-12.72	-21.53
Bubble tissue diffusion barrier							
1 tissue	6	-313.21	-329.41	-151.90	-90.88	-13.95	<u>-21.13</u>
2 tissue	9	-300.86	-303.91	-151.90	-88.59	-13.92	-21.13
3 tissue	13	<u>-293.03</u>	<u>-293.63</u>	<u>-151.89</u>	-87.86	-13.92	-21.13

* The greatest likelihood for each dataset is underlined. The number of parameters (separate decompression procedures) for the perfect models are listed.

Figure 7 summarizes the altitude data analysis using the modified Haldane, diffusion equilibrium, and diffusion barrier models with one, two, or three tissues. The x-axis is the number of tissues, and the y-axis is the maximum log likelihood. For comparison, the likelihood of the null model appears as a horizontal line at the bottom of the figure and the likelihood of the perfect model appears at the top. Likelihoods below the band marked "0.05 confidence level" are significantly different from the perfect model. A *solid line* connecting two adjacent points indicates a significant difference between two models, while a *broken line* indicates no significant difference. The diffusion barrier models performed best followed by the modified Haldane and diffusion equilibrium models, but all were statistically indistinguishable from the perfect model. While there was no statistical justification for preferring the diffusion barrier models over the simpler Haldane models, the model of choice for calculating decompression procedures would seem to be the one that best fits the data.

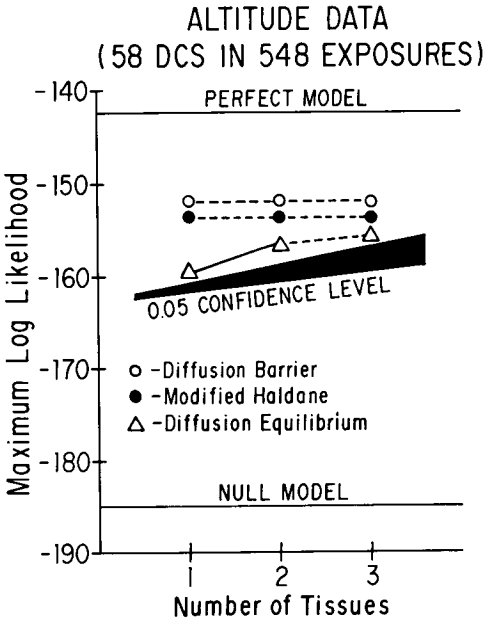

Fig. 7. Maximum likelihoods for various decompression models applied to the altitude data (5).

APPLICATION OF LIKELIHOOD TO DIVING DATA

Likelihood was applied to several sets of decompression trials, and the results are shown in Table 1. Dataset G contains development tests of the U.S. Navy Exceptional Exposure schedules (9). The DCS incidence was 46% in 46 decompression trials from a depth of 140 fsw with bottom times ranging from 90 to 360 min. These data were best described by a two-tissue Haldane model.

Dataset F contains 86 saturation-excursion exposures on nitrogen-oxygen. These dives tested surface interval safety after direct ascent to sea level from saturation (10). The DCS incidence was 8%. Dataset E includes trials of the U.S. Navy Standard Air schedules where the DCS incidence was 5% in 568 man-dives (11). Datasets E and F were best fit by a two-tissue diffusion equilibrium model.

Dataset D contains the 548 NASA and Air Force altitude exposures described earlier (5). The DCS incidence was 11%. A single-tissue, diffusion equilibrium model fits this data best.

Dataset C includes 47 multilevel, no-stop, repetitive air dives in which there was no DCS (12). Dataset A includes 1033 single, no-stop dives on air or

nitrogen-oxygen where the DCS incidence was 12% (13). Dataset A and Dataset AC, which combined A and C, were fit best by a three tissue, diffusion equilibrium model.

The bubble models exhibited similar multitissue behavior as the Haldane models. For some datasets, a single-tissue Haldane model performed better than many single-tissue bubble models. Multitissue bubble models outperformed multitissue Haldane models for all datasets except G. For short or repetitive exposures (A, AC, D), the diffusion barrier model was superior to the diffusion equilibrium model. During long or single exposures (E, F, G), a diffusion barrier did not seem to be important. Datasets A, AC, and E to a lesser extent, for example, required multitissue models whereas single-tissue models sufficed for datasets D, F, and G. Bubble models with a diffusion barrier described short or repetitive exposures (datasets A, AC, and D) better than diffusion equilibrium models. This might be expected in repetitive diving where diffusion would limit both bubble growth and resolution. These results show that a particular model may describe one type of diving better than another and that decompression procedures for a given dive type should be calculated by the model that best describes a similar body of data.

Figure 8 shows an analysis of all 2328 exposures in datasets A-G. This figure is similar to Fig. 7 for the altitude data. With a single tissue, all three models performed equally well. As the number of tissues increased, the diffusion-barrier model performed best followed by the modified Haldane and diffusion equilibrium models. No model, however, accurately described the combined data. It is concluded once again that a model should be limited to data it describes accurately and that calculated decompression procedures should not stray far from these data.

Let us limit the data to the 568 trials of the UNS Standard Air schedules (11) and examine the DCS risks predicted by the two-tissue diffusion equilibrium and three-tissue diffusion barrier models whose maximum likelihoods are the same (Table 1). Figure 9 shows the Standard Air decompression schedules for a 60-min dive to 150 fsw. The x-axis is time in minutes. The left-hand y-axis is depth in fsw, and the right-hand y-axis is the percent DCS risk. The *solid line* is the dive profile, and the *dotted line* is the risk predicted by the three-tissue, diffusion-barrier model. This risk rose gradually as gas diffused into the bubble. The maximum risk was 10% and occurred 90 min after surfacing. The *broken line* is the DCS risk for the two-tissue diffusion equilibrium model. When a bubble was present there was an instantaneous increase in risk when the depth decreased. A maximum risk of 12% occurred immediately upon surfacing.

As delayed DCS symptoms are frequently observed, the occurrence of the maximum risk immediately upon surfacing is worrisome. This is an even greater problem in altitude exposure where DCS may not occur until hours after decompression. This paradox is more sharply defined in Fig. 10 which shows the DCS risks for a 55-min air dive at 150 fsw with decompression according to the USN Surface Oxygen Table. The diffusion equilibrium model predicts

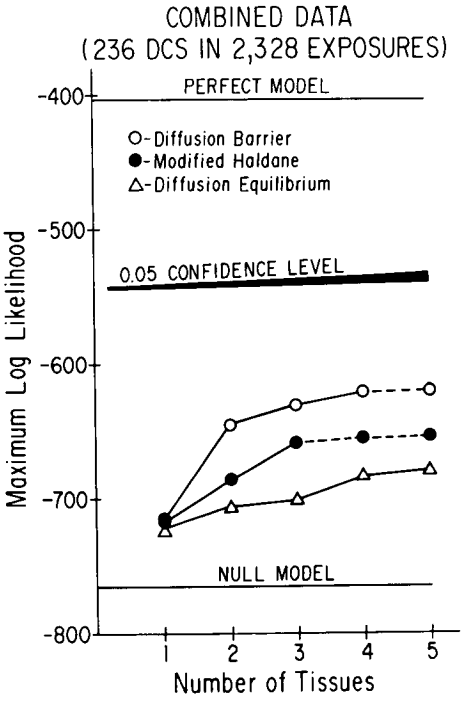

Fig. 8. Maximum likelihoods for various decompression models applied to datasets A to G.

that the risk increases to 48% during the 5-min surface interval and returns to zero upon recompression to 40 fsw. This occurs because gas diffuses into or out of the bubble instantaneously upon pressure change. In the diffusion barrier model, on the other hand, gas diffusion is slow, and the 5-min surface interval is too short to cause appreciable bubble growth. The delayed occurrence of the maximum risk upon surfacing is a consequence of the slow diffusion of gas into the bubble. The "risk models" discussed by Weathersby et al. (4) have a similar characteristic, but Haldane models lack this behavior.

The ability to predict DCS risk carries with it the obligation to choose an acceptable risk. Table 2 shows the influence of risk on decompression time after a 60-min air dive to 150 fsw. Schedules were calculated for risks of 0.1 to 3% using the three-tissue diffusion-barrier model with parameter values determined from the USN Standard Air trials (11). It is desirable that the DCS risk should be very small, which can make decompression time impractically long. If immediate recompression were available, one might be willing to accept risks of 1 or 2%. If treatment were not possible for several hours, on the other hand, risks of 0.1 to 0.5% would be preferable. These choices are further complicated because risk is influenced by factors other than depth and bottom time, and these factors can increase decompression time by as much as 300% (6). Thus, it is mandatory that decompression procedures be tested under the conditions of expected use.

The objective of this report was to demonstrate the use of likelihood in

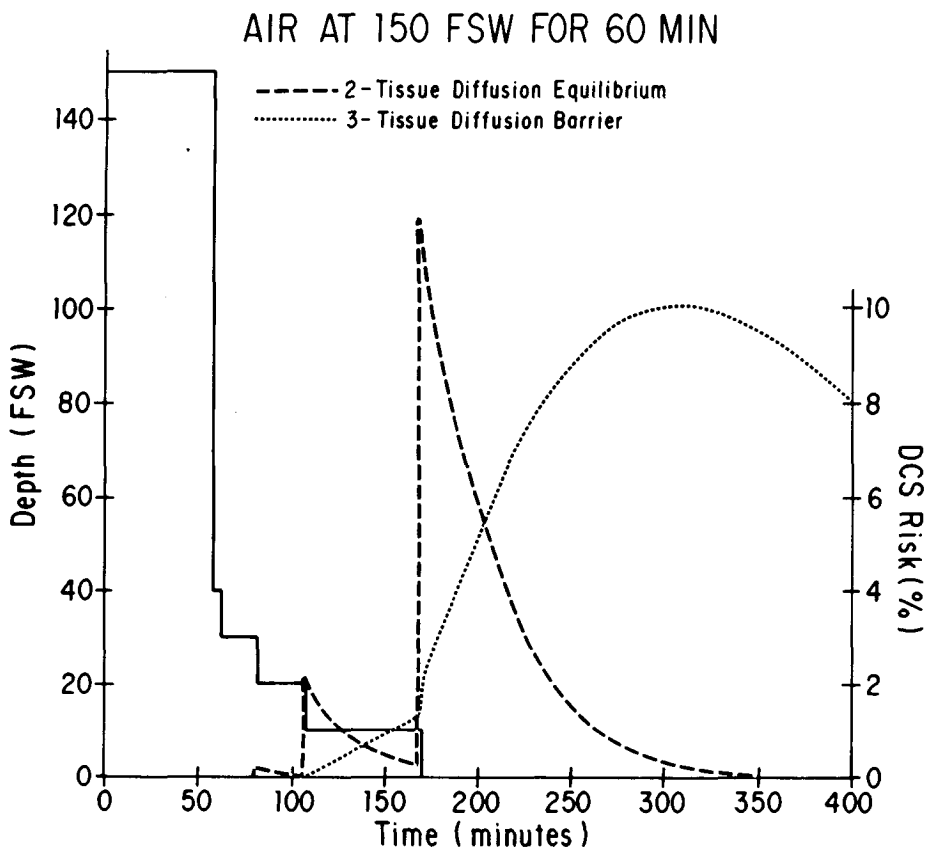

Fig. 9. DCS risk after a 60-min air dive to 150 fsw as predicted by the two-tissue diffusion equilibrium and three-tissue diffusion barrier models. The model parameters were determined from trials of the USN Standard Air schedules in dataset E (11).

decompression calculations. Imagination and ingenuity are the only factors limiting the models to which likelihood may be applied. The successful employment of likelihood, however, ultimately depends on the availability of a large body of well-documented decompression data. It is proposed that a standard decompression data bank be established and maintained by an independent body such as the Undersea and Hyperbaric Medical Society. This data bank would contain the results of dive trials judged to have been conducted under acceptable conditions and reliably reported. Information in the data bank would be generally available in digital form by telephone transmission or on floppy disk. Researchers could evaluate their decompression models using likelihood and the standard data. This would provide an objective mechanism for the evolution of decompression procedures toward greater safety and improved efficiency.

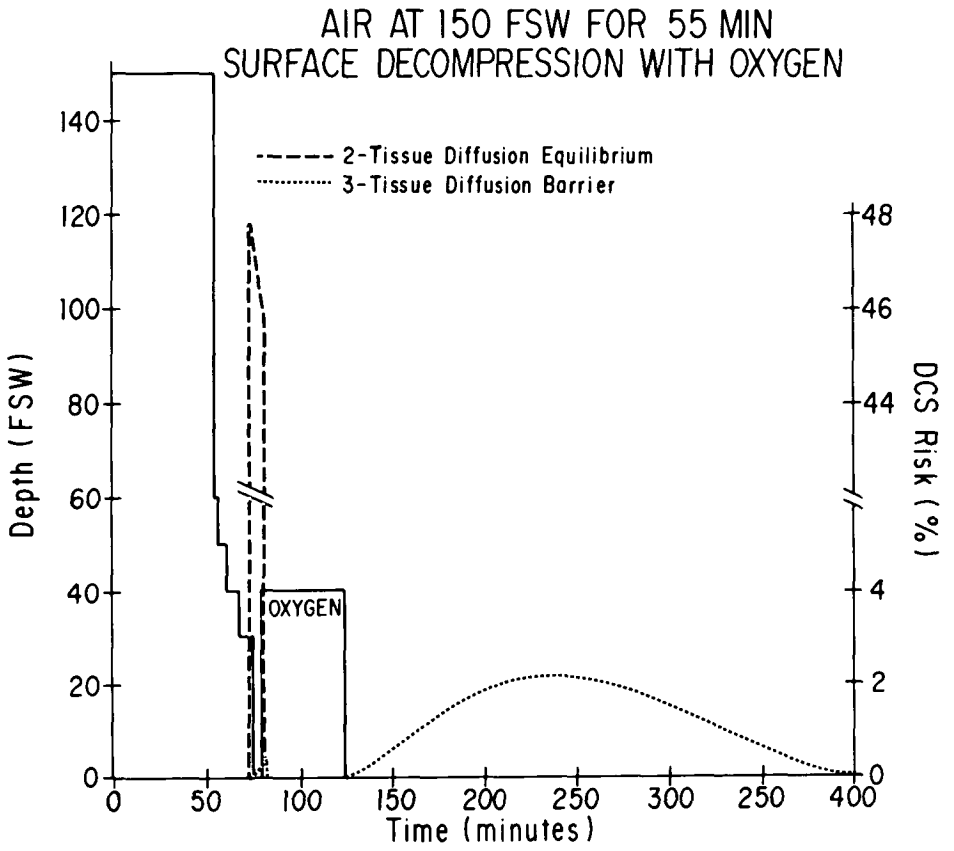

Fig. 10. DCS risk after a 55-min air dive to 150 fsw with decompression according to the USN Surface Decompression Using Oxygen procedure. Risks were predicted by the two-tissue diffusion equilibrium and three-tissue diffusion barrier models using model parameters determined from trials of the USN Standard Air schedules in dataset E (11).

Table 2
The Effect of DCS Risk on Decompression Time for a 60 min Air Dive to 150 fsw*

DCS Risk, %	Stop Time, min		
	20 ft	10 ft	Total
3	90	130	220
2	90	150	240
1	90	180	270
0.5	60	230	290
0.1	60	320	380

* The schedules were calculated with the three-tissue diffusion barrier model using the USN Standard Air Trials (Des Sranges 1957) to determine the model constants. Stop times are to the nearest 10 minutes to give the shortest total time.

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ANALYSIS OF DOPPLER ULTRASONIC DATA FOR THE EVALUATION OF DIVE PROFILES

B. C. Eatock and R Y. Nishi

The development of decompression tables or evaluation of dive profiles has traditionally been based on the observation of decompression sickness (DCS). Acceptance or rejection of a given profile usually depends on the incidence of DCS observed in experimental dives. Proving the safety of profiles with a reasonable degree of confidence, however, requires many more experimental dives than are normally feasible. There is no standard criterion for rejecting or accepting experimental profiles, although Homer and Weathersby (1) have recently recommended systematic procedures based on probabilistic considerations. A better method of evaluating dive profiles to supplement the traditional method is the use of the Doppler ultrasonic bubble detector to detect venous gas emboli (VGE), i.e., bubbles. There are two main advantages in using Doppler monitoring:

1. It provides more data than observing the incidence of DCS. We have observed bubbles in about 60% of all experimental dives—about 10 times more frequently than DCS (2). It is often possible to use bubble observations to compare the results of 2 dives, even in the absence of DCS. If no bubble results were available, it would have to be concluded that there were no observed differences. It is unlikely that an experimental dive program would be approved today if a high incidence of DCS were considered likely. Thus, as decompression procedures become safer, decompression testing could involve profiles that produce few or no cases of DCS, hence making the bubble results more important.
2. Bubble results are more objective than DCS results. The occurrence of DCS is sometimes not well defined because diagnosis might rely on a subjective report of invisible symptoms. Although success in detecting bubbles depends on the vigilance of highly trained observers, and bubbles

can sometimes be hard to detect, the results cannot be hidden by the diver. Mild symptoms could be ambiguous and a diver might not report symptoms if there were a perceived penalty for doing so. Conversely, an anxious diver might report having DCS when the pain had another origin.

The main disadvantage of Doppler ultrasonic bubble detection is that it is time consuming and labor intensive. For a typical bounce dive, the divers should be monitored periodically for several hours after the dive. The work becomes tedious, but demands concentration. A new term has been coined describing the condition that can afflict the observers—Doppler toxicity (E.D. Thalmann, personal communication).

This paper discusses the problems of analyzing bubble data and how to use the data to evaluate decompression profiles. There are at least two reasons why these problems exist.

1. The meaning of the data is open to different interpretations because the occurrence of bubbles does not directly lead to observable symptoms of decompression sickness (3-5). Although large numbers of VGE are not necessarily accompanied by DCS, the risk of DCS does seem to be increased. Conversely, the risk of DCS seems to be small when few or no bubbles are detected after careful observation (2, 6). If the association between bubbles and DCS were better established, the criterion for accepting or rejecting profiles could be expressed as a function of bubbles observed.
2. Doppler data are categorical, so *parametric* statistical techniques (e.g., mean, standard deviation, analysis of variance) cannot be used for analysis. Many scientists are unfamiliar with methods for handling this class of data, and are apt to apply inappropriate techniques. Some methods for handling categorical data (7, 8) will be recommended and illustrated here with some examples from experimental dives done while testing the new Canadian Forces decompression tables (9).

REVIEW OF PROCEDURES FOR DOPPLER ULTRASONIC BUBBLE DETECTION

To understand Doppler bubble data, it is necessary to be familiar with procedures for obtaining valid data (10). The objective is to obtain a history of bubble production for each subject in the dive.

Because it is generally not possible to monitor the divers continuously, the timing of the observations is important. For most nonsaturation dives, observations should begin soon after the divers reach the surface, and should be repeated at half-hour intervals for at least 2 h. We have often observed delays of 1 h or more before bubbles were detected, and have observed elevated bubble counts for periods longer than 6 h after severe dives less than 1 h in duration (5). For decompressions longer than 20 min it may be useful to monitor during the decompression if possible. For saturation dives it is essential to monitor during the decompression.

The choice of monitoring sites on the body is important. Ideally, it would be desirable to count all the bubbles formed anywhere in the body. With the Doppler method, however, only moving, intravascular bubbles can be found. By monitoring the right ventricle (the precordial site) an estimate of the rate of bubble production for the entire venous system can be obtained, assuming that the bubbles persist long enough to reach the heart. Monitoring the heart can be difficult, because the movement of the heart and the turbulent blood flow within provides a large background clutter that tends to mask bubble signals. To compensate, veins such as the jugular, subclavian, inferior vena cava, or femoral could also be monitored. Bubble signals at these sites are unambiguous; however, they should be monitored as a supplement only and not as an alternative to the precordial site, because they do not cover the whole body. Experience has shown that bubbles can sometimes be detected in the heart when they cannot be found in any of these veins. Conversely, bubbles can sometimes be detected in the veins when they cannot be detected in the heart, presumably because they are hidden by the heart signal.

A third consideration is the position of the subject; for example, standing or lying down for the evaluation. Either position produces good results, so the choice can be dictated by convenience. The evaluations are often done for two conditions—one with the subject at rest and another after the subject has moved in some defined way. For example, for precordial evaluation, a standing subject may be asked to do a deep knee-bend—squatting down and standing up, in a smooth, continuous motion. For the subclavian site the subject may be asked to clench his fist. The movement case is useful because it often produces a shower of bubbles that are easily identifiable. Also, there may be none detected for the rest case, so the movement case may provide a richer source of data.

The instrument used by most investigators is a simple, continuous-wave (CW) Doppler ultrasonic device operating at 5 MHz (3, 5, 11, 12) developed specifically for detecting bubbles. (Representative manufacturers are: Sodelec, Marseilles, France; the Institute of Applied Physiology and Medicine, Seattle, WA; and Techno Scientific, Woodbridge, Ontario, Canada.) CW Doppler instruments developed for clinical use tend to lack sensitivity and durability. Pulsed Doppler ultrasound units (13) have the advantage that penetration depth of the received ultrasonic signal can be selected, thus eliminating some unwanted signals from other ranges. In practice, however, the advantage does not seem to be great. CW devices are adequate and are easier to use.

It is essential to remember that bubbles may be missed for several reasons. Small bubbles may not scatter enough ultrasonic energy to be detected over the background. With Doppler ultrasound, it is not possible to deduce the size of the bubbles in vivo, but acoustical studies (14) suggest that they must be of about 100 μm in diameter or larger. Also, the investigator may simply have looked for the bubbles at the wrong time or in the wrong place. In general, it is not possible to prove the absence of bubbles; on the contrary, it is only possible to infer their existence from signals presumed to be scattered

by bubbles.

The number of bubbles detected can be estimated, but a true count is beyond the capability or patience of human observers. It has proved extremely difficult to produce a machine that counts bubbles reliably because the detection process involves pattern recognition. Bubbles can occasionally be so numerous that it is impossible to distinguish their individual contributions to the acoustic signal. To circumvent this problem, signal classification schemes have been developed, based on rough estimates of the number of bubbles the observer is able to identify. Two similar schemes seem to be in current use: Spencer's (3) and Kisman and Masurel's (10, 15, 16). Both methods use the approximate number of bubbles heard in each heart beat. The K-M Code also considers the percentage of heart beats with that approximate number of bubbles and the amplitude of the signal compared to the background cardiac sounds. The K-M Code produces a three-numeral classification, e.g., 213, that is reduced to a global bubble grade, e.g., I+, comparable to Spencer's grade. The final results are usually expressed as grade 0, I, II, III, IV. Here we have chosen to represent the grades with Roman rather than arabic numerals to emphasize that these grades represent a classification, not a number.

PROPERTIES OF DOPPLER ULTRASONIC BUBBLE DATA

Data can be classified into at least four types (7): nominal, ordinal, interval, or ratio:

1. Nominal data are categorical and can be classified or placed in categories based on attributes, such as occupation or sex. The DCS or no-DCS outcome of a dive experiment is an example of nominal data.
2. Ordinal data are also categorical but a ranking can be established between the categories, such as the ranks in the navy or academic letter grades. The outcome of a diving experiment by Doppler bubble grades is a good example of ordinal data.
3. Interval data are numerical. These data can be compared to a constant-interval scale. An example of interval data would be temperature measurements made on the Celsius scale. Bubble grades are not interval data because the relationship between grades is not linear (and cannot easily be linearized).
4. Ratio data are also numerical. In addition to an interval scale a true zero can be defined. An example of ratio data would be temperature measurements made on the Kelvin scale.

Any statistical test assumes that the observations are independent, i.e., that the result of one observation does not have an effect on the result of the next. Parametric statistical tests require that the data be measured on an interval or ratio scale. The appropriateness of parametric tests also depends on the validity of other assumptions about the data:

1. The data are assumed to be drawn from normally distributed

(Gaussian) populations.

2. The populations are assumed to have equal variances.

Examples of parametric tests are the t test and the F test. The F test (analysis of variance) assumes additionally that the means can be modeled as linear combinations of effects.

Arithmetic operations, such as means and variances, are permissible on numerical data but are meaningless on categorical data. (What is the mean of a captain and a commander? What is the variance?) For nominal data, only equivalence (one-to-one) transformations are permitted. For ordinal data, rank-preserving transformations are also permitted. Applicable statistics for categorical data include the median, quartiles, and the χ^2 test. Ordinal data can also be analyzed with tests based on rank.

Doppler ultrasonic bubble data do not satisfy the basic assumptions required to use parametric statistics. Bubble scores are not normally distributed (2) and they are not measured on an interval scale. Fortunately, Doppler ultrasonic bubble data do satisfy the assumptions required for many nonparametric statistical tests. These tests are valid and they are more likely to produce significant results than invalid application of parametric tests.

EXAMPLES

Figure 1 shows an example of data recorded from a diver showing the time course of the bubbles. However, there is too much information to conveniently summarize the results of the dive for all divers. The peak or maximum bubble grade observed for each site monitored and for both rest and movement cases may be used to summarize the results. These can then be tabulated or shown in a histogram of the results, e.g., Table I and Fig. 2. This gives an idea of the decompression stress associated with that dive. For example, if 50% of the divers had peak bubble grades of II or more, the dive might be classified as stressful. (What constitutes a stressful dive is an open question.) A linear plot with bubble grade as an axis would be inappropriate because it would imply a linear relationship between the bubble grades. This would be misleading.

If several different dives are to be compared, the results can be displayed as in Table 2 or, to give a more visual idea of the differences, in multiple histograms such as Fig. 3. If there seem to be differences, it is reasonable to ask whether they are the result of chance variation or of real differences in the underlying phenomenon that produces bubbles, i.e., are the differences significant?

This is similar to the common statistical problem of comparing the results of 2 or more different treatments. When the results can be measured on at least an interval scale, and when the samples can be assumed to have been

DCIEM DOPPLER DATA

Diver 1051 Date 830425 Profile 36150 AIR
Diver's Role WWB Start Time 1300 (Wet) Dry
Sex M Dive No. DR0208 Water Temp. 10.26
Monitor DE Series Name DCIEM 83-B Work Type BIKE

Eval		Heart		Shoulders				Other
	Time	Rest	Move	L/Rest	L/Move	R/Rest	R/Move	IVC
1.	80	232	242 242 242	0	0 (122) 0	0	0 0 0	L. LEG 323 III- R. LEG 213 I+
	Depth	Grade						
	0	III-	III		II			
2.	Time 110	132	242 242	0	112 112 112	0	0 0 0	
	Depth 0	Grade II	III		I			
3.	Time 139	232	(232) 242 232	0	0 (212) 0	0	0 0 0	
	Depth 0	Grade III-	III-		I			
4.	Time 169	0	(222) 232 222	0	0 0 0	0	0 0 0	
	Depth 0	Grade	II					
5.	Time 216	0	222 222 -	0	0 0 0	0	0 0 -	
	Depth 0	Grade	II					
6.	Time 248	0	0 0 0	0	0 0 0	0	0 0 0	
	Depth 0	Grade						

Fig. 1. An example of a data sheet for Doppler ultrasonic bubble detection. The results were described using the K-M Code (10, 15, 16). The form shows the time course, in minutes, of bubble evolution for 1 diver.

Table 1
Peak Bubble Grades for a Dive to 36 msw for 50 Min on Air

Diver	Role	Precordial R/M*	Time,** min	LS† R/M	Time, min	RS†† R/M	Time, min	DCS
1018	S	II/I	87	III/II	87	III/II	87	
1051	WWB	III-/III	80	0/II	80	0	—	
1052	WWS	III/IV	207	II/III-	116	II/II	207	
1053	L	II/III	199	II/I	140	0	—	
1054	DR	0	—	III/III-	110			
1055	DR	III+/IV	100	—	—	—	—	Type I

*Rest or movement; **time from the start of decompression; †left subclavian; ††right subclavian; S = standby diver; WWB = wet, working, bike; WWS = wet, working, swim; L = team leader; DR = dry, resting.

drawn from normal and homoscedastic populations, the test to use is the *t* test (7). When these assumptions do not apply, as is true for Doppler ultrasonic data, a nonparametric test must be chosen. Ideally, the populations used to test the different treatments should be the same or matched for all the important properties. This is the case of two or more related samples. In decompression research it is often not possible to obtain matched samples, so it is necessary to use tests that are applicable to the case of two or more *independent* samples.

The Kruskal-Wallis one-way analysis of variance based on ranks is applicable (7). This procedure tests the null hypothesis that the k samples come from the same population. It assumes that the variable under study has an underlying, continuous distribution and that the results can be ranked on an ordinal scale. Doppler ultrasonic bubble data meet both of these requirements. In this procedure the observations are ranked, the smallest being given the rank one. Ties are allowed. Then the sum of the ranks for each of the samples is calculated. The procedure tests whether the sums are so disparate that the different samples are unlikely to come from the same population. (It uses the sum of the ranks in a test statistic which, if the null hypothesis is true, is distributed as χ^2 with k-1 degrees of freedom.)

For example, we would like to know whether the different scores obtained for the three decompression procedures, air, in-water oxygen, or surface decompression with oxygen, for the dives to 36 msw for 50 min used in the previous examples are significantly different. The Kruskal-Wallis test (7), with $k = 3$ was used to test the results for each of the 6 measurement conditions, i.e., precordial, rest and movement, and right and left subclavian rest and movement. The test pointed to differences between the three decompression methods, with probability of error (i.e., incorrectly rejecting the null hypothesis) ranging from $P = 0.0015$ to $P = 0.0064$. Next, it is reasonable to

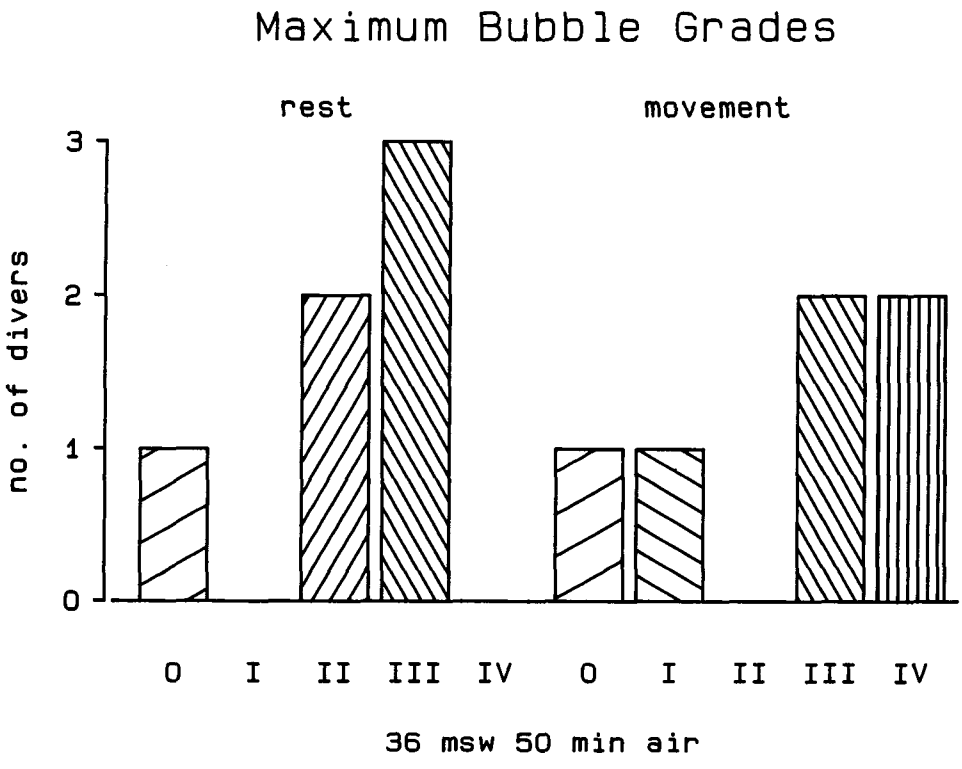

Fig. 2. A histogram of maximum precordial bubble grades for both rest and movement for a dive to 36 msw for 50 min on air.

ask where the significant differences lay, i.e., to compare the three methods pairwise. [It is not valid to do only three pairwise comparisons, leaving out the overall comparison, because this would inflate the probability that the null hypothesis would be incorrectly rejected (7).] When this was done it was found that the differences between decompression on air only and decompression using in-water oxygen were insignificant in 5 of the 6 cases (the left-subclavian movement case being the one exception). The differences between air decompression and surface decompression with oxygen were significant for all 6 cases, with values of P ranging from $P = 0.0001$ to $P = 0.0106$. The differences between the two methods of oxygen decompression were significant at $P < 0.05$ for 4 of the 6 conditions, the exception being the left subclavian results. It therefore seems justifiable to conclude that, for this profile, from bubble results, the two oxygen methods were at least as safe as the standard air method, and for surface decompression, safer.

Several other nonparametric tests are available. The test to use depends on the characteristics of the samples. If matched samples are possible, for example, it might be advantageous to use the Friedman two-way analysis of variance based on ranks (7).

Table 2
Maximum Bubble Grades for Dives to 36 msw for 50 min

Monitoring Site	Dive Mode	No. Man Dives	Man-Dives with Maximum Bubble Grade										No. of DCS
			At Rest					After Movement					
			0	I	II	III	IV	0	I	II	III	IV	
Precordium	Air	19	6	1	6	6	0	6	1	1	9	2	3
	IW	31	15	3	6	7	0	11	1	3	12	4	0
	SD	44	33	3	6	2	0	28	2	3	10	1	1
Left subclavian vein	Air	18	8	2	3	5	0	6	4	2	5	1	2
	IW	31	25	3	3	0	0	24	4	3	0	0	0
	SD	44	38	4	2	0	0	32	10	2	0	0	1
Right subclavian vein	Air	18	9	2	5	2	0	9	4	3	2	0	2
	IW	31	22	1	5	3	0	22	1	5	3	0	0
	SD	44	39	3	1	1	0	38	4	1	1	0	1

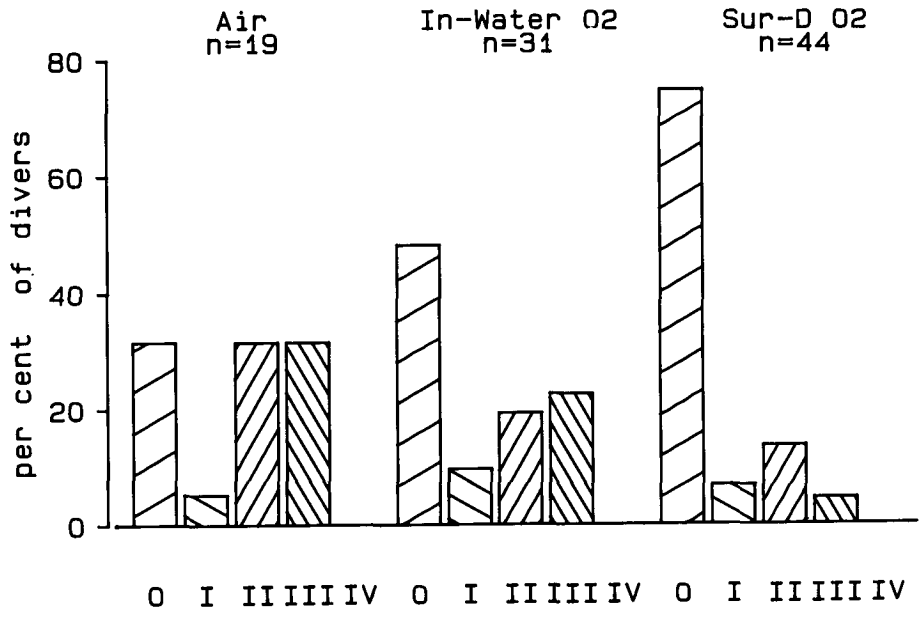

Fig. 3. A histogram showing the frequency of maximum bubble grades for dives to 36 msw for 50 min using three methods of decompression: air, in-water oxygen, and surface decompression with oxygen.

The χ^2 test is a useful nonparametric test that can be used to compare the relationship between two variables based on proportions. For example, an interesting question concerns the relationship between bubbles and DCS. The association between DCS, or bubbles, and such factors as smoking, alcohol consumption, obesity, fatigue, and exercise could be tested. The data can be nominal. The test is invalid, however, if any of the expected frequencies are zero, and should not be used if more than 20% of the cells have expected frequencies less than five. When the expected frequencies are small and the data can be cast in a 2 × 2 contingency table, the Fisher exact probability test may be useful (7).

For example, 66 divers were classified as either bubblers or nonbubblers, depending on whether they tended to have many more bubbles, more frequently than their fellow divers. There were 14 bubblers and 52 nonbubblers. Based on their answers to a questionnaire, the divers were also classified as smokers or nonsmokers, drinkers or nondrinkers, and exercisers or nonexercisers. Using $P = 0.05$ as the level of significance the χ^2 test showed the relationship between bubblers and smokers to be significant ($\chi^2 = 3.95$). Table 3 shows the 2 × 2 contingency table for bubblers and smokers. There was no clear relationship between alcohol consumers and bubblers ($\chi^2 = 0.475$). Because there was only one exerciser who was classified as a bubbler, the Fisher exact probability test with Tocher's modification (7) was used in this example. The relationship between exercisers and bubblers was close to the borderline ($P \approx 0.08$), suggesting that a reexamination with a larger sample might be warranted.

Table 3
Contingency Table of Bubblers and Smokers

	Bubblers	Nonbubblers	Total
Smokers	9	16	25
Nonsmokers	5	36	41
Total	14	52	66

Another common question concerns the correlation between two variables. The calculation of the usual correlation coefficients also requires the calculation of means and standard deviations, so these correlation coefficients cannot be defined for Doppler bubble grades. It is valid to use a correlation coefficient based on ranks, however. Siegel (7) discusses four coefficients that could be useful. The basic coefficients are the Spearman rank correlation coefficient, r_s, and the Kendall rank correlation coefficient, τ. The latter can be generalized to a Kendall partial correlation coefficient, $\tau_{xy,z}$. The coefficient of concordance, ω, can be used as a measure of the agreement between observers. Thus it could be used to compare the results of two

independent observers classifying the same Doppler signals. As an example of the use of the correlation coefficients, it is interesting to compare the bubble scores for the 6 measurement conditions mentioned above. When this was done for the data summarized in Table 2, it was found that the precordial rest and movement results are highly correlated ($\tau \approx 0.8$). The right and left subclavian results are moderately well correlated ($\tau \approx 0.5$), and the precordial and subclavian results show a small positive correlation ($\tau \approx 0.3$). The Kendall and Spearman correlation coefficients do not give identical numbers, but are equivalent in information content.

Binomial statistics can be used to describe the outcome of decompression experiments when the outcome can be classified as one of two possible results. The problem becomes one of estimating the probabilities of the two results. Usually the probability of DCS is sought, as in the traditional approach, but the results could also be expressed as bubbles vs. no-bubbles, or perhaps few or no bubbles vs. many bubbles. It may be advantageous to use the bubble results if few or no cases of DCS were observed.

To estimate the probability of DCS, Homer and Weathersby (1) have recommended the method of maximum likelihood. Their recommendations could easily be applied to observations of bubbles by a simple dichotomization of the results, as above. An intermediate result (similar to questionable DCS) could be introduced as follows: Bubble grades 0 and I could be assigned an outcome of 0; bubble grades III and IV could be assigned an outcome of 1; and bubble grade II could be assigned the outcome 0.5. The method of maximum likelihood allows the experimenter to choose between competing hypotheses based on the results (17).

CONCLUSION

It is notoriously difficult to do experimental diving scientifically. The advent of more sensitive indicators of decompression stress, i.e., Doppler ultrasonic bubble detectors, has provided a richer source of data for analysis. Originally it was hoped that these could prove to be fool-proof diagnostic tools for predicting DCS. It is now understood that they are not (18, 19), and a better appreciation of how to use these instruments has emerged. In addition, it is necessary to improve our understanding of how to analyze the data that they furnish. When the categorical nature of the data is realized and the assumptions underlying the normal statistical tests are examined, it is seen that only nonparametric tests can apply. This is not a great restriction, however, because there are many such tests allowing considerable scope for analysis.

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ACCELERATED ONSET OF DECOMPRESSION SICKNESS IN SHEEP AFTER SHORT, DEEP DIVES

C. E. Lehner, D. J. Hei, M. Palta, E. N. Lightfoot, and E. H. Lanphier

Decompression sickness (DCS) takes three major forms: local discomfort, usually in a limb (bends); central nervous system effects (CNS-DCS); and pulmonary DCS (chokes). The risk of serious injury or death from DCS depends on the incidence of CNS-DCS and chokes. Recent findings (1, 2) point to the importance of the dive profile in governing the relative probability of these DCS forms. These animal studies demonstrated that spinal cord involvement among DCS cases became more prevalent after brief, simulated dives (> 4 ATA) without staged decompression than after comparable, longer dives at lower maximum pressures. The influence of dive duration and pressure on the relative proportions of these DCS types presumably reflects different rates of tissue perfusion, tissue gas solubility, and tissue architecture where the formation of bubbles or their presence causes injury.

We questioned whether similar factors also influence the onset time of DCS forms. Eckenhoff and Parker (3) investigated the latent period of bubble formation in human subjects following simulated saturation air dives. They found that increased pressure shortened the latency of Doppler-detected venous bubbles and skin itch (cutaneous DCS). We hypothesized that DCS latency would become shorter as dive duration decreases and maximum dive pressure increases to provoke similar levels of DCS incidence.

The purpose of this study is to examine the relationship of exposure duration, form of DCS, and the latency of DCS signs. Records of previous 0.5- to 24-h simulated air dives in sheep that involved no-stop decompression provided the basis for this evaluation. We analyzed reports of human DCS latency to determine whether the pattern of latency established in sheep also occurs in humans.

METHODS

Mixed-breed sheep were used for 2 series of simulated compressed-air dives in the high pressure chamber of the University of Wisconsin Biotron. Compression was at 25 fsw (0.76 atm)/min. Near-saturation conditions were tested by 24-h dives of 14 sheep with body weights of 79.2 ± 10.6 kg (mean ± SD). Bounce-dive conditions were tested by 0.5-h dives of 10 sheep with body weights of 52.5 ± 26.1 kg. Maximum dive pressures ranged from 51 to 62 fsw (2.54 to 2.88 ATA) in the 24-h series and from 109 to 138 fsw (4.31 to 5.18 ATA) in the 0.5-h series. No-stop decompression was at 60 fsw (1.82 atm)/min. We observed the sheep for signs of DCS for 4 h from the time sheep reached ambient pressure (0.97 ATA). The animals were also examined on the following day.

Significance of differences in the latency of each DCS form was tested by analysis of variance. DCS observations permitted a two-way analysis of variance that tested for DCS latency differences between dive durations and between the three major DCS forms. We tested the unbalanced data with a weighted squares of means analysis equivalent to a type III sums-of-squares procedure (4). Statistical tests were performed with SAS (5) statistical routines.

Decompression responses in sheep were compared with literature reports of DCS latency among divers and tunnel workers. Unfortunately, few reports of human DCS latency distinguish the observed DCS by its various forms so that comparisons were restricted to overall DCS latency. Hyperbaric exposures generally correspond to relatively brief exposures at greater pressures among divers and longer exposures at lower pressures among tunnel workers. Decompression responses in these groups were evaluated by plotting cumulative DCS incidence as a function of time to correlate the type of exposure with DCS latency.

RESULTS

Decompression in sheep produced three major DCS signs. Bends presented with intermittent weight bearing or with sustained lifting of the affected limb. CNS-DCS signs involved the spinal cord and typically presented with transient hindlimb paralysis or quadriplegia. Spinal cord DCS frequently resolved within less than 0.5 h and without apparent sequelae. Chokes was indicated by panting, or by labored breathing, or by other suggestions of dyspnea. Except in 2 cases that terminated in death, signs of chokes disappeared within 3 h. Decompression frequently provoked more than one form of DCS in the same subject. Sheep were recompressed to treat refractory DCS episodes.

Twenty-Four-Hour Dives

In 102, 24-h individual dives the total DCS incidence reached 86.3%. Chokes signs of some degree occurred in 77.3% of all individual dives that resulted in at least one form of DCS. Limb bends resulted in 71.6% and CNS-

DCS in 6.8% of DCS cases.

Half-Hour Dives

In 41, 0.5-h individual dives the total DCS incidence was 36.6%. CNS-DCS incidence increased to 60.0% of the DCS cases. Limb bends fell to 46.7% and chokes declined to 40.0% of DCS cases. CNS-DCS in the 0.5-h individual dives increased nearly 10-fold over its occurrence in the 24-h dives despite a higher DCS incidence in the longer dives.

Latency of Onset

Latency of DCS signs had distributions skewed to the right. These distributions were modified for analysis of variance by logarithmic transformation of the latency times. Transformed latencies showed no significant difference ($P > 0.05$) from the normal distribution by the usual Kolmogorov-Smirnov D statistic.

Decompression sickness latency differences between 0.5- and 24-h dives were highly significant ($P < 0.001$). DCS latencies showed an overall decline from 42.6 min in the 24-h dives to 20.2 min in the 0.5-h dives.

The overall ordering of latency between the three major forms of DCS remained the same in the 0.5- and 24-h dives (Table 1). Average latency was shortest in limb bends and longest in chokes. Latency differences among DCS forms were at a significance level of $0.06 > P > 0.05$.

Table 1
DCS Latency in Sheep After 0.5- and 24-h Air Dives

	Manifestation of DCS		
Dive Duration, h	Limb Bends	CNS	Chokes
0.5	15.9 ± 6.7*	20.4 ± 6.3	24.8 ± 10.0
	8-27**	15-34	10-34
	$n = 7$	$n = 9$	$n = 6$
24	36.5 ± 24.8	44.4 ± 19.1	48.0 ± 26.9
	5-123	14-65	12-120
	$n = 63$	$n = 7$	$n = 68$

* Means ± SD, min; ** Range, min.

Cumulative DCS incidence in sheep plotted as a function of time after the start of decompression shows that more delayed cases of limb bends and chokes follow 24-h compressed air exposures than after 0.5-h exposures (Fig. 1). Interestingly, all CNS-DCS episodes presented within 1.2 h postdecompression (Fig. 1).

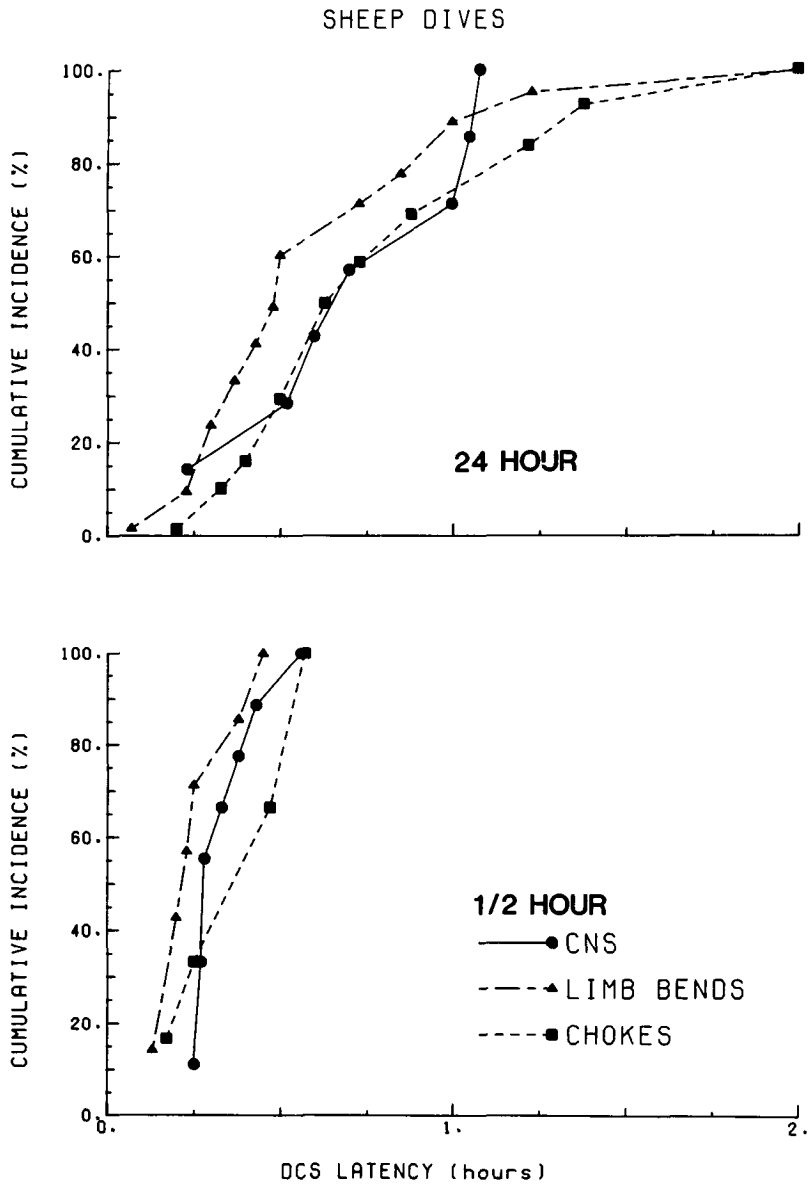

Fig. 1. Decompression sickness latency in sheep decompressed from 0.5- and 24-h hyperbaric exposures.

Human Latency

Decompression sickness latency responses in divers and tunnel workers overlap (Figs. 2 and 3). Variations in DCS latency can be partly attributed to the hyperbaric exposure conditions that also differ within these groups. DCS cases in divers reported by Kizer (6) from Hawaii and Slark's (7) diver population show a more rapid onset than DCS cases among Rivera's (8) population of U.S. Navy divers and those of Van Der Aue et al. (9) that include cases from saturation dives. DCS in tunnel workers reported by Keays (10) often had rapid onsets, and his data (Fig. 3) seem markedly similar to the early onset responses in most of the diver reports (Fig. 2).

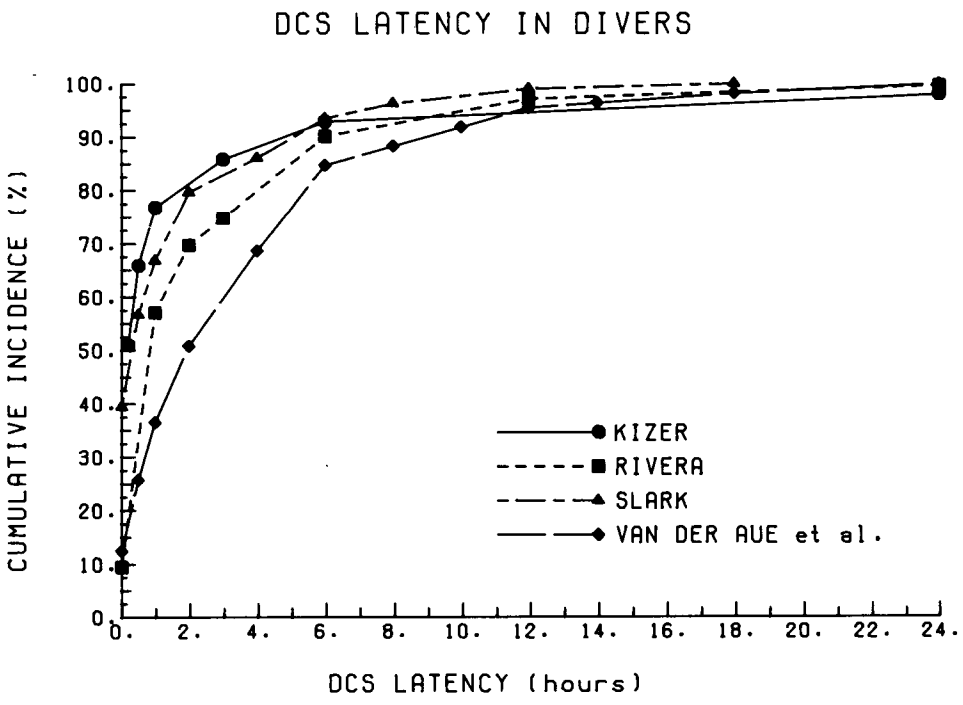

Fig. 2. Decompression sickness latency in divers reported by Kizer (6), Slark (7), Rivera (8), and Van Der Aue et al. (9).

We examined tunnel-worker responses to decompression more closely to assess trends in DCS latency. The 4 tunnel worker studies (10-13) evaluated in this comparison span 50 yr (Fig. 4). The cumulative percentage of DCS cases appearing within 1 and 3 h shows a historic trend with declining numbers of early onset cases in later years. This trend is more apparent in the 1st than in the 3rd h and presumably reflects changes in decompression procedures that provoke fewer DCS cases with a rapid onset.

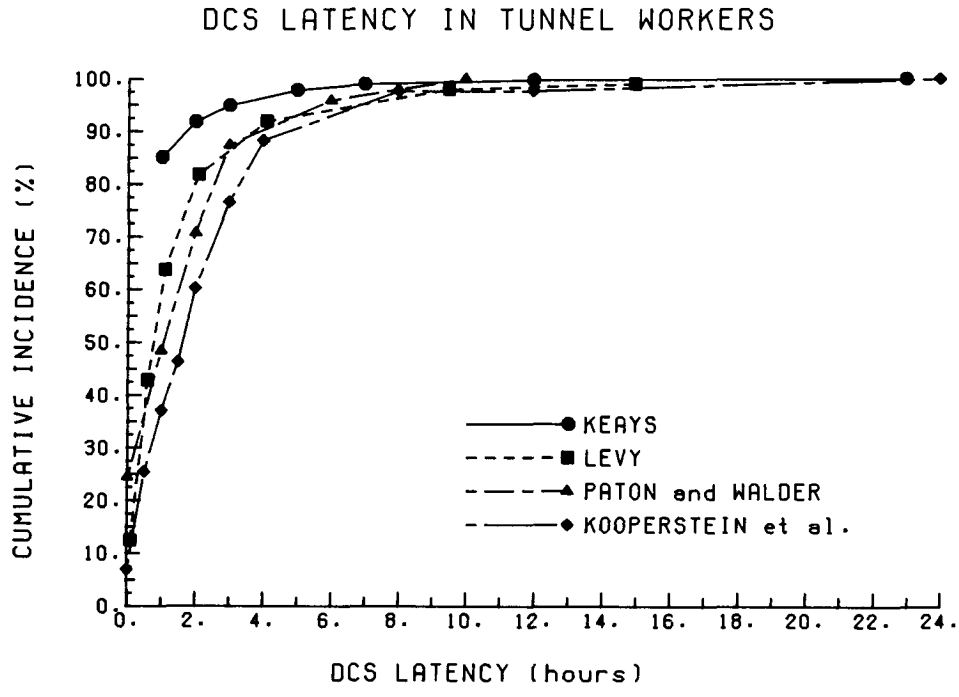

Fig. 3. Decompression sickness latency in tunnel workers reported by Keays (10), Levy (11), Paton and Walder (12), and Kooperstein and Schuman (13).

Hyperbaric Exposure and Latency

Human responses for comparison with sheep responses were selected from representative populations of divers and tunnel workers. The comparison is shown in Fig. 5. These curves of cumulative DCS incidence illustrate the rapidity of DCS signs provoked in sheep as compared with the slower onset of DCS in humans. Kizer's population of open-water divers contains decompression responses to dive profiles characterized by deep, relatively brief excursions. Decompression responses reported by Paton and Walder (12) come from tunnel workers decompressed from longer hyperbaric exposures at moderate pressure, often followed by slow decompression. These decompression responses in divers and tunnel workers come from a continuum of decompression profiles that range from short exposures at relatively high maximum pressures to long exposures at lower maximum pressures. Decompression of sheep and humans produced a similar pattern in DCS latency. Based on the latency values observed at the 50% cumulative DCS incidence, short exposures at greater pressures generated the highest proportion of rapid onset DCS (Fig. 5).

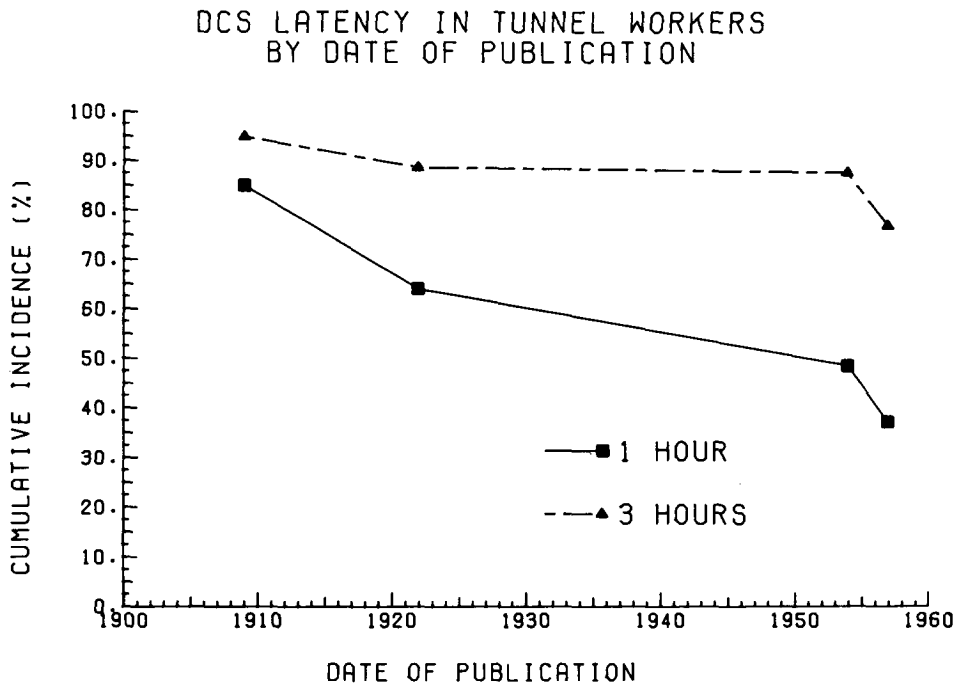

Fig. 4. Historic trend of declining rapid onset DCS at 1 and 3 h among tunnel workers reported by Keays (10), Levy (11), Paton and Walder (12), and Kooperstein and Schuman (13), respectively.

DISCUSSION

When a constant level of DCS incidence is maintained across dive duration, certain forms of DCS become more prominent, depending on the duration of the dive (2). For example, CNS-DCS appears more frequently among short, deep dives sometimes referred to as bounce dives. Conversely, long, shallow dives provoke a higher proportion of limb bends. The question raised by this study concerns the latency in the expression of each form of DCS and the influence of dive profile. Both the expression of DCS forms and their latencies should be closely interrelated because both are presumably controlled by the rates of inert gas uptake and elimination and by bubble effects that characterize various sites of tissue injury.

We found that latency in DCS varies according to the pressure and duration of hyperbaric exposure. Increases in either pressure or duration of hyperbaric exposure followed by direct, no-stop decompression increase the severity of decompression insult. Increased pressure decreases DCS latency after decompression. Eckenhoff and Parker (3) observed that increased exposure pressure, from 2.4 to 3.3 ATA (45 to 75 fsw) reduced the appearance time of pruritus and venous gas bubbles in decompressed humans from about 20

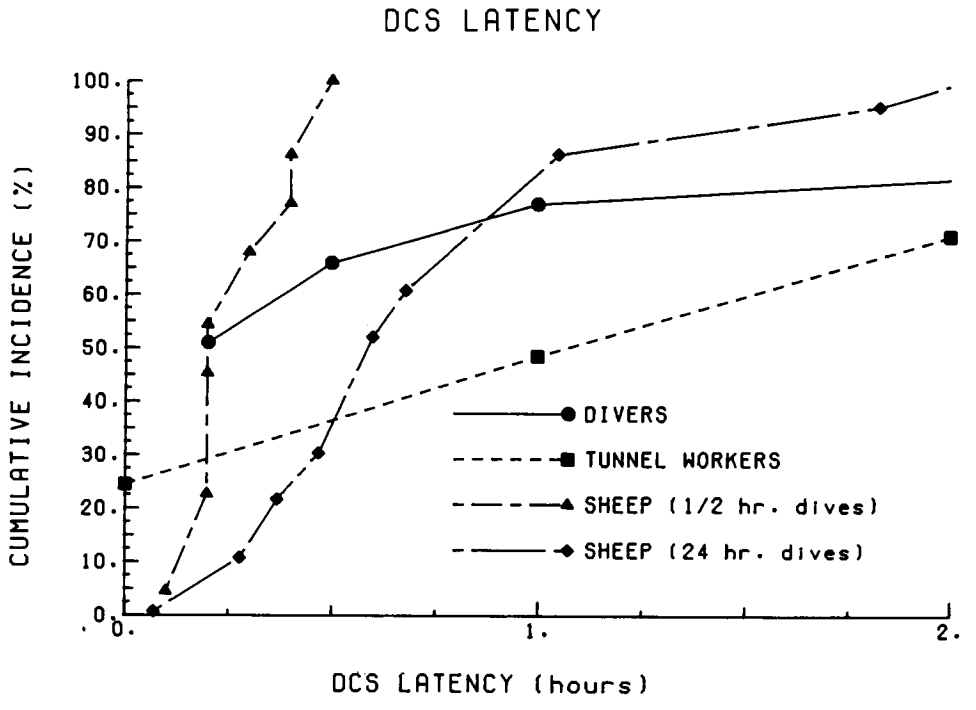

Fig. 5. Decompression sickness latency in humans and sheep during the first 2 h after decompression. Brief hyperbaric exposures provoke a higher percentage of rapid onset DCS in divers and in sheep (0.5 h) than after longer exposures in tunnel workers and in sheep (24 h).

min to 5 to 10 min. Venous gas bubbles are the apparent cause of chokes, and skin itch presumably results from subcutaneous bubble formation.

Decompression responses in sheep after 0.5- and 24-h hyperbaric exposures demonstrated that short exposures at greater pressure provoke DCS more rapidly. This finding should also be interpreted in light of the DCS incidence in these animal dives. The 24-h series provoked a high overall incidence of DCS (86%). Sheep responses in the 0.5-h series resulted in a lower DCS incidence (37%). If exposure pressure in the 0.5-h series had been increased to provoke the same DCS incidence as in the 24-h series, we speculate that DCS latency in the bounce dives would have fallen and thereby increased the latency differences between long and short dives.

Shorter durations of hyperbaric exposure require greater pressures to generate the same incidence of DCS. DCS latency decreases as dives become shorter based on the sheep responses reported in this study. Longer hyperbaric exposures at lower pressures provoke DCS with longer latencies.

Levy (11) in his study of New York tunnel workers observed that less than half of the 436 affected workers reported for treatment in the 1st h. Most studies of tunnel workers follow this pattern with important exceptions where the decompression insult was particularly severe. An example of severe decompression appears in the report by Keays (10) of an early New York tunnel project. Pressure was maintained at 40 psi (3.72 ATA) with a DCS incidence of 1.63% according to Keays. Levy's study (11) of East River tunnel workers in New York reported that pressures were comparably high, but those exposures provoked DCS in only slightly more than 0.1% of the man decompressions. Decompression of tunnel workers reported by Keays was abrupt and lacked the safety provisions for slower decompressions that characterize Levy's conditions and those in the studies by Paton and Walder (12) and Kooperstein and Schuman (13). The responses to abrupt decompression practices characterized in Keays' report show more in common with the fast onset cases of DCS typical in divers. In contrast, saturation dive cases of DCS in the Van Der Aue et al. study (9) represent responses to slow decompression conditions. Saturation dive exposures are similar to long, tunnel worker decompressions and presumably contributed many delayed-onset cases of DCS.

We speculate that slow decompression and the lower maximum pressures in the tunnel-worker studies eliminated most of the rapid DCS onset cases frequently observed in divers and in the no-stop decompressions of sheep. These findings indicate that lengthy hyperbaric exposures characteristic of tunnel work provoke delayed symptoms of DCS corresponding to longer DCS latency in sheep from the 24-h dives. Conversely, short hyperbaric exposures provoke DCS cases with a short latency especially if the decompression is abrupt as in the 0.5-h sheep dives.

Differences between animal and human responses to decompression may involve not only physiologic responses but also the conditions that affect data collection. Under less controlled conditions, human cases of DCS that resolve spontaneously may not be reported. This process will selectively filter and skew the human DCS data. If our experience with sheep DCS is extrapolated to humans, many early DCS cases resolve quickly and spontaneously without treatment. Such early DCS cases in humans could be underestimated. This would lead to more heavily weighting those DCS cases with sustained pain and loss of function. Such an effect could shift the cumulative DCS incidence curves of human responses further to the right and make human DCS latency appear even slower. Mechanisms that affect the decompression response such as allometric scaling (14) and other anatomical differences also probably contribute importantly to the species differences in DCS latency.

Implications of the shortened latency after short, deep dives with no-stop decompression concern submarine escape procedures and surface decompression as well as the clinical presentation of DCS cases. Rate of onset in serious DCS can determine the safety of procedures that require abrupt ascent and brief exposure to surface pressure followed by recompression. Shorter, deeper dives not only provoke a higher incidence of serious DCS but also

accelerate its onset. This indicates that submarine escape procedures, surface decompression, decanting, and any excursion to reduced pressure involve much greater risk of serious injury after short dives than after longer and shallower dives with the same overall DCS incidence.

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DEVELOPMENT OF SATURATION DECOMPRESSION PROCEDURES FOR NITROGEN-OXYGEN AND AIR HABITAT DIVING OPERATIONS

R. E. Peterson and R. W. Hamilton

As part of a development program to provide the U.S. National Oceanic and Atmospheric Administration (NOAA) with procedures for nitrogen-oxygen and air saturation-excursion diving from seabed habitats, saturation decompression schedules have been computed for storage depths from 35 to 150 fsw for the circumstance with no descending excursion history before the saturation ascent, and from 35 to 120 fsw for the circumstance with descending excursion history before the saturation ascent. This paper describes the factors considered and methods used in the preparation of these two sets of tables.

METHODS

One factor of considerable importance in the preparation of the saturation decompression procedures was the wide range in storage depths that had to be considered. While there is a substantial body of nitrox and air saturation decompression experience from scientific operations at shallow depths (1), there is only limited experience in the deeper range. Further, much of the available very-deep experience is highly unsatisfactory from the standpoint of saturation decompression (2, 3). This is in keeping with the principle that decompression calculation parameters which produce acceptable results in some time-depth domains do not produce acceptable results when the depth or, in the case of bounce dives, when the time of exposure is significantly increased (4, 5). Thus, an approach had to be found that could be used to calculate both safe schedules for very deep saturation based on experience from shallower depths, and also efficient schedules for the shallow storage depth range. The approach selected was to use not only an excess nitrogen pressure limit (M-

value) as an ascent constraint, but also to use an excess nitrogen (ΔP)-time integral function. This approach has been employed with satisfactory results to extrapolate from no-stop ascents in one-depth domain to ascents requiring decompression stops in a deeper-depth domain (4, 5). Additionally, the nature of this method is such that decompression schedules from shallower depths will naturally be more efficient than from deeper ones.

To establish ascent constraints for use in the decompression calculations, two saturation decompression procedures with useful track records were selected. One was the Hydro-Lab Schedule for storage at a depth of 42 fsw employed extensively in scientific diving operations (1). The other schedule (Table 1) was one that has been used for final saturation ascents from depths to 115 fsw following a number of construction and equipment testing operations. A summary of the available experience obtained with this schedule and some variants are given in Table 2.

Table 1
Schedule Used as Basis for Computation of Nitrox Saturation Decompression Procedures. Breathing Gas is Air

Depth, fsw	Ascent Rate, min/fsw
165-135	6
135-105	9
105-75	12
75-60	18
60-45	36
45-35	40
35-25	44
25-15	48
15-5	50
5-0	56

For both base schedules, the maximum excess nitrogen pressure and the ΔP-time integral were computed for a series of 16 half-time compartments using ordinary exponential, perfusion-limited inert gas uptake and elimination calculation methods (6). The greater value of each parameter for each compartment was selected to form the set of decompression ascent constraints. These are given in Table 3. The longest half-time, 1205 min, was selected on the basis of a previous analysis of half-times in nitrox saturation decompression procedures (7). The three slow half-times in relatively close proximity, 640, 670, and 720 min, were selected because each has been used for the slowest compartment in other air-nitrox compression computations. The faster

Table 2
Summary of Dives Performed With Schedule
Given in Table 1 and Variants of It

Storage Depth Range, fsw	Number of Dives	Number of Divers	Excursion Depth Range, fsw	Dive Duration Range, d
45-85	3	20	65-112	6-24
98-115	10	57	99-197	5-25

Table 3
Compartment Half-Time and Ascent Constraints Used
For Saturation Decompression Computations

Half-Time, min	Maximum ΔP, fsw	Maximum ΔP-Time Integral, fsw-min
5	0.99	0.48
10	0.99	0.96
20	0.99	1.88
40	1.35	4.11
80	1.81	14.39
120	1.97	25.32
160	2.05	37.02
200	2.10	83.81
240	4.24	487.38
320	9.55	3508.46
480	16.98	20,131.56
640	22.19	41,172.85
670	22.97	45,090.67
720	24.15	51,587.03
960	28.49	82,348.32
1205	32.63	113,319.14

half-times are typical of standard perfusion-limited, exponential nitrogen uptake and elimination computations. In practice, the ascent constraints for the slower compartments came from the longer, deeper schedule, whereas the ascent constraints for the faster compartments came from the shallower Hydro-Lab Schedule. This relationship was maintained in the table calculations as

described below where, though saturation schedules were being derived, the faster compartments had some influence on the formulation of the shallower tables.

To compute the decompression schedules, stop times were computed for holds at 5-fsw intervals starting at the depth 5 fsw shallower than the storage depth. The length of the stops was determined by the inspired oxygen pressure (Po_2) and the value of a constant, K, which relates ascent rate and Po_2 (8). The maximum ΔP for each half-time compartment and the total ΔP-time integral for the decompression were also computed. If any ascent constraint were exceeded, the value of K was reduced and the process repeated until arriving at the fastest schedule satisfying all constraints. The initial value of K was always set so that some ascent constraint would be exceeded. Other considerations in the calculations of the saturation decompression schedules were the ages and general condition of the prospective NOAA divers, the management of oxygen during the decompression, and the management of excursion history before the start of saturation decompression. Because the NOAA scientists could well be older and in poorer physical condition than the relatively fit, young individuals having experience with the deeper of the base schedules, it was considered prudent to introduce some degree of conservatism into the decompression schedule computations. In keeping with the successful NOAA OPS excursion computations (9), an extra nitrogen load was introduced by making the storage P_{N_2} 5 fsw greater than that of the depth for which the computation was being done.

To minimize saturation decompression times and gas composition logistics, air has been used as the decompression gas whenever possible. This approach is limited, however, by exposure to oxygen both during air excursions before commencement of the saturation and during the decompression itself. Prior experience from comparable nitrox saturation-excursion operations gives some indication of acceptable oxygen doses for general application. Following air excursions to 195 fsw from storage at 115 fsw, 6 divers undergoing an air saturation decompression employing oxygen breathing were exposed to a cumulative pulmonary toxic dose (CPTD) (10) of 1420 unit pulmonary toxic dose (UPTD) in about 41 h. Four of these men experienced typical, distinct symptoms of pulmonary oxygen poisoning which were generally resolved over the 1st wk postdive. Following air excursions to 165 fsw from storage at 100 fsw, 2 of 11 divers who underwent saturation decompression on the same schedule as mentioned immediately above noted severe dyspnea upon heavy exercise after reaching surface. These symptoms occurred after exposure to a CPTD of 1180 UPTDs over a period of about 38 h and disappeared over the first 72 h postdecompression. As the other 9 divers did not engage in as strenuous exercise as the 2 who reported symptoms, it was not possible to determine whether more of the men were affected in a similar way. Following air excursions to 195 fsw from storage at 115 fsw, 6 divers experienced no symptoms of pulmonary oxygen poisoning after undergoing an air saturation decompression which exposed them to a CPTD of 920 UPTDs over 27 h.

Based on the above and other experience in which saturation decompression CPTDs of less than 920 UPTDs have not produced pulmonary distress, even though the decompressions were preceded by long, relatively deep air excursions, it was considered reasonable to allow a CPTD of approximately 850 UPTDs for decompressions such as those that have been computed. Thus, whenever the oxygen dose of a schedule computed with air as the breathing gas significantly exceeded this amount, the breathing gas format was changed and the decompression recalculated. The alternative gas format was a fixed Po_2 of 0.6 atm to 60 fsw and air from that depth to the surface.

In the procedure given in Table 1, a significant descending excursion history (i.e., recent excursion PN_2 exceeded maximum storage PN_2) was managed by starting the saturation decompression immediately after the final excursion at a depth dependent on the excursion depth. Thus, the starting depth for saturation decompression could be deeper than the storage depth. While this procedure has proven effective and efficient in dealing with long, deep excursions before saturation ascent, compression to greater than the storage pressure was considered to be undesirable for use with seabed habitats, which are NOAA's primary mode of operation. Thus, another management approach which has the practical advantage of not requiring compression to pressures greater than the storage depth was utilized. This approach was the common one of requiring a holding period at the storage depth following the last significant descending excursion before starting the saturation decompression. Unfortunately, little quantitative and no generally applicable data are available upon which to reliably base the length of an optimal holding period. Thus, to retain operational efficiency in the derived procedures, the calculations were done by first computing the worst-case excursion situation for nitrogen loading in the slowest compartments, then computing the decompression schedule as described following that worst-case sequence of excursions and a holding period of 6 h at the storage depth. In use, however, the required hold at the storage depth following the last significant excursion was to be 12 h to compensate for reduced inert gas elimination should any phase separation occur as a result of an excursion ascent.

RESULTS

The decompression times and K values for the two sets of schedules calculated by the above methods are given in Table 4. The results are as would be expected, with the K values becoming progressively smaller and the decompression times relatively longer as the storage depth increases. Even with the holding period before the start of decompression following the last descending excursion, the decompression times for the significant-descending circumstance are notably longer than for the no-excursion circumstance, particularly for the deeper storage depths. From the standpoint of breathing gas management, the changeover from all air to the fixed-Po_2 and air format occurred at 110 fsw for the no-descending excursion schedules and at 105 fsw

Table 4
Saturation Decompression Times and Calculation Constants

Storage Depth, fsw	Without Excursions		With Excursions	
	Time, min	K	Time, min	K
35	95	5.9414	1180	4.9647
40	1145	5.7851	1325	4.9646
45	1275	5.6536	1485	4.8476
50	1400	5.6267	1645	4.7935
55	1520	5.5605	1800	4.6889
60	1670	5.3954	1935	4.6565
65	1810	5.2574	2065	4.6066
70	1940	5.1739	2175	4.5887
75	2060	5.0931	2290	4.5798
80	2180	5.0015	2465	4.4131
85	2295	4.9517	2665	4.2351
90	2495	4.6935	2820	4.1716
95	2690	4.5207	3000	4.0448
100	2895	4.3282	3215	3.8804
105	3105	4.1679	3645	3.5166
110	3325	3.9784	4460	3.1779
115	3920	3.7664	4960	2.9420
120	4505	3.3820	5440	2.7737
125	5100	3.0816		
130	5750	2.8248		
135	6255	2.6761		
140	6800	2.5308		
145	7335	2.4213		
150	7910	2.3112		

for the significant-descending excursion schedules.

As was intended, the computed schedules are conservative with respect to others used previously for practical air-nitrox operations. In the first preliminary trial, however, ascent from a 50-fsw storage depth following an extensive multiday, repetitive excursion battery (11) resulted in pain-only decompression sickness in 1 diver at 10 fsw. This was considered to be the result of failure of the overall procedure to deal adequately with phase-separated gas caused by the excursion ascents. In addressing this problem, extension of the 12-h holding period was considered to be an inferior solution because there was still no firm basis for selection of the optimal time, because a significant increase in the holding period would greatly reduce operational efficiency, and because

one or several small increases in the holding period would give little assurance of success in the limited trial dives scheduled. Thus, despite the increased operational complexity of compression to depths greater than the storage depth, safety and overall operational efficiency considerations made the approach of basing the starting depth of the saturation decompression on the recent excursion history the most attractive solution. This method was used in 2 subsequent trials from storage at 80 fsw and 110 fsw, both following extensive repetitive excursion programs. In each case, the saturation decompression was started at a pressure just greater than the P_{N_2} of the worst-case air excursion depth, and the decompression stops deeper than the storage depth were taken from the schedule given in Table 1 with air as the breathing gas. Preliminary results from these 2 dives confirm that such saturation decompressions with an initial recompression produce safe ascents and, overall, they required only the same time periods as the initial approach, including the 12-h holds.

CONCLUSIONS

A set of saturation decompression procedures providing for a relative increase in efficiency as the storage depth decreases has been formulated. With adjustments to facilitate starting deeper than the storage depth following significant descending excursions, the procedures have proven adequate in a limited trial series for further utilization in a careful program of field evaluation.

The method of utilizing a ΔP-time relationship as an ascent constraint for calculating saturation decompression procedures in different depth domains from those that form the basis of the calculations has practical merit. An acceptable approach for coping with a no-stop excursion ascent and a holding period at the storage depth before the start of saturation decompression has not yet been developed within this method.

It seems to be more efficient operationally, and fully compatible with the calculation method described, to start the saturation decompression immediately at a depth dependent on the excursion history, rather than employing a postexcursion holding period at the storage depth before commencing saturation decompression. The basis for this is believed to be the prevention or minimization of phase separation of gas taken into solution during the excursions.

An oxygen dose with a CPTD in the 800 to 900 UPTD range has proven to be acceptable for air saturation decompression from nitrox storage, even when preceded by an excursion program presenting significant oxygen exposure in its own right.

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PROCEDURES FOR TRIMIX SCUBA DIVES BETWEEN 70 AND 100 M: A STUDY ON THE CORAL GATHERERS OF THE MEDITERRANEAN SEA

D. Zannini and L. Magno

The rocky depths of some zones of the Mediterranean Sea are covered with red and pink coral (*Corallium rubrum*) that lives on reefs 35 to 150 m deep. In the course of ages, the calcareous boughs of this Antozoo have been gathered by coral-gatherers (Corallari) and made into jewelry. In the past, coral gathering was done by means of nets or other trawling devices (ingegni), but in recent times the diffusion of scuba has stimulated the desire to gather this precious product with one's own hands and to reach ever increasing depths.

Ten years ago (and unfortunately for some gatherers today) coral gathering with autonomous equipment was performed by breathing compressed air, even at 100 m depth, and by using decompression procedures that were quite arbitrary and empiric. In these procedures the danger of nitrogen narcosis was in some cases warded off by experience and self-control of the individual diver, but in all cases the time of diving was limited to a few minutes. Decompression sickness (Type II) or gas embolism very often caused serious casualties, some resulting in death. Sudden death that occurred in the sea bottom or near the surface was attributed to a mysterious source and was called "White Death."

In the 70s, with the use of heliox mixtures for commercial diving work, coral gatherers learned that by using them narcosis and some serious DCS problems could be warded off, and they adopted these techniques to reach longer times and greater depths, thus making their activity more profitable. From this practice, however, a new series of problems have arisen, especially during dives performed freely by one individual because he had to face: a) the cost and the scarce availability of the mixture; b) the storage of large bottles of helium on small boats; c) the heat loss caused by helium; d) an increased

amount of the helium to be carried in larger and heavier bottles for gatherings beyond 100 m with more than 20 min bottom time.

In the last few years, however, owing to the changed ecologic conditions and to the difficulties cited above, the advantages of gathering beyond 100 m have been greatly reduced. Consequently, on the basis of past experience in the use of heliox mixtures, coral gatherers now prefer to gather between 70 and 100 m, using procedures based on trimix (helium+nitrogen+oxygen), a mixture that has proved to be more economic and less taxing on thermal balance.

PURPOSE OF THE STUDY

The purpose of our study was to verify the possible advantages of the use of trimix and to prove the reliability of the decompression procedures adopted in bounce diving between 70 and 100 m, in particular where surface decompression and the use of oxygen are implied.

MATERIALS AND METHODS

For 2 yr, we have been following the dives of 10 coral gatherers who used a trimix mixture obtained by mixing 50% air and 50% He (resulting in: He 50%:N_2 40%:O_2 10%). Four divers used our trimix tables, 2 used heliox commercial tables (COMEX, etc.), and 4 used self-adapted tables. We have called this experiment: "Coral trimix procedure" or "Coral Trip."

Description of the Dive

In a standard procedure the dive is performed by 1 diver not connected with the surface. The standard duration is 15 to 20 min with a maximum of 30 min. The dive is executed with the usual equipment: cellular neoprene (6-7 mm) wet suit, wet socks, gloves, flippers, mask, regulator (demand valve), and a three-bottle scuba of about 40 liter pressurized to 220 atm and thus subdivided: 2 × 15-liter bottles containing trimix; 1 × 10-liter bottle containing air.

The diver starts the immersion breathing air until he reaches 10 m (he is obliged to do so because trimix is not physiologic within 10 m owing to its low percent of oxygen). Then he begins breathing trimix and after he has reached the bottom, starts gathering with a small spade and a basket. When the work is over, he starts the ascent at the established speed, and after reaching the first decompression stop he begins again to breathe air. He lets the warm water get in through the collar or sleeve of his wet suit to provide warmth. As soon as he reaches 12 m he breathes oxygen, O_2. He stops for 5 to 10 min at 9 m, and during this stay he gets rid of his equipment, with the exception of the wet suit. Then he rapidly reaches the surface, gets on the boat, and lets himself be compressed in a monoplace chamber where he performs the 9-m decompression stop and those that follow, breathing air or oxygen.

Criteria Utilized to Compute Decompression Tables

The "Coral Trip" is based on tables developed by us for this purpose (Table 1), which can be utilized both for water and surface decompression. The jump into the chamber from the 9-m stay has been suggested by us; but must be executed within 3 min and must not exceed 5 min. The tables have been calculated by means of an algorithm derived from the Schreiner-Kelley formula for linear and stepwise decompressions. According to this formula, the tissues implied are 16 with half-times different for helium and nitrogen. Saturation times are also different from those of desaturation for the same tissue and vary according to the depth of the dives. The same difference can be found for ΔM values. The speed of ascent from the bottom to the first step is 10 m/min (with this speed the first 5-6 tissues do not play a role in the calculation of the tables). The stops in O_2 are calculated, considering inert gas equal to 0 without any further lengthening of the desaturation times. The tables developed are similar to the USN He-O_2 table (surface-supplied decompression tables 1971) (Fig. 1). The whole set is formed by tables for decompression in O_2 or in air, and a table with a shortened decompression for emergency is added for use when contact is lost with supply vessels.

Table 1
Percentage of Decompression Sickness After Dives with Trimix Using Different Types of Decompression Tables

Tables	No. of Divers	No. of Dives	Decompression Sickness	
			Type I, %	Type II
Trimix	4	860	-	-
Heliox	2	570	0.17	-
Self-adapted	4	776	4.12	-
Total	10	2206	1.49	-

RESULTS

Our information shows that when trimix is used, it is sufficient to use warm water during the decompression stops in a humid environment to balance the heat loss. Even after 30 min of immersion at 100 m, neither distracting attention nor degradation of performance were found, suggesting that no evidence of nitrogen narcosis was present.

During the work (medium light) no breathing difficulties occurred while breathing dense gas. Results shown in Table 1 demonstrate that on a total of 2206 dives, decompression sickness of Type II has never occurred, and the number of bends was between 0 and 4.12 %, depending on the procedure used. No bends occurred in subjects who used trimix tables. Moreover, it appears

that divers who used heliox experienced no increased rate of decompression sickness, whereas those who used empiric tables or tables empirically modified, shortened or lengthened by themselves, had a higher number of casualties.

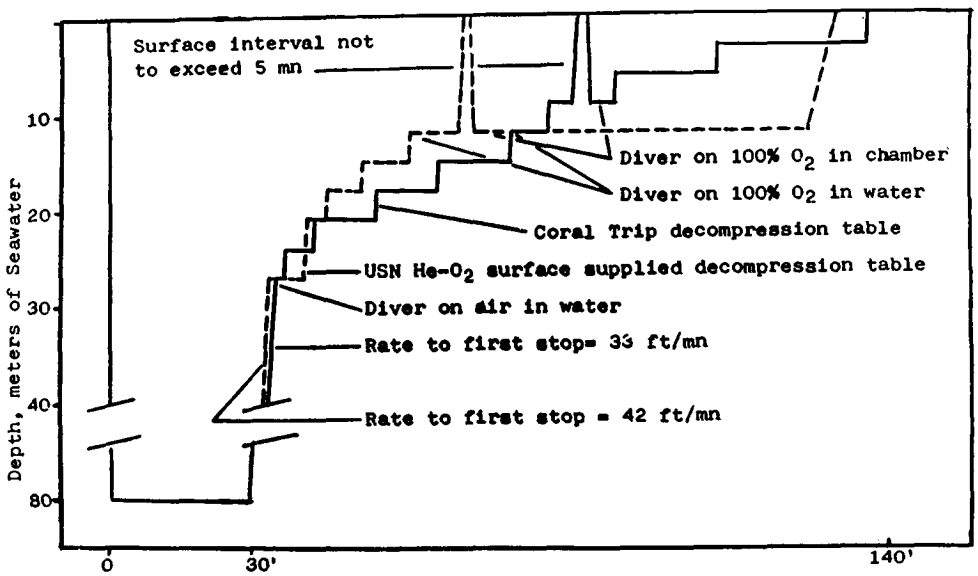

Fig. 1. Profile of trimix "Coral Trip" table for 80 m/30 min bottom time compared to USN He-O_2 surface supplied decompression table with 85% He:15% O_2.

DISCUSSION

From this experience, we assume that for bounce dives at no more than 100 m depth, trimix can be utilized for commercial diving work without any inconvenience and with good performance in medium-light work. The high percentage of nitrogen does not seem to modify substantially the decompression profiles in comparison with those of heliox mixtures. The advantages observed in the use of trimix for depths ranging between 70 and 100 m encourage us to extend the research to verify whether, with possible modifications in the percentage of air, trimix can be used for dives over 100 m.

Acknowledgment

We acknowledge the unique and courageous work of the Mediterranean coral gatherers whose contributions have assisted importantly in advancing scientific knowledge of underwater activities.

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LATE MANIFESTATION OF SPINAL CORD LESIONS IN DECOMPRESSION SICKNESS: HISTOPATHOLOGIC ANALYSIS OF AN AUTOPSY CASE

M. Kitano, K. Hayashi, M. Kawashima, S. Tokufuji, and A. Yamaguchi

Many cases of decompression sickness (DCS) with spinal cord damage have been reported in the medical literature (1-5). The pathogenesis of spinal cord damage in DCS remains a subject of controversy. The conventional view ascribes tissue damage to arterial bubble embolization with consequent obstruction of arterioles and capillaries (1, 2). Another view describes the venous obstruction leading to venous infarction as the major cause of spinal cord damage (3). Our previous reports (4, 5) described autopsy findings of cases with acute decompression sickness accompanied by acute spinal cord lesions that were limited to the white matter of the spinal cord, the acute damage of the spinal cord being intimately associated with venous thrombosis of the spinal cord parenchyma and widespread blood coagulation with and without fat embolism of the epidural veins. We concluded that the spinal cord damage was caused by severe retardation of venous flow.

Literature describing late manifestation of the spinal cord lesion is very rare (6, 7). It would be important to compare the findings of delayed changes in the spinal cord with those of early changes represented in the literature for discussing the pathogenesis of the spinal cord lesions in decompression sickness.

This paper describes pathologic findings in a spinal cord in an autopsy case who had suffered spinal cord damage from decompression sickness about 15 yr before death. The purposes of this study were to a) compare the findings of delayed changes with those of early changes represented in the literature, and b) to discuss the etiology of spinal cord damage in decompression sickness.

CASE REPORT

A 52-yr-old male died of malignant fibrous histiocytoma which developed at the site of bone infarction in the right femur (8). From 16 to 37 yr of age, he worked as a helmet diver and had two episodes of diver's disease. Conventional recompression treatment, called "Fukashi" among Japanese divers, was very effective, and complete remission occurred the first time at age 32 yr. However, during the second episode of decompression sickness at 37 yr of age, he suffered from spastic paraplegia in the lower extremities with gait disturbance, hypesthesia of the lower half of the body, and vesicorectal disturbances, after various medical treatments including recompression therapy based on U.S. Navy programs. He subseqently received bone implantation to the right femoral neck and head at age 41 and to the left humeral head at age 42, because of bone infarction.

Neurologic examinations done approximately 2 yr before death revealed no evidence of mental impairment and cranial nerve dysfunction except for muscle weakness in both legs, with moderate gait disturbance and muscle weakness in the left upper limb. Muscle stretch reflexes in the 4 limbs were exaggerated, but pathologic reflexes were not elicited. Sensory tests disclosed hypesthesia of all modalities up to the lever of T-7 dermatome.

At about 1.5 yr before death, an amputation of the right thigh with subsequent chemotherapy was done because of the malignant bone tumor of the distal half of the right femur (8). The cause of death was wide metastasis of the tumor to various organs.

PATHOLOGY OF THE SPINAL CORD

The autopsy was routine. The transverse sections of the spinal cord were prepared from each segmental level and were stained with hematoxylin and eosin (H & E) and special stains including Klüver-Barrera, Bodian's silver impregnation method, PTAH, Azan-Mallory, elastica-Van Gieson, Fontana-Masson's silver impregnation method for melanin, and Berlin-blue reaction for iron.

Histopathologic examinations disclosed marked degenerative changes of the spinal cord (Fig. 1). The bilateral lateral funiculi were more or less markedly damaged from C-5 to the sacral segments where the lateral corticospinal tracts were mainly affected (Figs. 2-5).

At C-4 level, although the right lateral funiculus was almost intact, the left lateral funiculus revealed a considerably marked tissue injury (Figs. 6 and 7). The left lateral funiculus showed extensive degeneration, with associated gliosis intermingled with several small cystic spaces containing serous fluid (Figs. 8 and 9) and many small blood vessels possessing thickened walls with cicatrization of the perivascular layers (Fig. 10). Slight aggregation of the melanin-granule-laden cells was found in the fibrotic perivascular layers (Fig. 11).

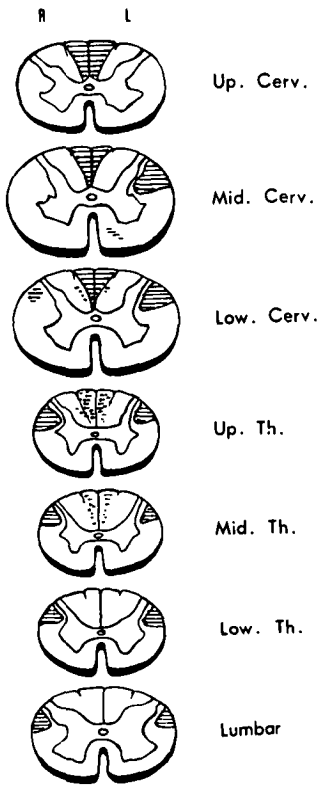

Fig. 1. Schematic diagram showing localization of lesions in the spinal cord.

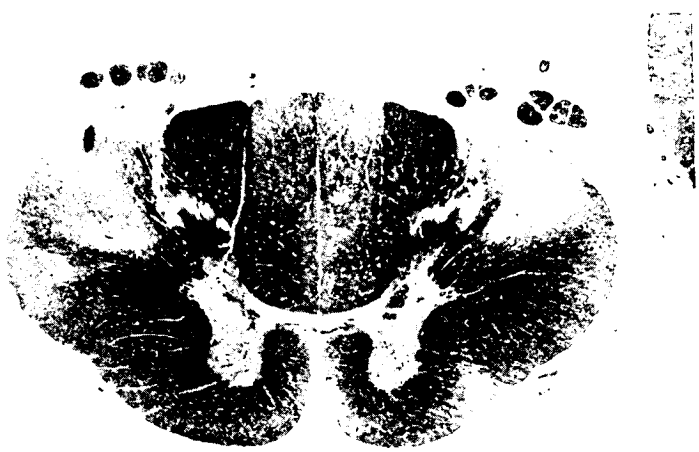

Fig. 2. Transverse section of the spinal cord at T-4 level. Secondary degeneration of the both lateral and posterior *funiculi* is observed. Klüver-Barrera. ×11.

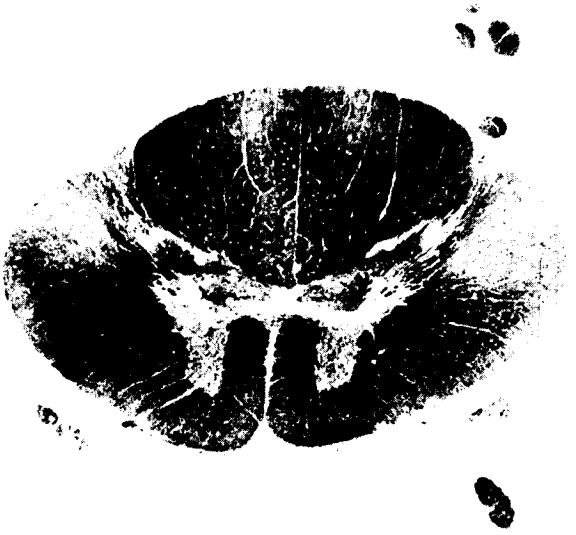

Fig. 3. T-7 Segment of the spinal cord. Klüver-Barrera. ×11.

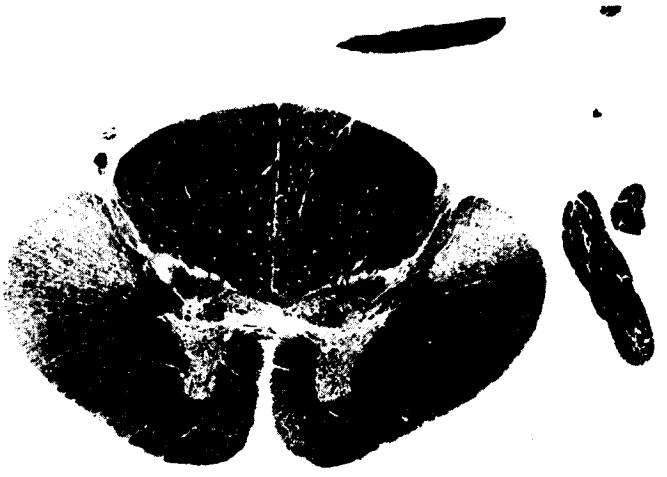

Fig. 4. T-10 Segment of the spinal cord. Klüver-Barrera. ×11.

Degeneration of the bilateral posterior funiculi, especially in the gracil fasciculi, was noted from T-7 to C-1. Small foci of degeneration were occasionally found in the anterior funiculi. The gray matter was almost normal in all segments (Fig. 12).

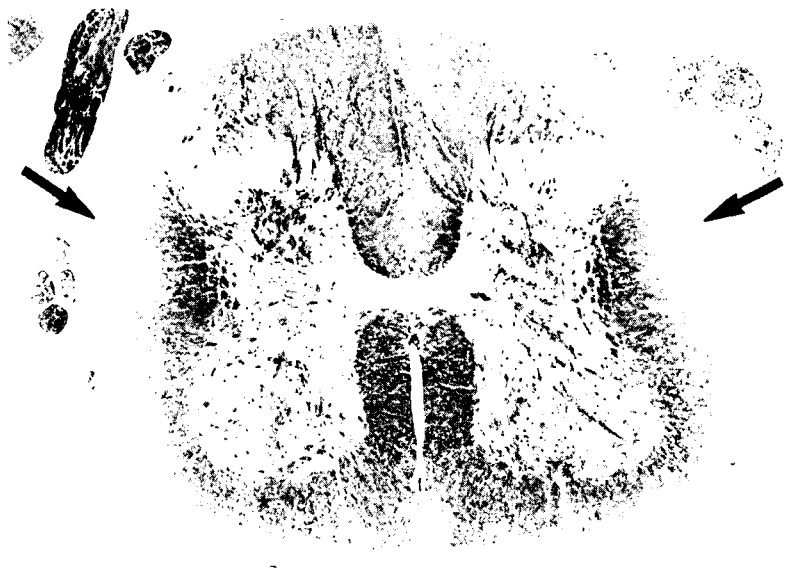

Fig. 5. Lumbar spinal cord. Secondary degeneration is noted only in the bilateral lateral funiculi (arrows). Klüver-Barrera. ×11.

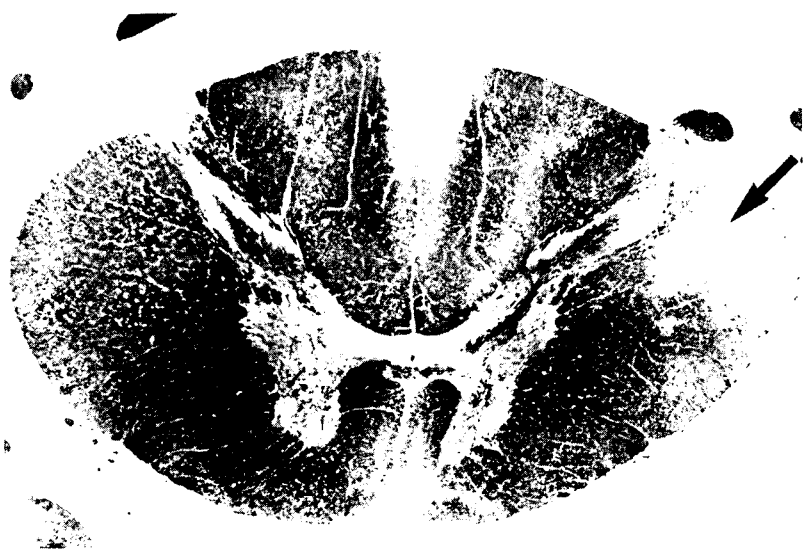

Fig. 6. C-4 segment of the spinal cord. The left funiculus (arrow) shows degenerative changes whereas the right is almost intact. Both the right and left posterior, and the left anterior funiculi are involved also in the degenerative changes. Klüver-Barrera. ×11.

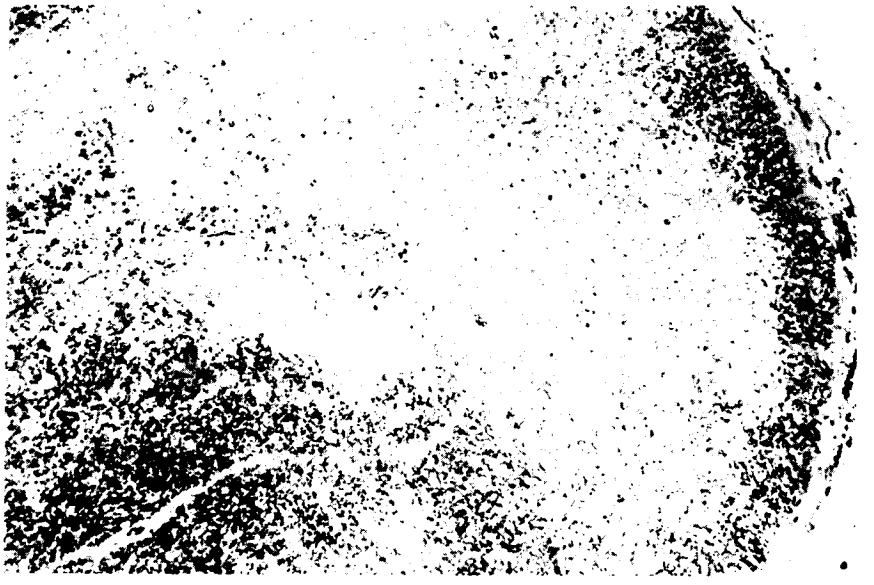

Fig. 7. The left lateral funiculus of C-4 segment. Higher magnified photo of Fig. 6. Klüver-Barrera. ×80.

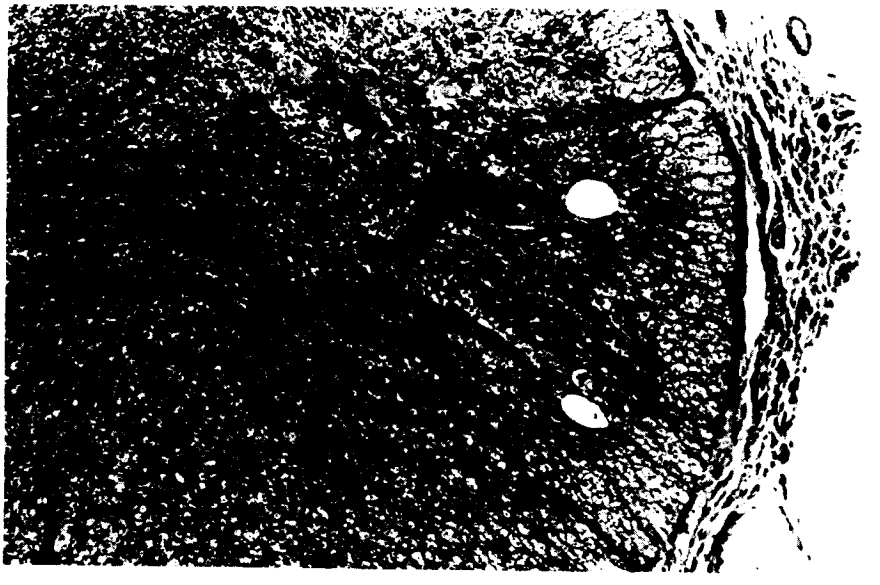

Fig. 8. The left lateral funiculus of C-4 possessing small cystic spaces. The cystic spaces are surrounded by gliotic tissue. PTAH. ×80.

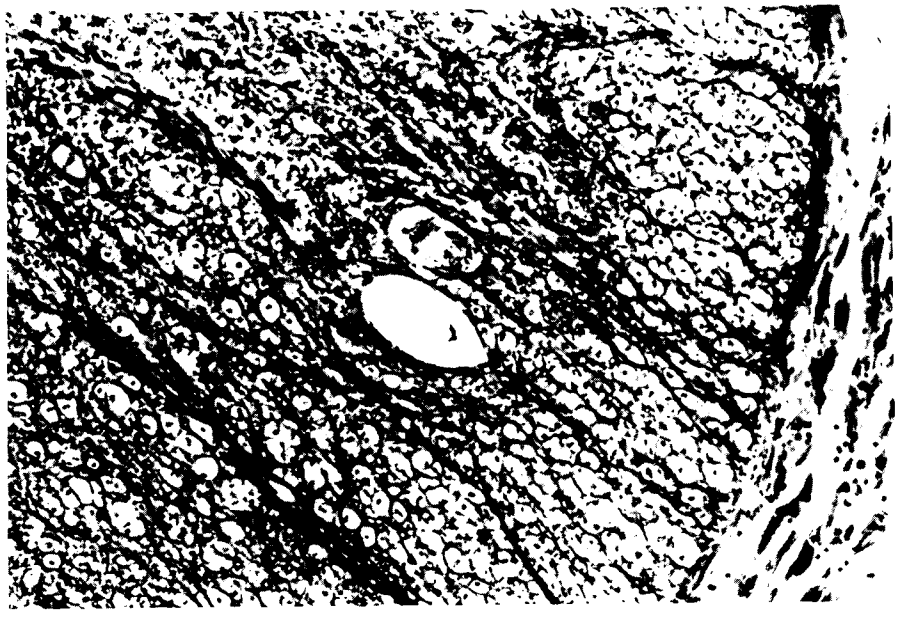

Fig. 9. Higher magnified photo of Fig. 8. The cystic spaces contain serous fluid. PTAH. ×200.

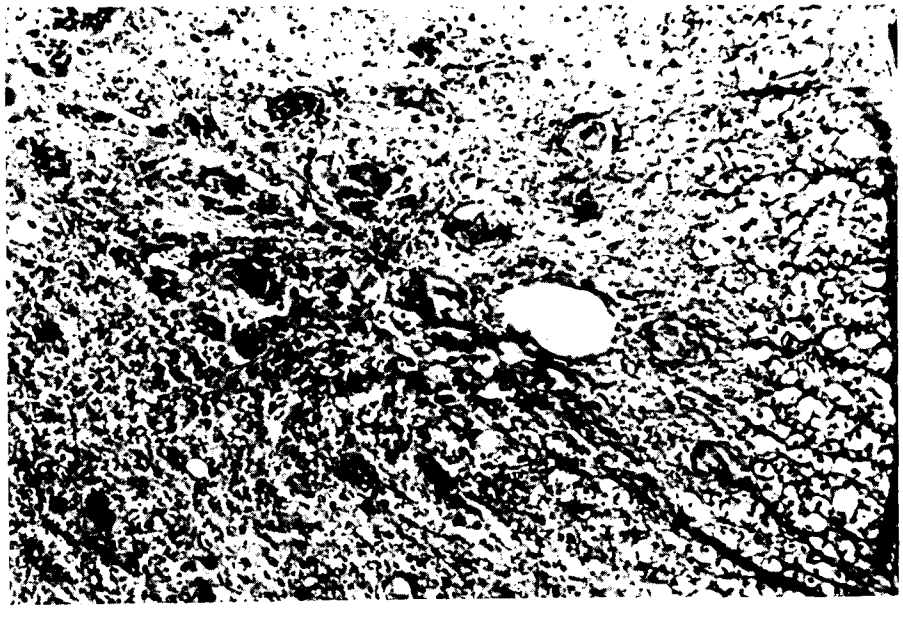

Fig. 10. The left lateral funiculus of C-4 segment intermingled with many blood vessels showing fibrous thickening of their walls and cicatrization of their perivascular layers. Azan-Mallory. ×80.

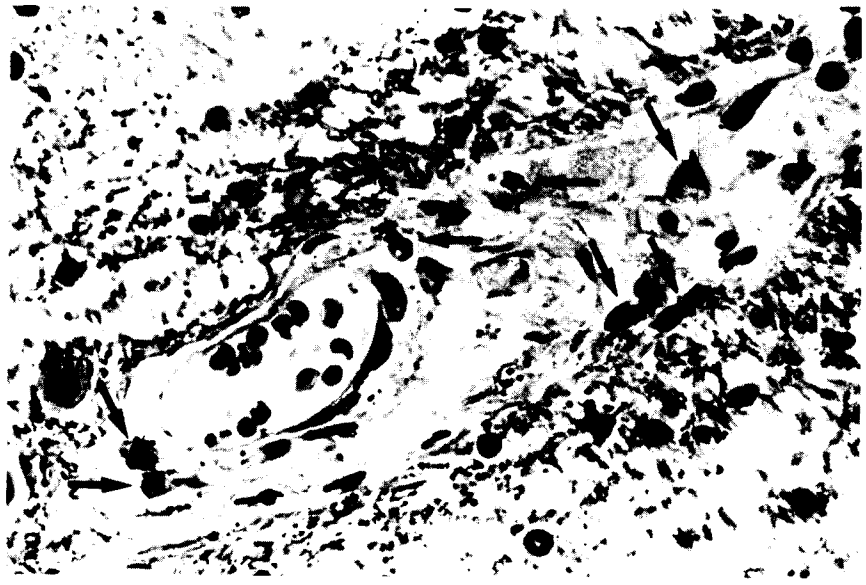

Fig. 11. A blood vessel with a thickened wall in the left lateral funiculus of C-4. Cells containing yellowish-brown granules (arrows), which show a positive reaction to Fontana-Masson's silver impregnation method for melanin, are in the cicatrized perivascular layer. H & E. ×800.

Fig. 12. Anterior horn of T-4. The gray matter is almost intact in the full length of the spinal cord. H & E. ×200.

DISCUSSION AND CONCLUSION

In this case, distribution of the lesions in the spinal cord was similar to other reported cases of acute decompression sickness (1, 2, 4) and seemed quite compatible with the clinical symptoms.

Only 2 autopsy cases describing late manifestation of spinal cord lesions associated with decompression sickness have been reported. Palmer et al. (6) reported a case in which death occurred approximately 3.5 yr after the onset of diver's disease. Lichtenstein and Zeitlin (7) reported on a case of 25-yr duration from the onset of caisson disease. The autopsies of the 2 cases disclosed that secondary degeneration of the ascending and descending tracts was the main event in the spinal cords. The present case also demonstrated that the main finding was secondary degeneration of all three funiculi, especially of the lateral and posterior funiculi, bilaterally.

In the C-4 segment, however, there were asymmetrical changes in the lateral funiculi, where the left lateral funiculus was more severely affected than the right. The left lateral funiculus of C-4 showed some characteristic changes in addition to the secondary degeneration, i.e., fibrous thickening of the walls of the blood vessels. We could not confirm whether most of these vessels were arterial or venous. Other characteristic changes were fibrosis of the perivascular spaces with aggregation of melanin-granule-laden cells, and formation of small cystic spaces with surrounding gliosis. It seemed appropriate to consider that necrotic changes preceded these changes in this area. Haymaker (1) and others (2, 4) previously stated that necrosis was found frequently in the spinal cord during the acute stage of decompression sickness.

Our previous reports described 4 autopsy cases of acute decompression sickness who died less than 15 d after the onset of diver's disease (4, 5). Two of the 4 cases showed marked necrotic changes in the spinal cords with the necrotic foci limited to the white matter, particularly in the lateral and posterior funiculi. Widespread thrombosis was associated and found in and around the necrotic foci and in the epidural veins. Therefore, based on a) the gray matter was not affected and b) widespread venous thrombosis existed, we hypothesized that the main cause of the spinal cord injury was a marked retardation of the venous return from the spinal cord parenchyma.

Although the histologic analysis failed to identify the affected blood vessels as arterial or venous or to find evidence of old thrombosis, the spinal cord damage observed in this case should have a close relationship to the changes occurring in the blood vessels. It seems reasonable to conclude that marked disturbance of the venous flow was the main cause of the spinal cord damage, because neuropathologic changes were confined to the white matter of the spinal cord, including the C-4 segment.

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AUDITORY AND VESTIBULAR DISORDERS DUE TO BAROTRAUMA

T. Nakashima, H. Yokoi, M. Ito, Y. Watanabe, M. Sato, and N. Yanagita

Rapid and large variations of atmospheric pressure occasionally cause aural barotrauma triggering vascular engorgement, bleeding, and formation of exudate in the middle ear, which results in barotitis media. Inner ear barotrauma causing perceptive hearing loss or vertigo has also been reported (1, 2). Recently, many clinical reports have appeared regarding round and oval window ruptures after atmospheric pressure change (3, 4). The purpose of this experimental study was to investigate both auditory and vestibular disorders due to barotrauma.

MATERIALS AND METHODS

Subjects for this study included 12 experimental white guinea pigs weighing 150 to 300 g. The animals were put in a pressure chamber in which pressurized oxygen infusion elevated the atmospheric pressure up to 2 ATA in 3 min and maintained it at that pressure for 10 min. Then the chamber gas outlet was opened to decrease the atmospheric pressure from 2 to 1 ATA in 30 s. At the pressure level of 2 ATA, the volume percent of oxygen and nitrogen was about 60 and 40, respectively. The chamber had two windows through which the movements of the animals could be observed. The direction and duration of the spontaneous nystagmus caused by the pressure change were investigated. Before and after the exposure to the pressure change, Preyer's reflex (acoustic pinna reflex), auditory brainstem response (ABR), and rotatory test were performed in 12 animals. These measurements were performed between 24 and 48 h after the exposure.

The threshold of the Preyer's reflex was measured using a PA audiometer (Nagashima). The audiometer was set up for the evaluation of Preyer's reflex and the animals were placed in the testing box. A speaker was mounted on top

of the testing box. The stimulus was a 500-ms tone burst, and the intensity was increased until the clear movement of the pinna was obtained in both right and left ears. Moreover, the size of the movements of the right and left pinnas were compared at more intense stimuli.

For recording the ABR, the animals were anesthetized with 30 mg/kg Nembutal i.p. All animals tolerated well this temporary restraint. Needle electrodes were inserted s.c. at the vertex and at subauricular regions near both external auditory canals. The animals were stimulated monaurally, and the results from both ears were evaluated. The positive electrode was placed at the vertex, the negative one at the stimulated ear, and the one for reference at the contralateral ear. As for the auditory stimulus, clicks were presented at a rate of 1/75 ms while masking the contralateral ear with noise. The responses to 1024 stimuli were averaged by a signal processor (Sanei 7T17). The threshold and the latency of the ABR were evaluated.

In rotatory tests, electronystagmograms were obtained without anesthesia, using an electric turntable. After the animals were wrapped and their eyes covered to avoid visual suppression, electronystagmograms were obtained using needle electrodes inserted near the external canthi of both eyes. Postrotatory nystagmus was inducted by abruptly stopping the turntable after turning the animals clockwise or counterclockwise. The speed of the rotations was 6 or 8 revolutions in 20 s. The rotatory test was repeated 4 times at intervals under the same speed at clockwise and counterclockwise rotations. The number and duration of nystagmus during and after rotation were evaluated.

Forty-eight hours after the exposure to pressure change, the animals were deeply anesthetized and decapitated. Then both right and left tympanic bullas were removed and opened. The state of the middle ear including tympanic membrane and inner ear windows was observed using an operating microscope. Four ears of 2 animals were investigated serially using hematoxylin and eosin staining. The other inner ears were analyzed by surface preparation technique after fixation and decalcification. The round window membrane was evaluated from both outside and inside the membrane.

RESULTS

After the atmospheric pressure change, spontaneous nystagmus occurred in 8 out of 12 animals. The animals that had spontaneous nystagmus turned their heads and rotated toward the direction of the slow component of the nystagmus (Fig. 1). The directions of the nystagmus were almost horizontal but in 2 cases a rotatory component was clearly observed. The duration of the nystagmus ranged from 30 s to 6 min. Under these experimental conditions the spontaneous nystagmus began during decompression. Auditory dysfunction, investigated by ABR and Preyer's reflex, appeared in animals that had spontaneous nystagmus but not in those that did not. The state of the auditory dysfunction was different between the right and the left ears. These results are summarized in Table 1.

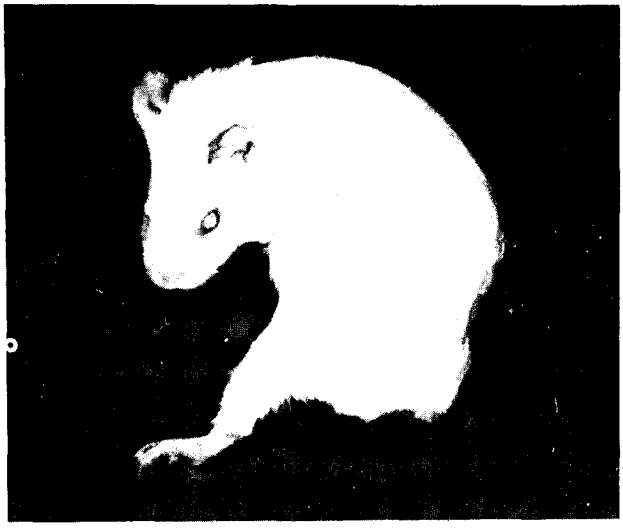

Fig. 1. A guinea pig that had spontaneous nystagmus during the atmospheric pressure change. This animal showed horizontal nystagmus to the right and rotated in the counterclockwise direction.

With regard to the wave form of the ABR, waves I, II, III, and IV were clear; wave V was not clear (Fig. 2). The elevation of the threshold of ABR was recognized in 11 ears as shown in Table 1. Unilateral change of ABR was recognized in 5 and bilateral change of ABR in 3 out of 8 animals that had spontaneous nystagmus. The auditory dysfunction was more evident in the ear toward which the slow component of the spontaneous nystagmus moved. The latencies of the waves were prolonged in ears that showed the elevation of the threshold. In 13 ears that did not show the elevation of the threshold, the latencies were the same as those before the exposure.

A significant correlation occurred between the results of ABR and Preyer's reflex. Table 1 also shows the change of Preyer's reflex. The elevation of the threshold of Preyer's reflex was observed in the ears where the change of ABR was recognized. Two animals showed total disappearance of Preyer's reflex at both right and left pinnas. In 6 animals, the difference between the sizes of the movements of the right and left pinnas was observed in Preyer's reflex. The movement of the pinna due to Preyer's reflex was weaker on one side where the elevation of the threshold of ABR was stronger compared to the contralateral side.

Regarding the rotatory test, Fig. 3 shows an example of an electronystagmogram during and after rotation. Immediately after rotation, nystagmus began and was followed by a no-nystagmus period. After stopping the turntable, postrotatory nystagmus appeared in the opposite direction. Except for 1 animal, the number and duration of the nystagmus did not change significantly by the exposure to pressure change. In comparing the number and duration of

Table 1
Effects of Alterations of Atmospheric Pressure

Animal Number	Occurrence of Spontaneous Nystagmus		Elevation of Threshold of ABR, dB	Elevation of Threshold of Preyer's Reflex
1	-	right ear	0	-
		left ear	0	-
2	+	right ear	15	+
	(to the right)*	left ear	40	++
3	-	right ear	0	-
		left ear	0	-
4	+	right ear	0	+
	(to the right)	left ear	30	++
5	+	right ear	0	+
	(to the right)	left ear	30	++
6	+	right ear	0	+
	(to the right)	left ear	30	++
7	+	right ear	5	+
	(to the left)	left ear	0	-
8	+	right ear	70	+++
	(to the left)	left ear	40	+++
9	+	right ear	10	+
	(to the left)	left ear	0	-
10	-	right ear	0	-
		left ear	0	-
11	+	right ear	30	+++
	(to the left)	left ear	25	+++
12	-	right ear	0	-
		left ear	0	-

* Direction of the fast components are in parentheses.

In elevation of theshold of Preyer's reflex, + = elevation of more than 4 dB in the average of the elevation in 8000, 10,000, and 12,000 Hz; ++ = weaker movement of the pinna than the contralateral one; +++ = total disappearance of the movement of the pinna in Preyer's reflex.

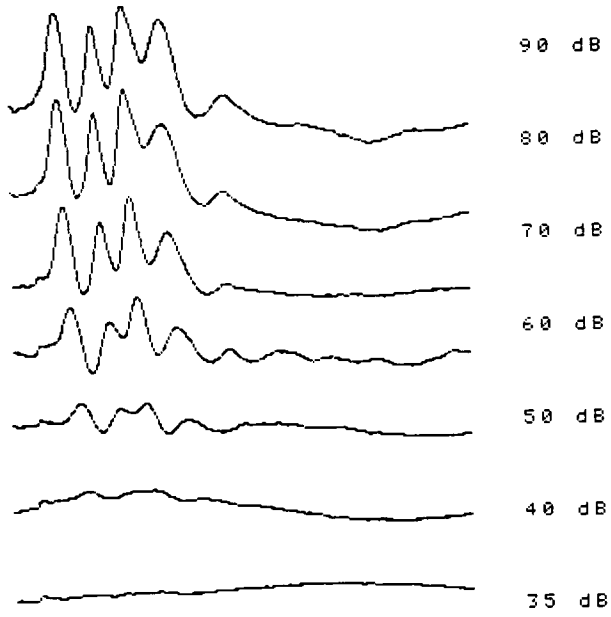

Fig. 2. An example of ABR after the exposure to the pressure change. The threshold of this ABR is 40 dB.

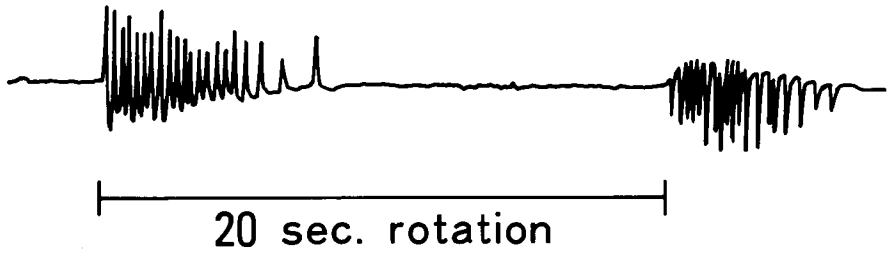

Fig. 3. Electronystagmogram during and after the rotation. The period of rotation indicated by horizontal line was 20 s.

nystagmus from clockwise rotation to those from counterclockwise rotation, 1 animal showed right directional preponderance and 1 animal showed left directional preponderance both before and after exposure to the pressure change. The remaining 10 animals showed no directional preponderance either before or after the exposure.

Various degrees of hemorrhage in the middle ear space were observed in 21 out of 24 ears. There was a tendency for auditory dysfunction to be greater in the ears that had more hemorrhage in the middle ear space, but there was no clear correlation between middle ear hemorrhage and auditory dysfunction. For example, the right ear of animal 9 which had only 10-dB elevation of the threshold of ABR as shown in Table 1, had the most severe hemorrhage in the middle ear space.

Inner ear hemorrhage was observed in most ears, although the amount of hemorrhage was less than that of the middle ear. Blood was commonly demonstrated in the scala tympani of the basal turn close to the round window. Hemorrhage in the round window membrane was observed in more than half of the ears. Findings attributed to the rupture of the round window membrane were recognized in four ears. Occasional adhesion of blood clots to part of the round window membrane made it difficult to judge the presence or absence of ruptures. There was a tendency for the inner ear hemorrhage to be more severe in ears with rupture of the round window membrane. However, round window membrane rupture did not necessarily occur in ears that showed auditory dysfunction. In animal 6, which showed only unilateral elevation of the threshold of ABR as shown in Table 1, the round window membrane rupture was recognized only in the contralateral ear that showed no change of ABR. Thus, no direct relationship existed between the extent of auditory dysfunction and changes of the round window membrane.

The degeneration of the organ of Corti was observed in 10 out of 11 ears that had the elevation of threshold of ABR (Fig. 4). The degeneration of the organ of Corti was the most significant cause for the auditory dysfunction. Rupture of the tympanic membrane and the oval window could not be recognized. No degeneration could be observed in the cells of stria vascularis and vestibular end organs.

DISCUSSION

With regard to inner ear barotrauma, it was thought that pressure was transmitted directly to the cochlear partition producing structural and functional damages to the sensory hair cells of the organ of Corti. Hando et al. (5) studied the morphologic changes caused by barotrauma in guinea pigs, using a scanning electron microscope, and observed degeneration or disappearance of the stereocilia, mainly in outer hair cells.

In our experiments, spontaneous nystagmus occurred toward the contralateral side of the most impaired ear. According to Ewald's law, ampullifugal endolymph flow might have occurred around the crista of the horizontal semicircular canal on account of the transmission of impulses from the cochlea in the impaired ear. It is reasonable to conclude that the different effects between the right and left vestibular end organs may be the cause of the difference of the direction of the nystagmus.

Fig. 4. The organ of Corti demonstrated by the surface preparation technique. The damage of the outer hair cells of the organ of Corti is shown. Original magnification ×1000.

SUMMARY

To investigate auditory and vestibular disorders caused by barotrauma, 12 guinea pigs were exposed to compression and decompression between 1 and 2 ATA in a pressure chamber. During atmospheric pressure change, spontaneous nystagmus occurred in two-thirds of the animals. Auditory dysfunction, which showed a difference between the right and left ears, was confirmed by auditory brainstem response and Preyer's reflex in animals with spontaneous nystagmus. Hemorrhage in the middle ear space was recognized in 21 out of 24 ears. Rupture and/or hemorrhage of the round window membrane were observed in more than half of the examined ears. However, these changes of the round window membrane were not direct causes for the auditory dysfunction. Degeneration of the organ of Corti was the most significant cause for auditory dysfunction.

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SESSION 5: POSTER PRESENTATION
HYDROSTATIC EFFECTS;
SATURATION DIVING; THERMAL PROBLEMS IN DIVING

9th International Symposium on Underwater and Hyperbaric Physiology
Undersea and Hyperbaric Medical Society, Bethesda, MD, 1987

IDENTIFICATION OF INDIVIDUALS SUSCEPTIBLE TO DECOMPRESSION SICKNESS

C. A. Ward, P. K. Weathersby, D. McCullough, and W. D. Fraser

Previous theories that have been advanced to predict the occurrence of decompression sickness (DCS), have made the assumption that all individuals are physiologically the same. A "critical" parameter that is associated with the pressure profile is then usually proposed and if this parameter is exceeded, it is assumed that DCS ensues for all individuals (1). Critical parameters proposed previously have included the number of bubbles formed in the circulatory system (2), a gas volume (3), the bubble formation rate (4), and a tissue supersaturation (5). These approaches are open to criticism because a common observation in studies of DCS has been that individual susceptibility to DCS varies widely. For example, in a group of individuals undergoing the same pressure profile only a fraction of them will have any symptoms of DCS.

Weathersby et al. (1) have proposed an approach that could potentially account for the observed variation in susceptibility to DCS. They assume that all individuals are physiologically the same and that the initiation of DCS is a random event. The probability of DCS on a particular pressure profile is assigned using the maximum likelihood method (6), and is the same for all individuals.

Ward et al. (manuscript submitted) have proposed an approach in which it is unnecessary to make the assumption that all individuals are physiologically the same. They propose to measure the sensitivity of the complement system of an individual to activation by gas bubbles, where the gas is the same as that which the individual is to breathe on the pressure profile. They then assume that those individuals who are more sensitive to complement activation are the ones who are more susceptible to DCS. The only parameter about the pressure profile considered is whether it is severe enough to produce Doppler-detectable bubbles in the circulatory system of the individuals.

The basis for the complement-sensitivity approach is that many of the symptoms of DCS can be generated as a result of activation of this sequentially acting enzyme system. First, pruritus is a common complaint of divers who suffer DCS. Ivanovich et al. (7) have found that the severity of pruritus can be correlated with the degree of complement activation, and Yancey et al. (8) have shown that severe pruritus can be generated by injecting intradermally purified C5a, a fluid phase metabolite of complement activation. Second, plasma loss and pulmonary edema have often been observed to accompany severe DCS (9, 10). The infusion of purified C5a into guinea pigs was found to lead to increased alveolar capillary permeability and hemorrhagic pulmonary edema (11). Third, there have been numerous observations of changes in the number of circulating platelets, leukocytes, and erythrocytes in man and several other animal species resulting from decompression (12). These changes could easily be brought about by activation of the complement system. For example, Polley and Nachman (13) have shown that platelet aggregation is stimulated by C3a, another fluid phase metabolite that results from complement activation. In addition to initiating pruritus, C5a strongly induces leukocyte aggregation, and activation of the complement system produces a membrane attack complex which can lysis erythrocytes. Thus, if the complement system is activated by air bubbles produced during the decompression phase of a pressure profile, it could be the mechanism by which all these symptoms of DCS are brought about. Air bubbles have been shown to activate the complement system in vitro in both rabbit (14) and human plasma (Ward et al., manuscript submitted). Further, if some individuals are less sensitive to complement activation when air bubbles are present in their circulatory system than are others, then it could provide an explanation for the variation in susceptibility that has been observed.

Another approach to "accounting" for the variation in susceptibility of individuals is through ultrasonic Doppler monitoring. Often, individuals who undergo the same pressure profile are assigned "grades" (15) as a result of Doppler monitoring, indicating that they have different numbers of bubbles present in their circulatory system. If all individuals are physiologically the same, then DCS would be expected to occur with all individuals when they are subjected to a pressure profile that results in them having the same bubble grade. Thus even though a group of individuals underwent the same pressure profile, they could show different susceptibilities to DCS because they had different numbers of bubbles present in their circulatory system. If this possibility is valid, then one would expect to find a correlation between the bubble scores on a set of pressure profiles and the incidence of DCS.

Thus, at present there are three methods by which one might attempt to account for the variation in susceptibility of individuals to DCS: a) the maximum likelihood method (1), b) the complement-sensitivity method (Ward et al., manuscript submitted), and c) ultrasonic Doppler monitoring (15). We report the results of a study in which all three methods were applied to the same set of pressure profiles. Only the complement-sensitivity method is found

to account for the observations.

MATERIALS AND METHODS

Fifteen healthy male individuals were involved in this study. Blood samples were collected from each as described below and the individuals were subjected to a total of 25 pressure profiles. The experimental protocol and pressure profiles used in this study were approved by the Human Ethics Committee at the Defence and Civil Institute of Environmental Medicine, Downsview, Canada. All subjects provided written, informed consent documents. The maximum likelihood method has been used to predict the probability of DCS occurring on the pressure profiles considered (16), i.e., P(DCS).

To apply the complement-sensitivity method to the set of air dives that are to be considered below, it is necessary to determine the degree of complement activation that occurs when the plasma of each individual who is to undergo the pressure profiles is incubated with air bubbles. The technique by which this measurement can be made has been previously described (Ward et al., manuscript submitted). Briefly, it involves the following from each individual: a blood sample of 15 ml is collected in heparin and the plasma is separated from the cells and stored at -70°C until the day on which it is to be examined for sensitivity to complement activation by air bubbles. After thawing on that day, a plasma sample is divided into two portions. One portion, a volume of 1.5 ml, is placed in a 1.65-ml polypropylene tube and, after capping, it is vigorously shaken to introduce bubbles. The tube is then placed in a device that can rotate the tube end-over-end while it is being incubated. During each rotation, it is thumped by the device to ensure that the bubbles remained present throughout the rotation period. The other portion is also placed in a 1.65-ml polypropylene tube; however, it completely fills the tube. After capping, this tube is also placed in the rotating device and incubated while under rotation, but it is not thumped. There were no visible bubbles present in it. This latter portion of plasma served as the control for the experiment. Both tubes were incubated simultaneously for 30 min.

Afterward, both the plasma sample incubated with air bubbles and the control sample for each individual were assayed for complement activation by measuring the concentration of C5a present in each with commercially available radioimmunoassay kits (Upjohn, Kalamazoo, MI). The kit actually assays for C5a des Arg. However, if C5a is formed, it will be quickly converted to C5a des Arg in equimolar concentrations. Thus we assume the concentration of C5a des Arg was the same as the concentration of C5a that was produced during the incubation.

Several days after a blood sample had been taken, each individual was subjected to a pressure profile while breathing air. The maximum pressure to which an individual was exposed varied from 36 meters sea water (msw) or 0.36 MPa to a maximum of 72 msw. The decompression profiles followed the *DCIEM 1983 Decompression Model for Standard Air* (17). All pressure profiles

considered in this study were severe enough to produce bubbles that were detectable with Doppler ultrasonic monitoring at the precordial position immediately after the individual had been moving. The ultrasonic Doppler monitoring technique and method by which the bubble grade was assigned is described in (15). If an individual complained of symptoms of decompression sickness and was treated for DCS, he was taken as having experienced DCS.

RESULTS

The concentration of C5a measured in both the plasma samples incubated with air bubbles and in the control samples of plasma are shown as the first two bars in Fig. 1. Although the average concentration of C5a in the plasma samples incubated with air bubbles is larger than that in the controls, the difference is not significant because the error bars overlap. However, the reason for this is the wide variation in the concentration of C5a that was measured for the different individuals. For example, in the plasma samples incubated with air bubbles, the C5a concentration varied between 2.0 and 66 ng/ml, and there was an order of magnitude variation in the concentration of C5a that was measured in the control sample for this group of individuals.

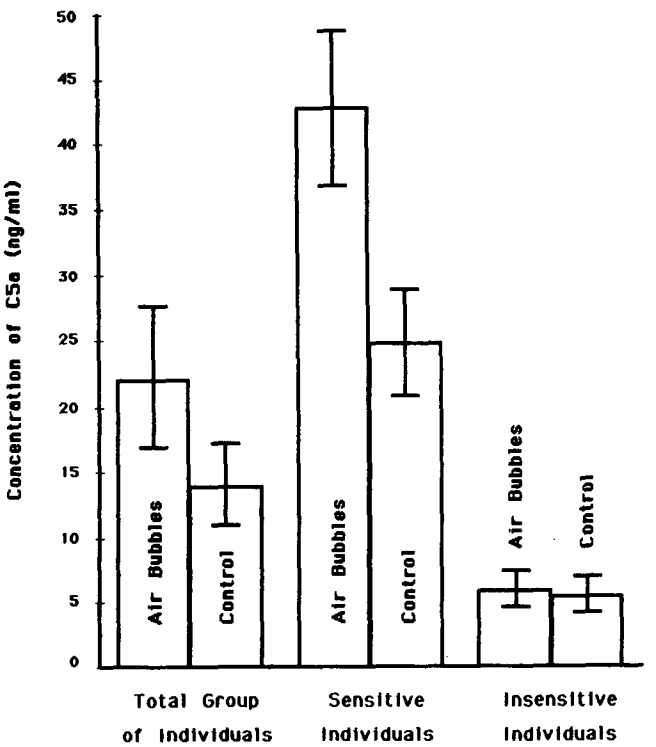

Fig. 1. Degree of complement activation (C5a) measured in plasma samples incubated with air bubbles and in controls. The two bars to the left are for the total group of 15 individuals, the central pair is for the individuals that were classified as sensitive ($n = 7$) and the other is for those classified as insensitive ($n = 8$).

Accordingly, the individuals were divided into 2 groups: sensitive and insensitive. The sensitive group was taken to be those individuals who produced a C5a concentration of greater than 25 ng/ml in their plasma samples incubated with air bubbles; the insensitive group as the ones who produced less.

The results obtained for the sensitive group are summarized by the second pair of bars in Fig. 1. For this group of individuals, the larger concentration of C5a measured in the plasma samples incubated with air bubbles is significantly greater than that in the control samples ($P < 0.01$ on the paired t test). By contrast, the measured concentration of C5a in the plasma samples of the insensitive individuals that were incubated with air bubbles are not significantly different from the samples that were incubated as controls, as is clear from the third pair of bars in Fig. 1.

The pressure profiles to which the sensitive individuals were subjected are listed in Table 1 and for the insensitive individuals in Table 2. The first column indicates the individual, the second indicates the pressure profile, and the third gives the probability of DCS occurring on that particular profile, according to the maximum likelihood method (16). The fourth and fifth column of these tables indicate the bubble grade recorded for the particular individual who experienced the pressure profile and whether the individual experienced DCS, respectively.

Table 1
Complement Activation and Sensitivity to Decompression Sickness

Sensitive Individuals				
Individual Number	Pressure Profile, msw/min	Maximum Likelihood, P(DCS)	Bubble Grade	DCS?
2	45/40	0.048	2.7	Yes
	36/50	0.042	4.0	No
6	45/40	0.048	3.0	Yes
	45/40	0.048	2.0	Yes
14	45/40	0.048	3.3	Yes
1	45/40	0.048	3.0	No
	36/50	0.042	2.7	No
9	45/40	0.048	1.0	No
	54/30	0.036	3.0	No
13	45/40	0.048	3.7	Yes
16	45/40	0.048	2.7	No
	Total:	0.504	Avg, 2.8	5 Yes; 6 No

Table 2
Complement Activation and Sensitivity to Decompression Sickness

Insensitive Individuals				
Individual Number	Pressure Profile, msw/min	Maximum Likelihood, P(DCS)	Bubble Grade	DCS?
4	36/50	0.042	3.0	No
10	63/30	0.060	2.7	No
12	54/30	0.036	3.0	No
	72/40	0.102	3.0	No
3	36/50	0.042	2.0	No
	63/30	0.060	3.3	No
	72/40	0.102	3.3	No
15	36/50	0.042	3.0	No
	72/40	0.102	3.0	No
11	72/40	0.102	3.0	No
8	63/30	0.060	3.3	No
	72/40	0.102	3.3	No
7	63/30	0.060	3.0	No
	72/40	0.102	4.0	No
	Total:	1.014	Avg, 3.1	0 Yes; 14 No

For the sensitive individuals, the total probability for DCS occurring in the series of pressure profiles to which they were subjected, calculated according to the maximum likelihood method, is given at the bottom of Table 1, along with the average bubble grade and the number of cases of DCS for this group of individuals. The corresponding quantities for the group of insensitive individuals are shown in Table 2.

DISCUSSION

As may be seen from the results shown in Fig. 1, there is a remarkable difference in the sensitivity of this group of individuals to complement activation that results from the incubation of their plasma with air bubbles. For example, the average concentration of C5a in the plasma samples of sensitive individuals incubated with air bubbles was more than 7 times larger than that of the insensitive group. A similar difference is seen in the plasma samples incubated as controls. The concentration of this anaphylotoxin in the plasma samples of the sensitive group incubated in absence of any air bubbles, i.e., as controls, was more than 4 times larger than that of the insensitive group. Both of these results would indicate that the complement system of sensitive individuals is more easily activated than that of individuals classified

as insensitive. We would also emphasize that for this group of insensitive individuals, there was no measurable activation of the complement system by the air bubbles.

To determine whether this clearly identifiable difference in sensitivity to complement activation between the 2 groups indicates a difference in susceptibility to DCS, the incidences of DCS occurring after the sets of pressure profiles may be considered. If the average bubble grade for sensitive individuals (2.8) or insensitive individuals (3.1) is considered, then the insensitive group of individuals would have been expected to experience more cases of DCS. Similarly, a comparison of the maximum likelihood predictions on the two sets of profiles indicates that DCS was more likely to occur on the profiles to which insensitive individuals were subjected. It was 0.504 for the pressure profiles of the sensitive individuals and 1.014 for the insensitive individuals. Thus, both the bubble grades on the pressure profiles and the maximum likelihood prediction would lead to the expectation of DCS occurring a larger number of times with insensitive individuals than with sensitive individuals. As can be seen from the total number of cases of DCS for each group of individuals, the observations were contrary to this expectation.

Only the sensitivity of the complement system of the respective groups of individuals would lead one to expect that DCS would occur more frequently with the sensitive group of individuals. As may be seen from the results in Tables 1 and 2, it is this prediction that is in agreement with the data. The sensitive group experienced DCS after 45.4% of their profiles, whereas the insensitive group did not experience any cases of DCS.

If one examines the statistical significance of the observed susceptibility of sensitive individuals to DCS on the basis of the 25 dives in this series, one can conclude that sensitive individuals are more susceptible ($P < 0.01$ with the χ^2 test).

Since the maximum likelihood approach was developed by assuming all individuals to be physiologically the same, the data in Tables 1 and 2 raise questions about this assumption. The complement sensitivity approach has identified one physiologic parameter that is considerably different between these 2 groups of individuals (Fig. 1), and it appears that for this set of pressure profiles and for these 2 groups of individuals it is an important difference. To date, only this one study has been conducted and it involved a total of 5 cases of DCS.

The average bubble grade assigned to individuals undergoing these pressure profiles shows no correlation with the incidences of DCS. Nonetheless, Doppler-detectable bubbles were present in each case. The results could be interpreted as meaning the complement system of each individual mediates DCS. Thus, for DCS to occur, both the presence of bubbles and the requisite sensitivity of the complement system to activation by air bubbles are required.

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CHANGES IN MICROCIRCULATION AND THE APPEARANCE OF BUBBLES IN LARGE BLOOD VESSELS DUE TO DECOMPRESSION STRESS

M. Nodera, Y. Gotoh, and I. Nashimoto

It is well known that rapid decompression after high pressure exposure causes bubble formation in tissues or blood which will induce decompression sickness. Concerning this fact, a number of investigators have attempted to observe the bubbles in microcirculation experimentally (1-4).

In 1968, Buckles (1) reported, from direct microscopic observation, bubble formation in the microvessels of a hamster's cheek pouch following decompression. Heimbecker et al. (2, 3) also observed remarkable changes in the microcirculation of hamsters before bubble formation following decompression after exposure to high pressure. Wells et al. (5) observed erythrocyte aggregation in the microvessels and bubbles that passed through the capillaries after decompression in the mesentery of a dog. However, no references were found in these studies to relate changes in microcirculation and appearance of bubbles in large blood vessels, such as femoral artery and vein or vena cava.

The purpose of this study was to find such relationship, which seems to be largely concerned with the pathophysiology of decompression sickness.

METHODS

Sixty male golden hamsters (*Mesocricetus auratus*) weighing 100 to 120 g were used. The animals were anesthetized with sodium pentobarbital (6 mg/100 g) for microscopic observation and then exposed to 12 ATA in a small hyperbaric chamber.

After 40 min the animals were decompressed to surface at a rate of 1 m/s. Immediately after surfacing, the cheek pouch of the hamster was carefully spread over a glass slide (1-mm thick), and kept under wet conditions. To

avoid the disturbance of microcirculation due to injury, no surgical operation was carried out in the procedure.

The microcirculation of the cheek pouch was observed by a microscope (Nikon, Optiphot or industrial microscope-Optiphot; ×40 to ×400). Pictures were simultaneously recorded with a CCD video camera (Hitachi, VK-888) and video recorder (National, NV-9850). The pictures were taken with a motor-driven camera (Nikon, F-3) for detailed subsequent analysis. The microscope was slightly modified to take the photograph and the video-image simultaneously with the microscopic observation.

Based on our previous observations (6), the animals that survived over 40 min and those that died within 40 min were regarded as "non-fatal" and "fatal," respectively. Forty minutes after surfacing, the surviving animals were given a surgical dissection to observe the bubbles in femoral vein and artery, posterior vena cava, and the microvessels of the intestine. The large vessels were observed with a stereo microscope (Olympus, SZ-Tr, x15 to x20). Post-mortem examinations were performed immediately on those animals who did not survive.

RESULTS

Tables 1 and 2 summarize the results.

Fatal Cases

Nineteen of 60 hamsters were killed by rapid decompression. Severe stasis was the most conspicuous change in the microcirculation of the cheek pouch after surfacing (Fig. 1).

Table 1
Microcirculatory Changes and the Appearance of Bubbles in Cheek Pouch of 60 Hamsters after Surfacing

Group	Microcirculatory Changes		Bubbles
Fatal 19	19	stasis WBC sticking white thrombi	19
Nonfatal 41	9		0

When severe stasis appeared in an early stage, white blood cell (WBC) aggregation and the appearance of white thrombi were not observed. Bubbles appeared first in the arterioles after the changes of microcirculation, and then in the venules (Fig. 2). The bubbles in the arterioles entered quite rapidly into the capillaries and expanded them. They grew in the microvessels which were free of blood. In moderate cases, adherence of WBC to the walls of venules

(WBC sticking) and appearance of white thrombi in venules were found before stasis. In fatal cases, these microcirculatory changes occurred within 10 min after decompression, and recovery was not seen.

There were two patterns of growth of bubbles in the vessels. One was an increase in volume of a single bubble and another was the fusion of bubbles. In either case, the growth of bubbles progressed quite rapidly after the appearance of bubbles in the microvessels.

In the postmortem examinations, a lot of bubbles were formed in the blood vessels in every part of the body. Figure 3*B* shows many bubbles that appeared in intestinal microvessels and free of blood.

Table 2
State of Microcirculation in the Cheek Pouch and
Appearance of Bubbles at Certain Locations in Animals That Survived

	Microcirculation in Cheek Pouch		Bubbles	Location
Nonfatal animals 41	normal	32	0	
	abnormal	9		
	4 (recovered)		0	
	5 (not recovered)		5	microvessels of intestines (5) vena cava (2) femoral vein (0) femoral artery (0)

Nonfatal Cases

No bubbles were seen in the microvessels of cheek pouches of 41 surviving hamsters. In 32 of 41 animals, no changes were found in the microcirculation and no bubbles formed in vessels. In 4, slight stasis appeared in the microcirculation of cheek pouches at an early stage, and disappeared after a while. These microcirculatory disturbances were restored within 30 min after decompression. No bubbles were found in the femoral vein and artery, vena cava, or intestinal microvessels.

In the remaining 5 animals, microcirculatory changes in the cheek pouches were observed. The changes were similar to those found in the hamsters that were killed by rapid decompression. There were small gas emboli in the intestinal microvessels in these 5 (Fig. 4 *A*, *B*). The bubbles continued to increase in volume for 70 min after surfacing, and disturbances of microcirculatory flow were observed in several microvessels. Bubbles in the vena cava were observed in 2 of the 5 animals. These bubbles were much smaller than those found in fatal cases. No bubbles were found in the femoral vessels.

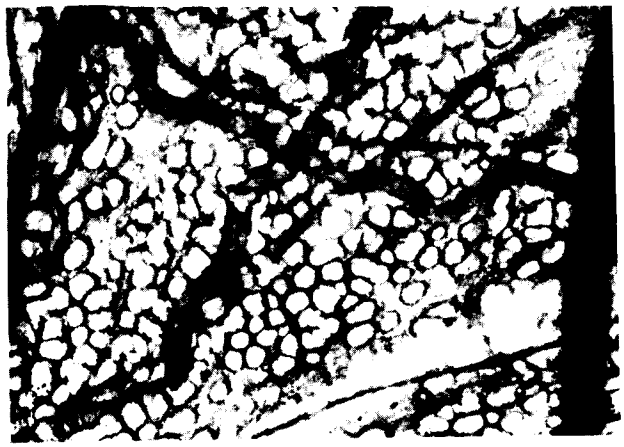

Fig. 1. Severe stasis of venules of the hamster's cheek pouch. ×100.

Fig. 2. Gas emboli in microvessels of cheek pouch. ×100. They appeared first in arterioles after the occurrence of stasis in venules and capillaries.

DISCUSSION

It has been suggested recently, that not only the disturbances by gas embolism but also multiple physiologic changes, such as the red blood cell (RBC) agglutination, stasis, and the adherence or aggregation of circulating platelets, may be involved in the pathogenesis of decompression sickness (DCS) (5, 7, 8). In addition, several authors described microcirculatory disturbances before the appearance of bubbles in microvessels (3, 6). Our findings in DCS cases are consistent with their conclusions. Furthermore, we observed conspicuous WBC sticking and the appearance of white thrombi (platelets) before the occurrence of bubble formation.

Philp et al. (9) demonstrated the interaction of platelets and bubbles in DCS. They also observed (10) platelet aggregation (platelet thrombi) in the venules of the lungs of DCS animals. The present results suggest that decompression stress causes aggregation or adhesion of circulating platelets as well as the agglutination of RBC and stasis. Although the origin of this phenomenon

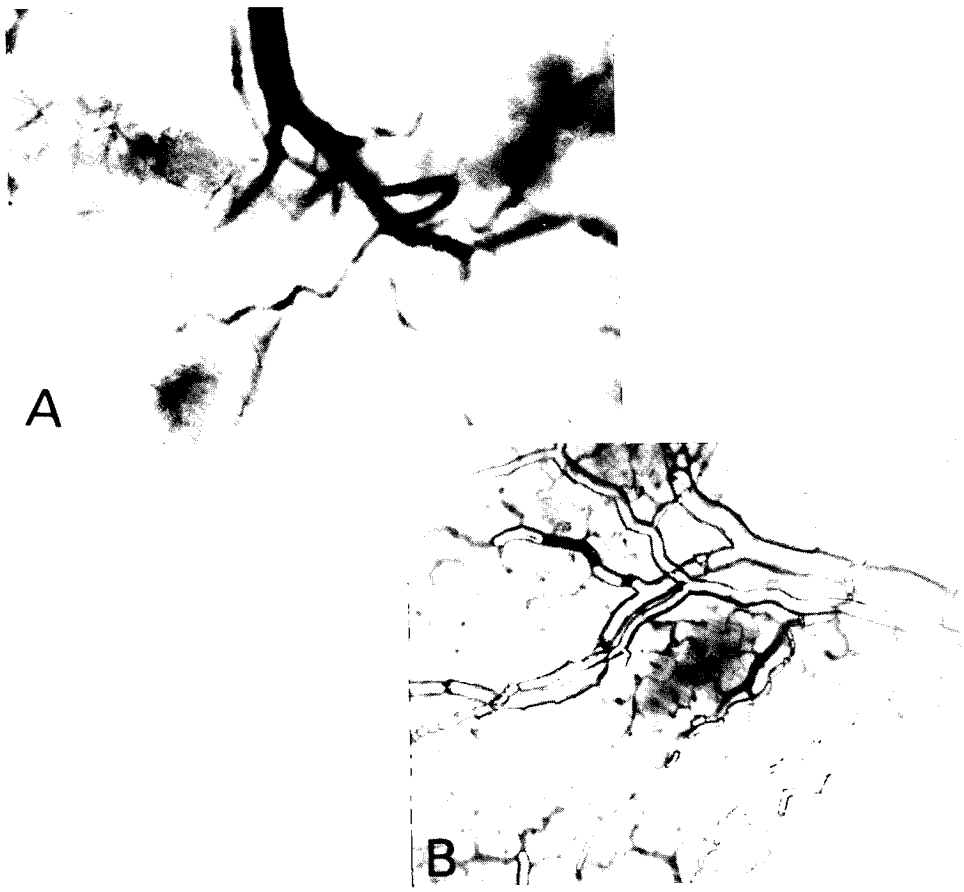

Fig. 3. Intestinal microvessels of the hamster. A normal finding. ×40. B appearance of many bubbles. ×40.

has not been revealed by microscopic observation, it seems undeniable that undetectable bubbles formed before the microcirculatory changes.

In the animals that died after decompression, many bubbles were observed in various parts of the vessels. On the other hand, in the surviving animals, bubbles did not appear in the microvessels of the cheek pouch or in femoral vessels, but did appear in the intestines of 5 animals and in the vena cava of 2 animals.

In summary, a) changes in the microcirculation occur before bubbles appear in large vessels; b) the response of animals to decompression stress is affected by where the bubble appears as well as by the quantity of bubbles; and c) "nonfatal" bubbles also cause microcirculatory disturbances.

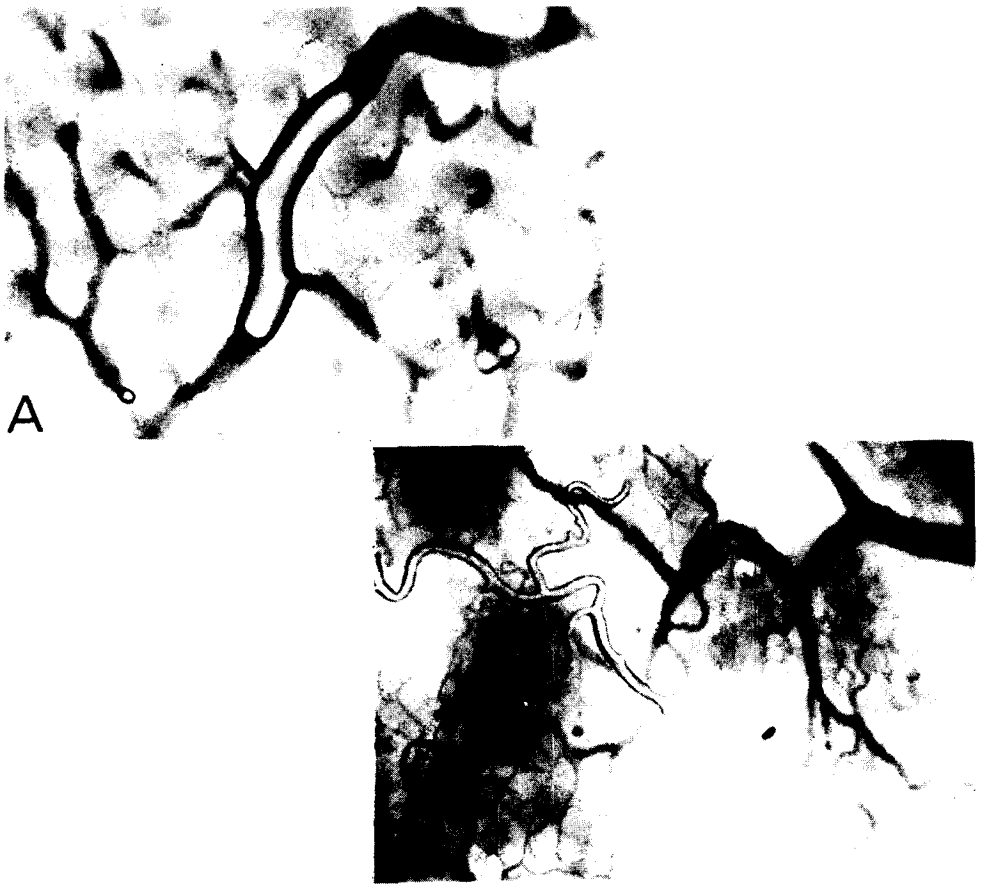

Fig. 4. Gas emboli in intestinal microvessels. A, ×100. B, ×40.

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AB INITIO SIMULATION OF INERT GAS TRANSPORT IN THE HUMAN

M-C. Liou and E. H. Wissler

> *Decompression following conventional diving, despite the elaborate mathematical models and sophisticated analysis discussed in previous chapters, has been unsatisfactory and, on occasion, assuredly not safe for any diver.*
>
> *A. R. Behnke, 1975*

Decompression sickness is a medical problem associated with activities in which man is subjected to a significant reduction in pressure. Typical situations in which this malady occurs are diving, rapid ascent in high performance aircraft, and extravehicular activities in space. Although the etiology of decompression sickness is undoubtedly related to separation of gas from tissue and fluids in the body, the precise mechanism remains unknown more than a century after the work of Bert (1). Nevertheless, a number of mathematical models have been developed to aid in the construction of decompression tables. These highly empirical models contain a large number of adjustable parameters that have little direct relationship to physical or physiologic quantities. Given the complex nature of decompression sickness, that undesirable state of affairs is not likely to change soon.

Research on the underlying nature of decompression sickness has been hampered by the difficulty associated with human experimentation. By far the largest number of studies has involved evaluation of various decompression schedules, often with simultaneous monitoring of vascular bubbles using Doppler techniques. Unfortunately, a clear correlation between the occurrence of venous bubbles and decompression sickness has not been established. A few studies have been conducted to measure rates of inert gas uptake and clearance in human subjects under various conditions, although it is still difficult to relate the results of such studies to the occurrence of decompression sickness. Animal studies have provided additional information about bubble formation

under various conditions and the resulting pathology in blood and tissue.

For the most part, mathematical models have been developed for the purpose of constructing decompression tables, and accordingly, have been tested against decompression data. Although that is a useful exercise, it does not provide a great deal of insight into the nature of decompression sickness. The model described in this paper is different from others in that it focuses attention on inert gas uptake and clearance, with the objective of gaining a better understanding of fundamental phenomena without necessarily carrying the analysis to the point of developing improved decompression schedules.

METHODS

A previously developed human thermal and cardiovascular model was extended to include transport of helium and nitrogen within the body. The original model accounted for human morphology by subdividing the human geometrically into 15 major elements representing the head, upper and lower trunk, and proximal, medial, and distal elements of each extremity, as illustrated in Fig. 1. Each of these major elements is further subdivided into 15 cylindrical shells, for which properties are assigned in a manner appropriate to the distribution of aqueous and adipose tissue, blood in major vessels, and bone. Temperature, concentrations of oxygen, carbon dioxide, lactate, helium and nitrogen, and local metabolic and perfusion rates are computed at 10-s intervals for each of the 225 tissue elements and the 15 arterial and venous pools. Additional cylindrical shells can be added to simulate various garments as may be appropriate.

Metabolism serves as a source of heat, carbon dioxide, and lactate and as a sink for oxygen. The local metabolic rate is determined by the intensity and kind of exercise and the intensity of shivering. In a given region, metabolism may occur via either the aerobic or anaerobic pathway depending on metabolic and perfusion rates and the arterial oxygen tension. Glucose is used as a representative substrate in this model.

Metabolically produced heat is either stored in tissue increasing its temperature, conducted to region of lower temperature, or carried away by circulating blood. This process is described by the heat conduction equation, which is merely an energy balance constructed for an infinitesimal element of material. The set of partial differential equations that constitute the thermal model are solved numerically using well-established, finite-difference techniques to compute temperatures throughout the body at 10-s intervals. Since that procedure, which allows quantities such as the perfusion and metabolic rates to vary with position and time, has been described in considerable detail in several previous publications (2, 3), it will not be discussed in this paper. Suffice it to say that the thermal model has been carefully checked against experimental data for conditions ranging from immersion in cold water to exercise in a hot, humid environment and found to produce reasonable results (3).

Transport of chemical species is considerably more complex than transport of heat. Figure 2 provides a schematic representation of a typical property region containing both aqueous and adipose tissue and the arteries, veins, and capillaries for the region. Inasmuch as bubble formation occurs whenever the

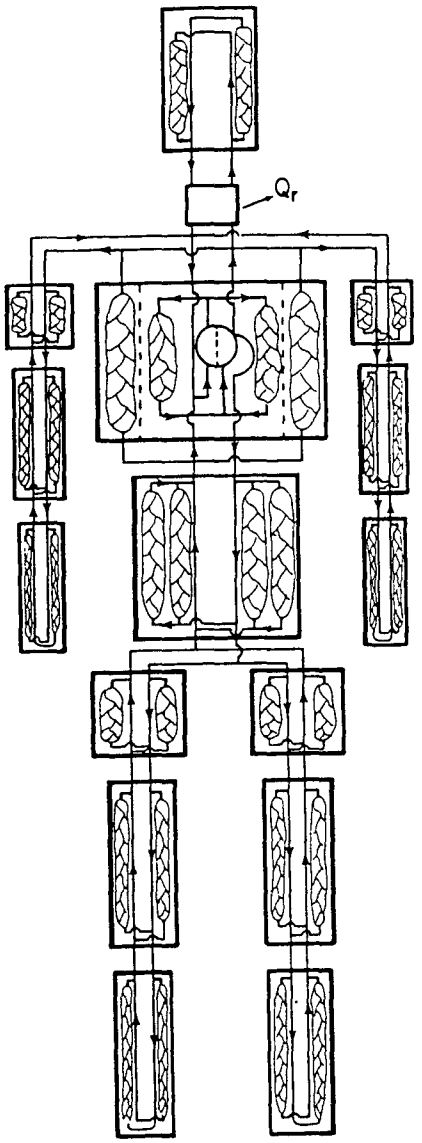

Fig. 1. Schematic representation of the physical configuration of the model.

sum of the dissolved gas tensions for all components (O_2, CO_2, H_2O, He, and N_2) exceeds the pressure-dependent threshold for bubble growth, a separated gas phase is also shown in each tissue compartment. For a given element (aqueous or adipose tissue or blood), one can write a material balance stating that the rate of accumulation of each soluble substance is the sum of the rate of production owing to metabolic reactions, the rate of transport into the region by circulating blood, and the rate of transfer from any included gas space to tissue (or fluid). The rate of production of a given species may be positive, negative, or zero, depending on whether the substance is a product, reactant, or nonparticipant in the metabolic process. Similarly, the rate at which each component is carried into the region by circulating blood may be either positive or negative depending on relative gas tensions in tissue and blood. In the case of gas transport between tissue and a bubble, diffusion of each component is driven by the difference between the dissolved gas tension or blood and the partial pressure of the component in the bubble.

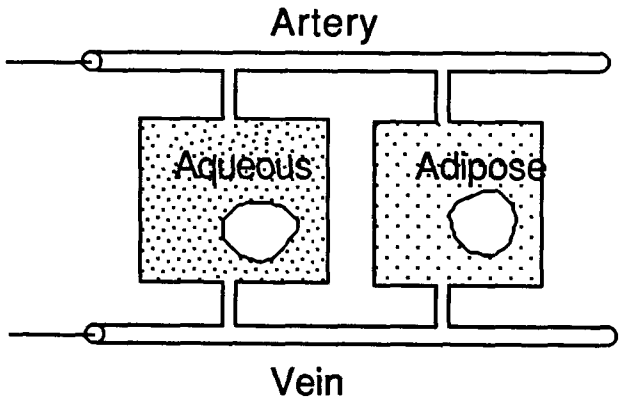

Fig. 2. Representative element of tissue containing large arteries and veins, aqueous and adipose tissue, capillaries in each region, and a separated gas phase in each region.

Specific features incorporated into the various material balances are summarized below.

Tissue

Aqueous and adipose compartments are treated separately in each region.

Oxygen

Since equilibrium is assumed to exist between oxyhemoglobin and oxygen

in perfused tissue, the material balances for oxygen in capillary blood and tissue can be combined into a single differential equation. The rate of accumulation of oxygen in a given tissue compartment and its associated capillary blood is equal to the net rate of convection into the region minus the rate of metabolic consumption, which is determined by the metabolic rate and pathway. If the oxygen tension in a given capillary bed falls below a limiting value, anaerobic production of ATP occurs. In any separated gas phase, the partial pressure of oxygen equals the oxygen tension in tissue.

Carbon Dioxide

Equilibrium is also assumed to exist for carbon dioxide in blood and tissue and, therefore, the capillary blood and tissue material balances can be combined. The rate of accumulation of carbon dioxide in either tissue compartment and its associated capillary blood is equal to the rate of convection of carbon dioxide into the region plus the rate of production owing to metabolism. If separated gas exists in the region, the partial pressure of carbon dioxide is equal to the carbon dioxide tension in tissue.

Lactate

Whenever the anaerobic pathway is active, lactate is produced in muscle. The rate of accumulation of lactate in tissue equals the rate of production minus the rate of diffusion from tissue to blood. Diffusion of lactate between aqueous tissue and blood occurs at a rate proportional to the difference in concentrations. Lactate is consumed as a metabolic substrate in muscle in addition to being converted into glycogen in liver when sufficient oxygen is present.

Helium

The rate of accumulation in each tissue compartment is equal to the rate of transfer from blood to tissue minus the rate of transfer from tissue to separated gas regions, whenever they are present.

Nitrogen

The rate of accumulation in each tissue compartment is equal to the rate of transfer from blood to tissue minus the rate of transfer from tissue to separated gas regions, whenever they are present.

Capillary Blood

Oxygen

See the preceding statement for oxygen in tissue.

Carbon Dioxide

See the preceding statement for carbon dioxide in tissue.

Lactate

The rate of accumulation of lactate in capillary blood equals the rate of diffusion from tissue to blood plus the net rate of convection into the region. Lactate is readily distributed throughout aqueous tissue by convection.

Helium

The rate of accumulation of helium in capillary blood equals the rate of convection into the region plus the rate of diffusion from tissue to blood.

Nitrogen

The rate of accumulation of nitrogen in capillary blood equals the rate of convection into the region plus the rate of diffusion from tissue to blood.

Arterial and Venous Pools

All Components

Since no allowance is made for metabolism or diffusion of any component across the walls of larger vessels, the rate of accumulation in an arterial or venous pool is equal to the net rate of convection into the pool. In the case of veins, flow into the pool includes drainage from the capillary beds of the region.

Pulmonary Capillaries and Alveolar Gas

All Components

A material balance for each component equates the net rate of convection by blood into the lungs to the rate of clearance through the respiratory tract. The end-capillary tension of each volatile component is assumed to be equal to the partial pressure of that component in the alveolar gas space. Two percent of the blood flow is assumed to be shunted through unventilated portions of the lungs. All bubbles are assumed to be cleared by the lungs.

Ligament

Oxygen and Carbon Dioxide

As a first approximation, metabolism in ligaments and tendons can be neglected and, therefore, the oxygen and carbon dioxide tensions are equal to corresponding tensions in adjacent tissues. The assumption is made that these tissues are not perfused with blood.

Helium and Nitrogen

These two inert components are assumed to diffuse into connective tissue, such as ligament, from surrounding perfused tissue. Since the diffusion length can be the order of several millimeters, the time constant for uptake or clearance of inert gas from these tissues is rather long. Although the amount

of gas involved is not large, formation of bubbles in ligaments and tendons during decompression from saturation can, nevertheless, be significant.

Notes: a) All rates have associated signs which are assumed to be positive in the balances as written but may, in fact, be negative; the validity of the balances is not affected by the sign of any term. For example, since oxygen is actually transported by convection from the lungs to the system, the net rate of convection of oxygen by blood into the lungs is negative. However, the rate of clearance of oxygen through the respiratory tract is also negative and, therefore, the balance for oxygen remains valid. Similarly, the net rate of convection of helium or nitrogen into a capillary bed and the rate of diffusion from blood to tissue will be positive during compression and negative during decompression, but the material balances are valid in either case, if the correct sign is used. b) Diffusion of helium or nitrogen across the capillary wall is driven by the difference in gas tensions in tissue and blood, which are defined in terms of the concentrations of dissolved gas and the solubilities in each region. c) When a separated gas phase exists, diffusion of helium or nitrogen across the gas-tissue interface is driven by the difference between the gas tension in tissue and the partial pressure in the bubble. d) The mass transfer coefficient for diffusion of inert gas across a capillary wall or blood-gas interface is proportional to the diffusivity of gas in tissue. It also depends on the geometry of the system defined in terms of the size of the capillary and its associated tissue cylinder in the case of blood-tissue interchange, or the radius of the bubble in the case of gas-tissue interchange. e) It is assumed that separated gas in tissue can cross the capillary wall and enter the venous circulation at a rate proportional to the volume of separated gas in tissue, although the precise mechanism for transfer is not defined. f) Stable gas nuclei are assumed to exist in all tissue. They are assumed to be of uniform size and to grow whenever the sum of the dissolved gas tensions exceeds a critical threshold value. Equations defining the concepts stated above are presented in another publication (4).

The marching procedure used to solve the heat conduction equation and material balances that define this model permits one to simulate arbitrary dive profiles in which pressure, gas composition, temperature, and intensity of exercise all vary with time. Given the initial thermal state and amount of dissolved gas for an individual, the program computes temperatures and compositions at subsequent times. Although the original thermal-cardiovascularmetabolic model can now be executed on a large desktop computer, the addition of inert gas transport with bubble formation expands the computational requirements, and efficient execution of the program requires a rather large computer.

RESULTS

Simulations using the model were carried out for 10 cases previously

studied experimentally by 4 different investigators. A comparison of computed nitrogen clearances with measured values will be presented in the following sections. Physical properties used in these simulations are tabulated in Table 1.

Table 1
Physical Properties Used for the Simulations

Tissue	Muscle	Fat	Ligament
Density of nuclei, nuclei/ml	60,000	6000	40,000
Surface tension, dyne/cm	12.6	12.6	18.1 (12.6)
Diffusivity of N_2 ($10^6 \times cm^2/s$)	2.34	0.35	0.59
Mean diffusion distance for capillaries, mm	20	24	—
Radius of gas nuclei, μm	0.5	0.5	0.5
Surface stress of nuclei $2\gamma/r_b$, atmosphere	0.50	0.50	0.71 (0.5)

Several investigators have measured nitrogen uptake and clearance following an isobaric gas switch or during a bounce dive and the subsequent period of decompression. Experimental data provided by these studies are useful for evaluating parameters in the model and assessing its validity. It soon became apparent that certain kinds of experimental data were better suited than others for evaluating particular constants. For example, an upward excursion from saturation is very sensitive to the level of supersaturation required for growth of stable gas nuclei, while inert gas uptake and clearance during a pressure excursion lasting several hours are sensitive to the details of gas transport by circulating blood and bubble formation. Isobaric gas switches in which countercurrent diffusion is not significant provide information about perfusion and diffusion uncomplicated by bubble formation.

In 1937 Behnke (5) measured the rate of clearance of dissolved nitrogen from subjects who breathed 97% oxygen at 1 atm. Analysis of samples collected periodically from the circulating breathing gas supply provided data for estimating the amount of nitrogen eliminated from the body. These data are presented in Fig. 3 together with curves computed for 3 subjects having different percentages of body fat. Since the mean body weight and percent body fat of the 3 subjects studied by Behnke were 60.5 and 13.2 kg respectively, the upper computed curve should be compared with the experimental data.

Behnke reported his data as percent of initial body burden cleared as a function of time and, therefore, it is necessary to estimate the equilibrium amount of nitrogen dissolved at 1 atm. The experimentally derived curve shown in Fig. 3 is based on his assumption that 95% of the dissolved nitrogen is eliminated during the first 245 min of oxygen breathing, but computed results

indicate that nitrogen clearance was only 77% complete at that time. When Behnke's data are multiplied by the ratio 77:95, agreement between computed and measured values improves greatly, as shown in Fig. 4.

The three simulations presented in Fig. 3 indicate that the fractional rate of nitrogen clearance increases as the percentage of body fat decreases. Since the solubility of nitrogen is approximately 5 times greater in fat than in muscle, added fat provides an excellent reservoir for dissolved nitrogen, as illustrated by the values in Table 2. In addition, the diffusivity of nitrogen and the rate of perfusion by blood are both smaller in fat than in tissue. Hence, the three physical factors that influence clearance of inert gas from tissue all contribute to slower clearance from fat.

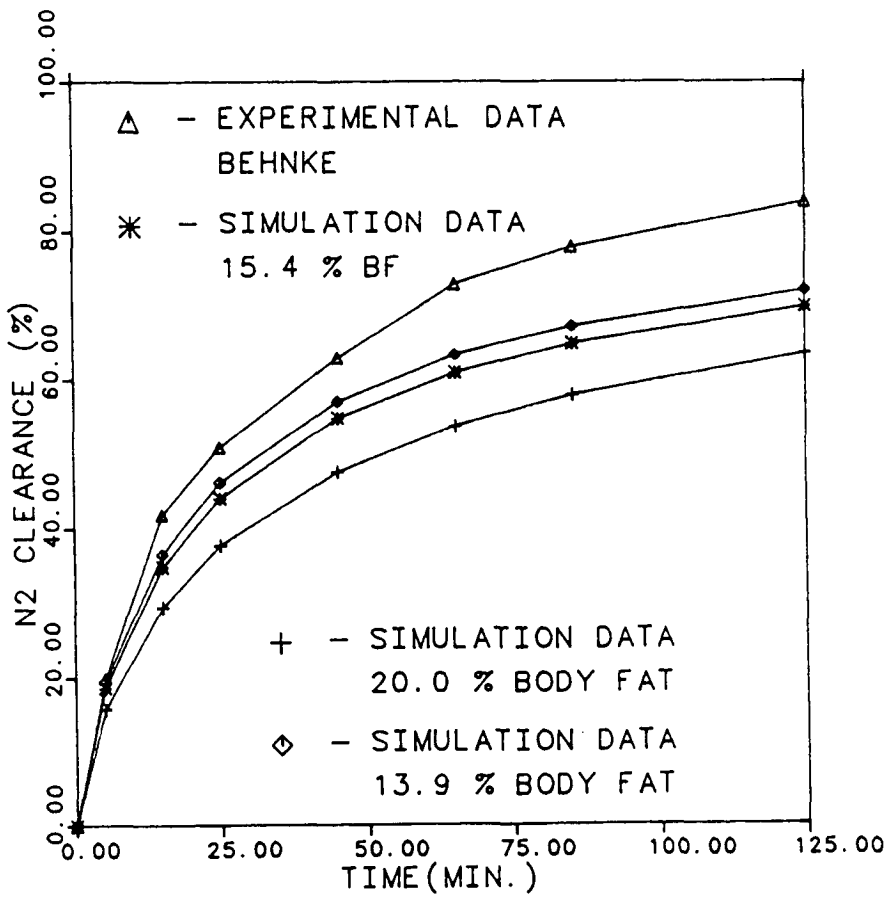

Fig. 3. Computed and measured nitrogen clearance while breathing oxygen at 1 atmosphere (5). Computed clearances are shown for 3 individuals having the same lean body mass, but different fat masses. The experimental curve is based on the assumption that 95% of the dissolved nitrogen is cleared in 245 min.

Although agreement between computed and measured results for Behnke's isobaric gas switch was as good as one could reasonably expect, several possible sources of discrepancy should be noted. The actual gas mixture used in the experiment contained from 2.5 to 3.0% nitrogen, while the simulations were conducted using 100% oxygen as the breathing gas. Uncertainty about the actual ambient temperature should also be noted, because subsequent experimental studies and simulations to be discussed later both indicate that temperature is an important factor in experiments such as these. The simulations were carried out using an ambient temperature of 25.5°C.

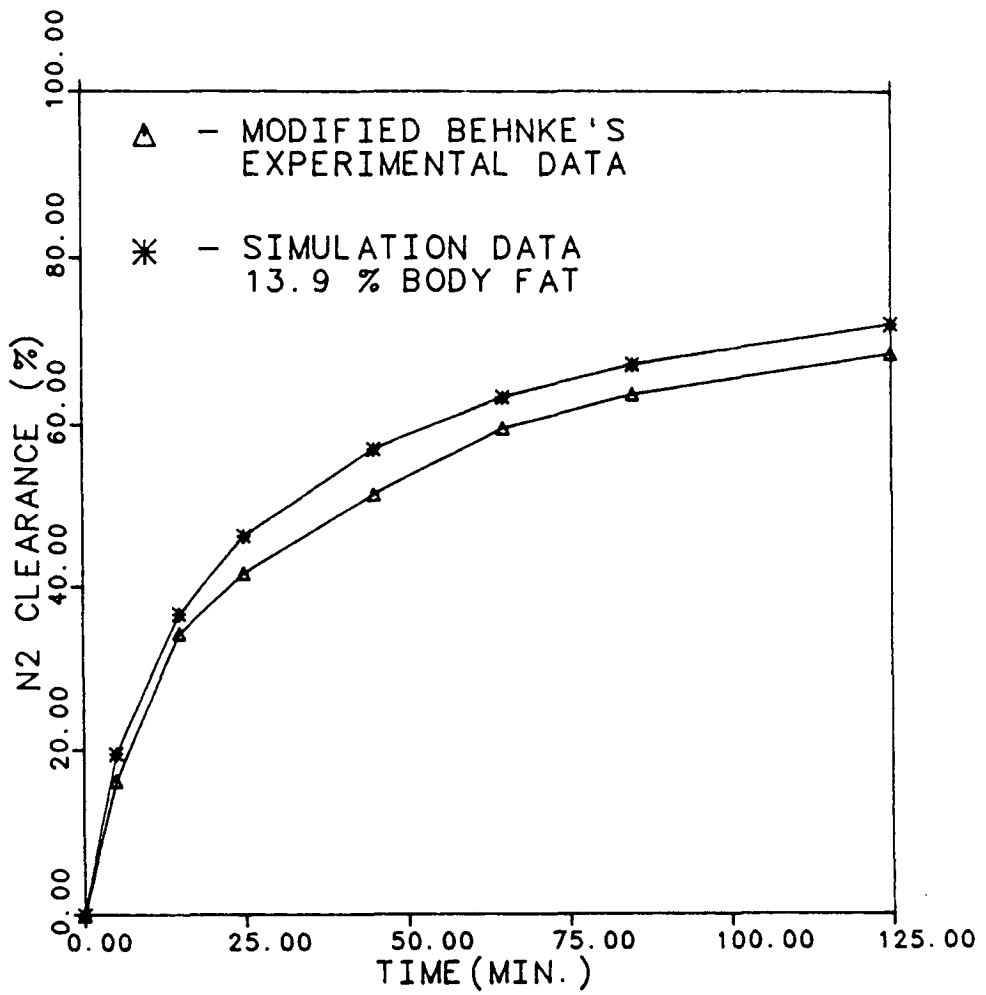

Fig. 4. Computed and measured nitrogen clearances while breathing oxygen at 1 atmosphere (5). The experimental curve is based on the assumption that only 77.6% of the dissolved nitrogen is cleared in 245 min.

In another series of experiments, Kindwall et al. (6) measured nitrogen elimination under three different conditions from divers who breathed compressed air for 40 min at a depth of 100 fsw, or approximately 4 atm. After the initial exposure in a dry hyperbaric chamber, these divers switched to an 80:20 heliox mixture which they breathed for 3 min from an open-circuit system to rinse compressed air from the lungs. Then they continued to breathe 80:20 heliox for 90 min while 5-min samples of expelled nitrogen were collected in Douglas bags. Nitrogen elimination during the lung-rinsing period and the first 2 min on the closed-circuit system was not measured. The three conditions studied were: an isobaric gas switch at 100 fsw, decompression at 50 fsw, and decompression at 10 fsw. Computed and measured results for the three conditions are presented in Figs. 5-7.

Bubble formation does not occur in tissue or blood during the isobaric gas switch at 100 fsw, for which computed and measured nitrogen clearances are presented in Fig. 5. The rate of clearance computed during the first 20 min of collection is approximately 40% greater than the measured rate, but during the remaining 70 min the two rates are in reasonably good agreement. Computed and measured clearances during decompression at 50 fsw are presented in Fig. 6 where it can be seen that differences in the amount of gas eliminated are never large, although the two curves have rather different shapes. The computed rate of elimination is larger than the measured rate during the first half of the clearance period, and smaller during the second half. Agreement between computed and measured clearances was excellent for the case of decompression at 10 fsw, as shown in Fig. 7.

Comparison of computed and measured data is facilitated by referring to Table 3 in which the amounts of nitrogen eliminated during the entire 90-min washout period are tabulated for the 3 cases. The experimental data of Kindwall et al. (6) suggest that intravascular bubble formation during decompression to 50 fsw enhances nitrogen clearance by increasing the gas-carrying capacity of blood. Simulation results are consistent with experimental data in predicting that nitrogen clearance is greater for decompression at 50 fsw than for either of the other 2 cases, although predicted differences are not as great as those observed in the experimental study. An additional case run for decompression at 50 fsw without bubble formation in blood yielded clearances

Table 2
Amount of Nitrogen Dissolved in the Human Body at 1 atm

Body weight, kg	133	135	141
Body fat, %	13.9	15.4	20.0
Dissolved nitrogen, ml at STP			
In muscle	258	259	262
In fat	353	387	502
Total	672	709	829

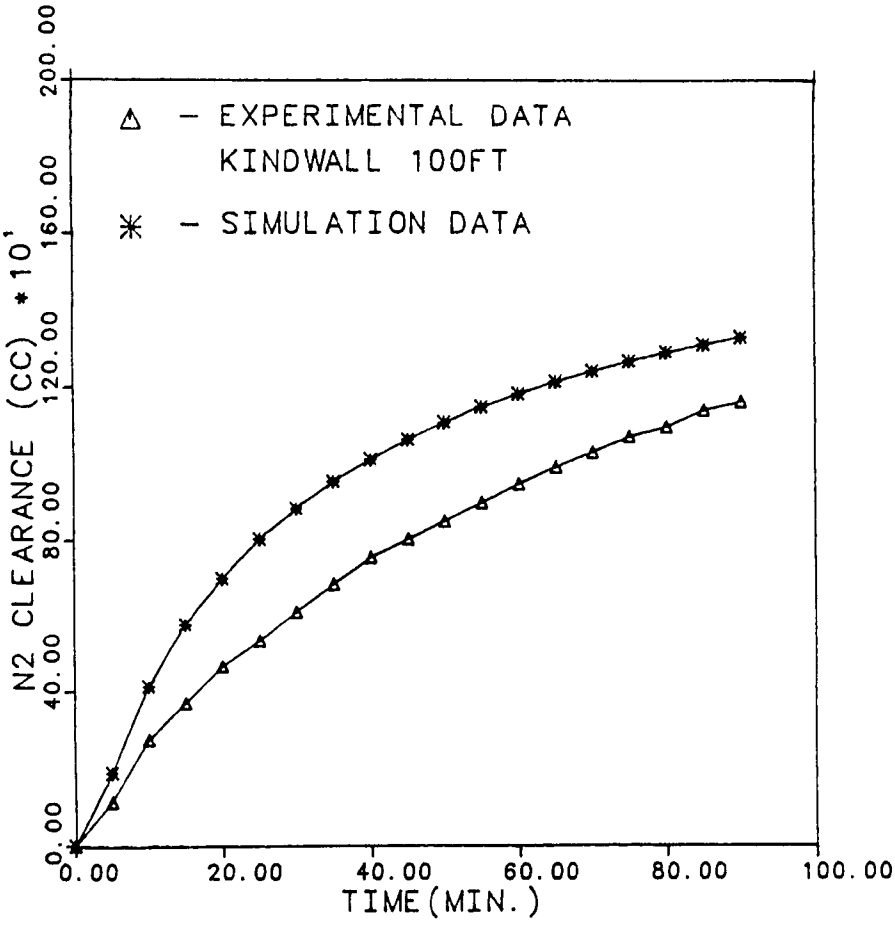

Fig. 5. Computed and measured nitrogen clearances while breathing 80:20 heliox at 100 fsw after breathing compressed air at the same depth for 40 min (6).

virtually identical to those for the isobaric gas switch; that is, about 13% lower than those with bubble formation. Hence, the computed results appear to support the postulate that bubble formation in blood can serve to facilitate inert gas clearance under very particular circumstances. However, that will only happen if bubble formation occurs more readily in blood than in tissue, which was one of the assumptions incorporated into the model.

One would expect the occurrence of greater supersaturation with attendant extravascular bubble formation during decompression at 10 than at 50 fsw. Computed results indicate that such bubbles are present in tissue after 90 min at 10 fsw but have been completely reabsorbed at 50 fsw. The amounts of nitrogen and helium within bubbles remaining after 90 min of decompression at 10 fsw are listed in Table 4 for both fat and muscle.

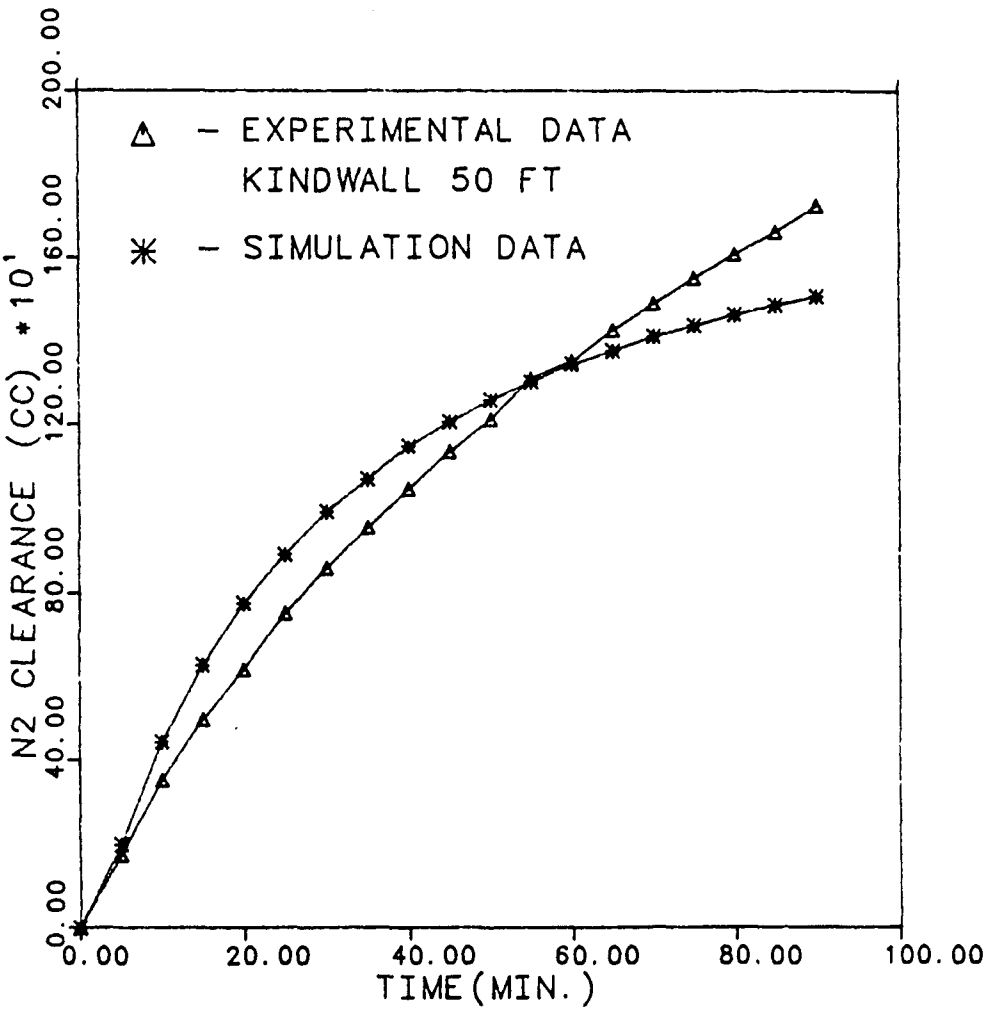

Fig. 6. Computed and measured nitrogen clearances while breathing 80:20 heliox at 50 fsw after breathing compressed air at 100 fsw for 40 min (6).

Comparison of computed and measured amounts of eliminated nitrogen is complicated in this case by several factors. One is that because the body weight and percent fat were not reported for the subjects, "reasonable" values had to be used. In addition, the chamber temperature was unknown. All of these factors are known to influence uptake and clearance of inert gas owing to a change in breathing gas properties.

In 1978, Low (7) repeated Kindwall's experiments with several important differences. The most significant was that Low's subjects were immersed to the neck in 35°C water instead of being in air. Another was that Low began to

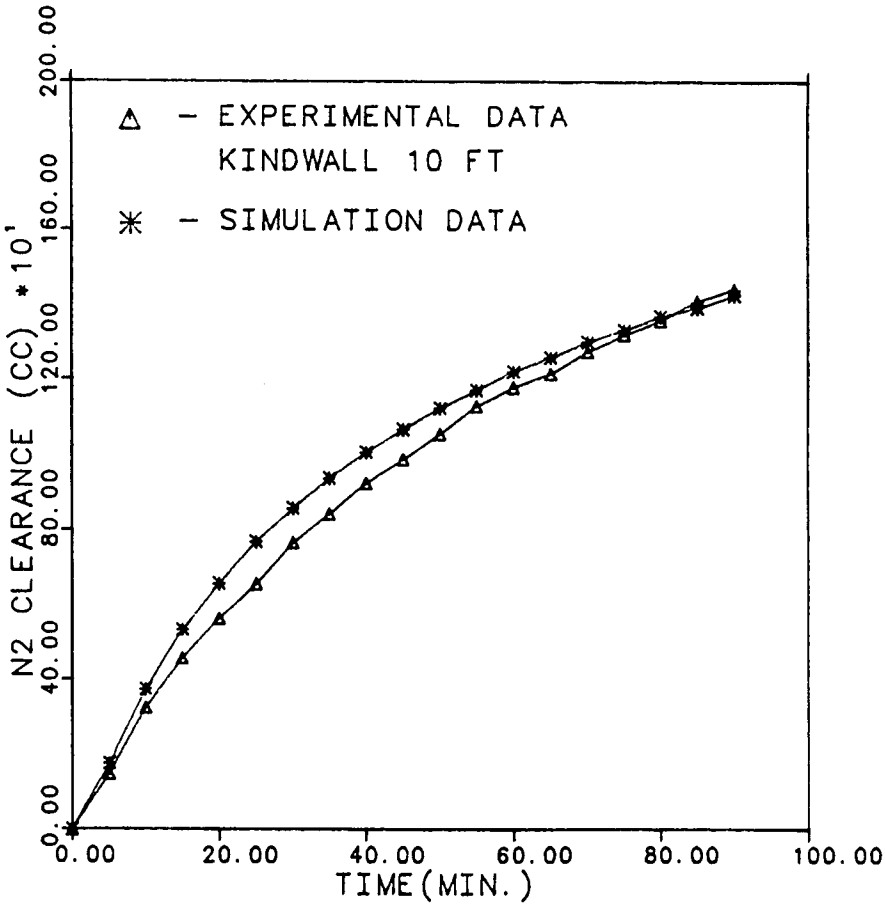

Fig. 7. Computed and measured nitrogen clearances while breathing 80:20 heliox at 10 fsw after breathing air at 100 fsw for 40 min (6).

collect expired gas after the 2-min transition period, instead of the 5-min interval used in the earlier experiments. Since the rate of elimination decreases rapidly during the first few minutes, minimizing loss of gas during the transition period is important.

Presented in Figs. 8-10 are computed results and mean measured nitrogen clearances for 1 subject who performed replicate dives under all three conditions. Excellent agreement between computed and measured values was obtained for the isobaric gas switch and decompression at 50 fsw, but computed clearances were consistently lower than measured values at 10 fsw. This particular subject exhibited a clear tendency toward increased clearance with decreasing depth, but 3 other subjects eliminated more nitrogen at 50 than at 10 fsw. As the values presented in Table 3 indicate, the model predicted that clearance should be only slightly greater at 50 than at 10 fsw.

Table 3
Measured and Computed Amounts of Nitrogen Cleared
During 90 Min of Decompression at Three Different Depths

	Kindwall's Study, ml at STP		Low's Study, ml at STP	
Depth, fsw	Experimental	Computed	Experimental*	Computed
10	1441**	1423	2314	1914
50	1726**	1509	2150	1996
100	1163†	1330	1953	1990

* Tabulated values are mean amounts measured for 1 subject (ML) who performed replicate dives at each depth. ** Average of five values reported by Kindwall et al. (6). † Average of two values reported by Kindwall et al. (6).

Table 4
Amounts of Separated Gas Remaining in Fat and Muscle
Following 90 min of Decompression at 10 fsw

Milliliter of gas at STP	Nitrogen	Helium
In muscles	39.6	128.2
In fat	12.7	91.8

The most significant feature of Low's study was his observation that immersion in 35°C water greatly increases the rate of nitrogen clearance, as observed previously by Balldin and Lundgren (8). While Low (7) speculated that enhanced clearance could be attributed to the effect of immersion on blood flow, it seems that thermal considerations are more important, because agreement between computed and measured values is reasonably good for both sets of experiments and the only significant difference between the two simulations was the higher temperature used in Low's case.

The third set of experiments to be discussed were conducted by Dick and Vann (9), who studied nitrogen elimination at the surface following standard (U.S. Navy Diving Manual), no-decompression, bounce dives to 60, 100, and 130 fsw. Bottom times while breathing air were 60, 25, and 10 min, respectively. Upon returning to the surface, subjects walked from the chamber to a closed-circuit breathing apparatus where nitrogen elimination was measured for 60 or 90 min with the subjects at rest; collection of expired gas began within 30 s after reaching the surface. Divers either rested or exercised moderately for 25, 15, or 5 min, depending on the bottom time. Three subjects participated in the study, but only 1 was studied at all three depths.

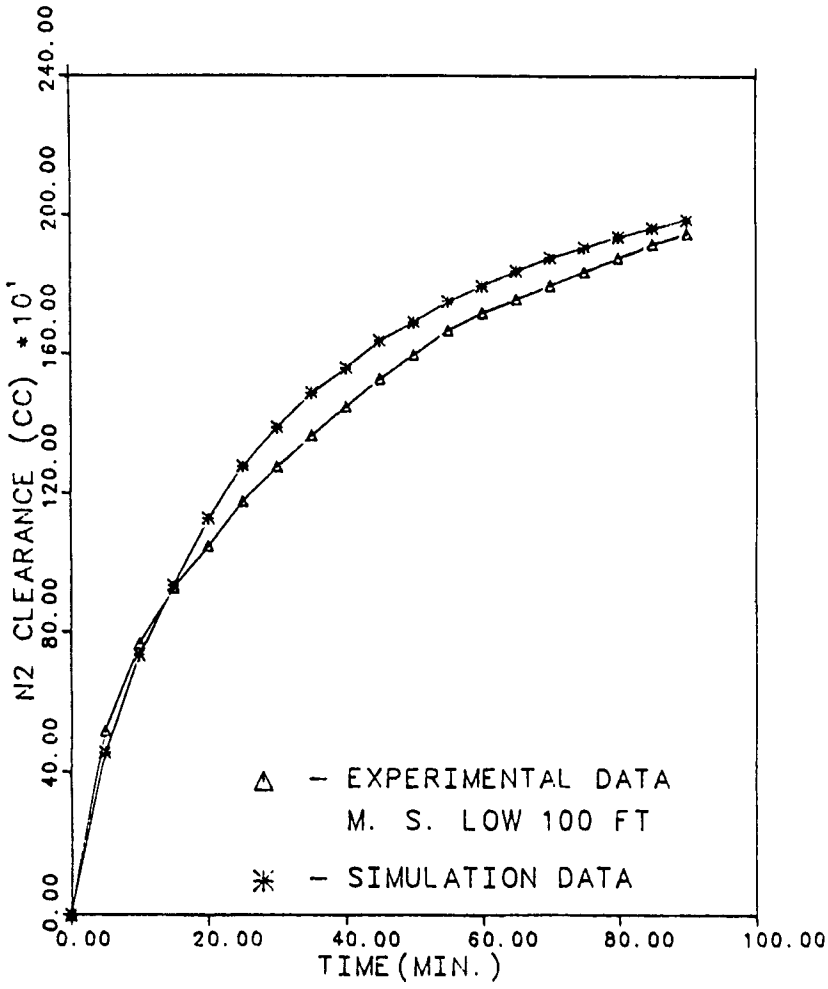

Fig. 8. Computed and measured nitrogen clearances while breathing 80:20 heliox at 100 fsw after breathing compressed air at the same depth for 40 min. Subject is immersed to the neck in 35 ° C water (7).

Computed and measured nitrogen clearances for the 3 dives in which no exercise was performed are presented in Figs. 11-13. Measured values are the means for the number of dives shown in Table 5. Data for this subject were quite consistent at 60 and 130 fsw, but at 100 fsw there was a range of approximately 200 ml in the total amount of nitrogen collected during the 60-min period.

The greatest discrepancy between computed and measured rates of clearance occurs for the 60-ft dive shown in Fig. 11. For the other 2 dives shown in Figs. 12 (for 78°F) and 13, there is excellent agreement between

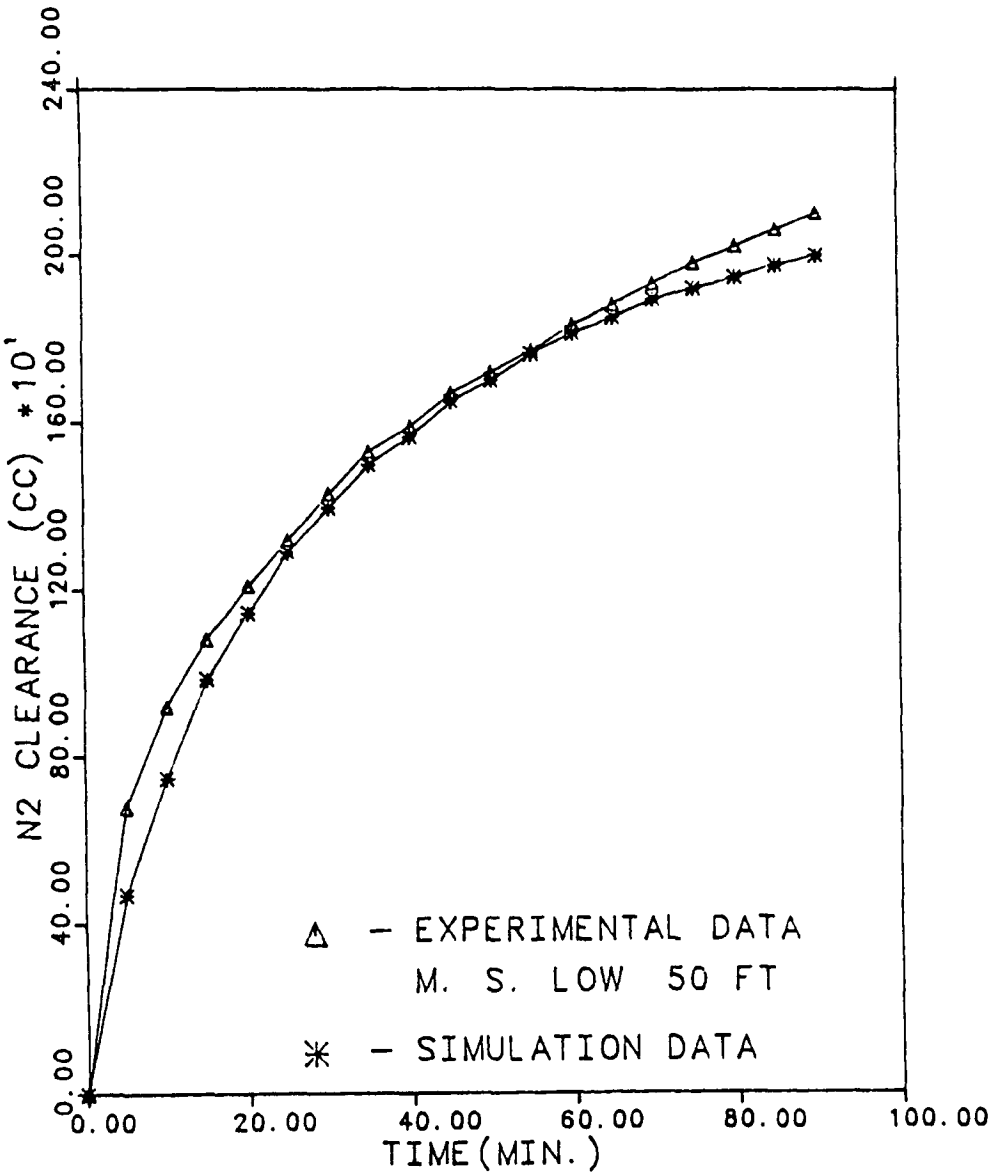

Fig. 9. Computed and measured nitrogen clearances while breathing 80:20 heliox at 50 fsw after breathing compressed air at 100 fsw for 40 min. Subject is immersed to the neck in 35 ° C water (7).

computed and measured values of the total amount of nitrogen collected, although all three computed curves have a somewhat different shape than the measured curves. In particular, computed rates of clearance tend to be too

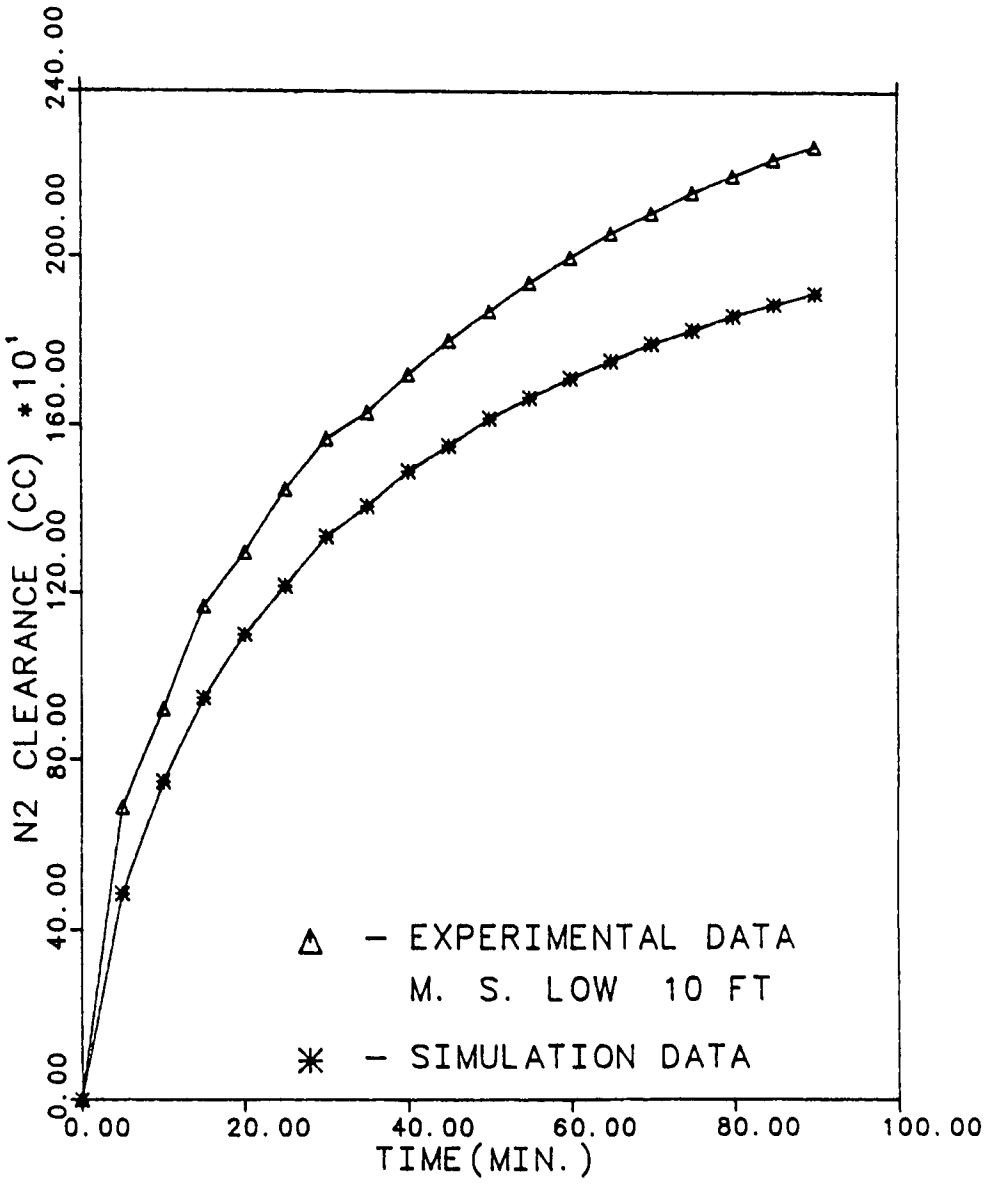

Fig. 10. Computed and measured nitrogen clearances while breathing 80:20 heliox at 10 fsw after breathing compressed air at 100 fsw for 40 min. Subject is immersed to the neck in 35 ° C water (7).

large during the first 6 min, too small during the next 25 min, and about right during the last 30 min. Measured clearance curves for some of the dives had a shape similar to the computed curves, but Dick and Vann (9) considered those cases to be exceptional.

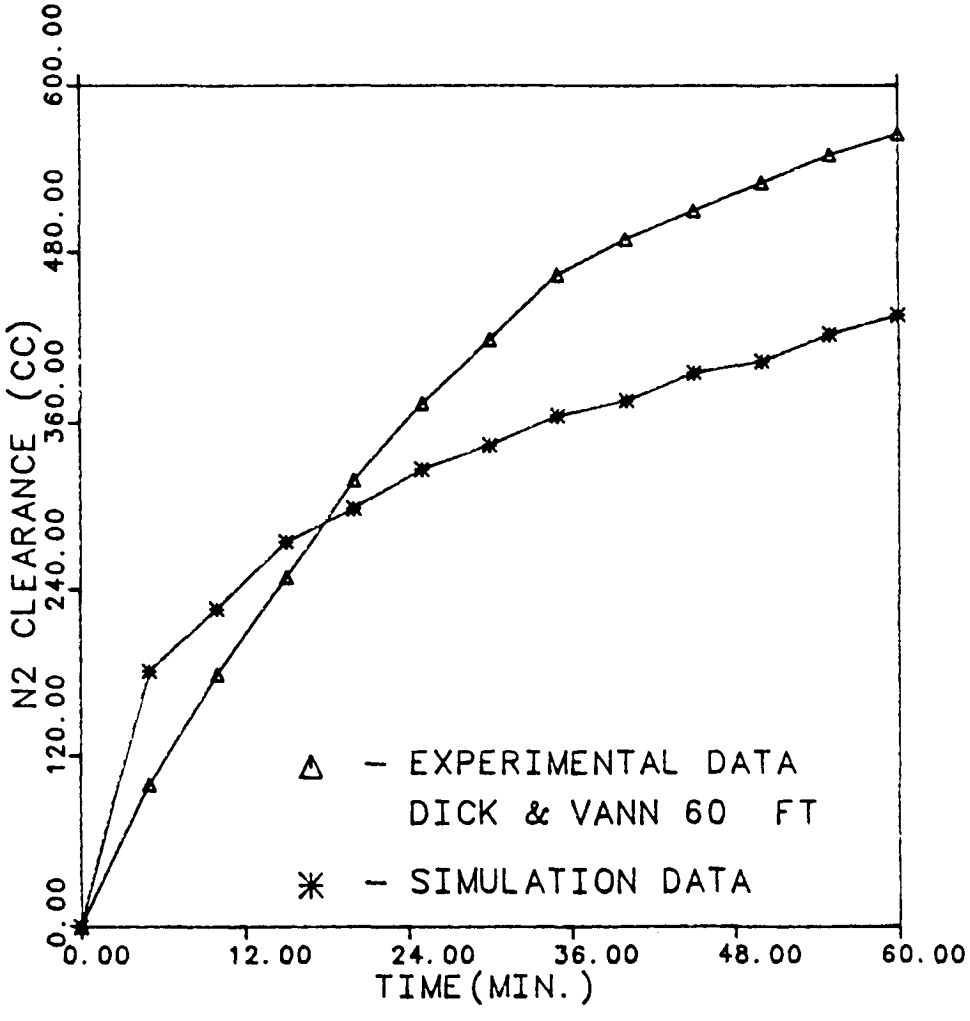

Fig. 11. Computed and measured nitrogen clearances during surface decompression following a bounce dive to 60 fsw for 60 min (9).

The rate of clearance is affected by a) the amount of gas dissolved during the dive, which depends on the body mass and percentage of fat, and the perfusion rate and diffusional resistance for each tissue; and b) bubble formation in tissue and blood. Values presented in Table 5 indicate that the amount of nitrogen dissolved is roughly comparable for the 3 dives, but the computed fraction cleared following the 60-ft dive is lower than for the other 2. This is readily explained by the greater bottom time at 60 ft, which allows

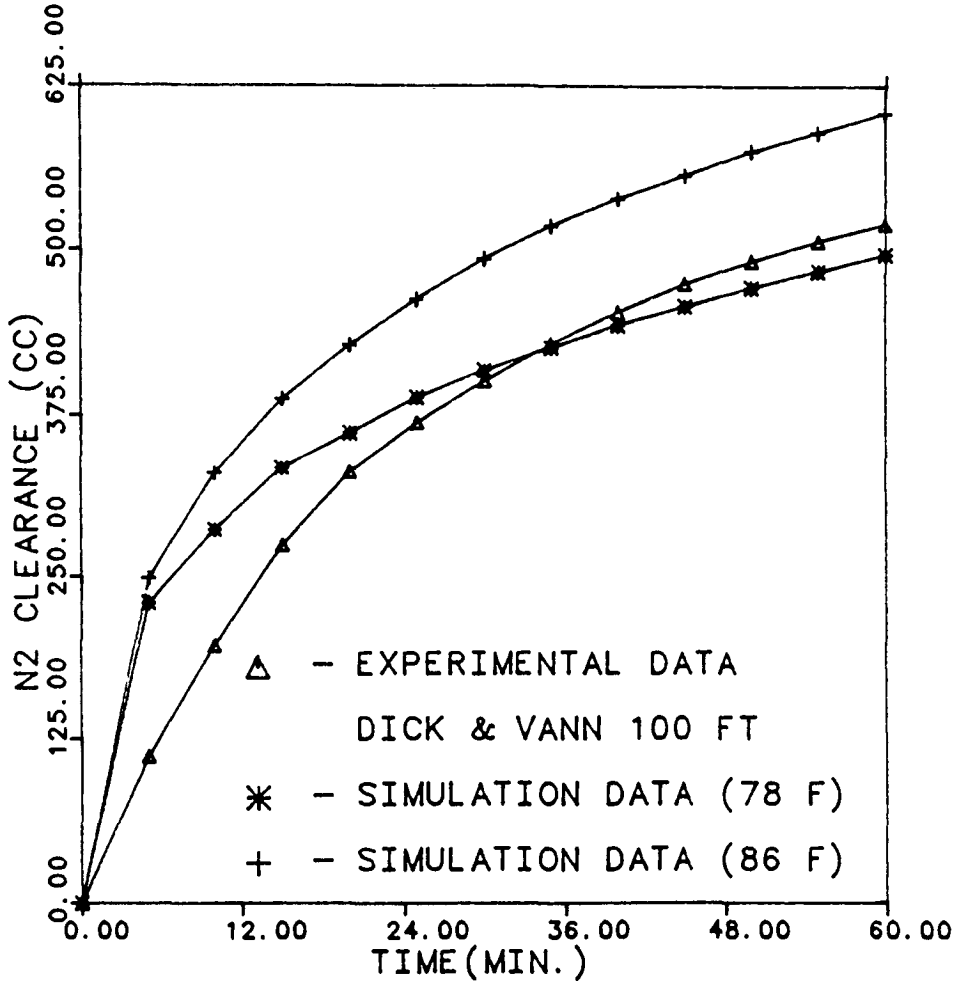

Fig. 12. Computed and measured nitrogen clearances during surface decompression following a bounce dive to 100 fsw for 25 min (9). Two different simulations are shown to illustrate the effect of ambient temperature.

more gas to dissolve in poorly perfused fat where it tends to remain for a longer time. However, there seems to be no obvious explanation for the experimental observation that the amount of nitrogen eliminated following the 60-ft dive is greater than for the other 2.

Additional computations were carried out to investigate the importance of several factors that influence inert gas transport. Probably the most important factor for these dives is extravascular bubble formation, which reduces the diffusional driving force from tissue to blood by lowering the gas tension in tissue. Figure 14 shows the rate of nitrogen clearance computed assuming no bubble formation in the 60-ft dive. When these results are compared with the

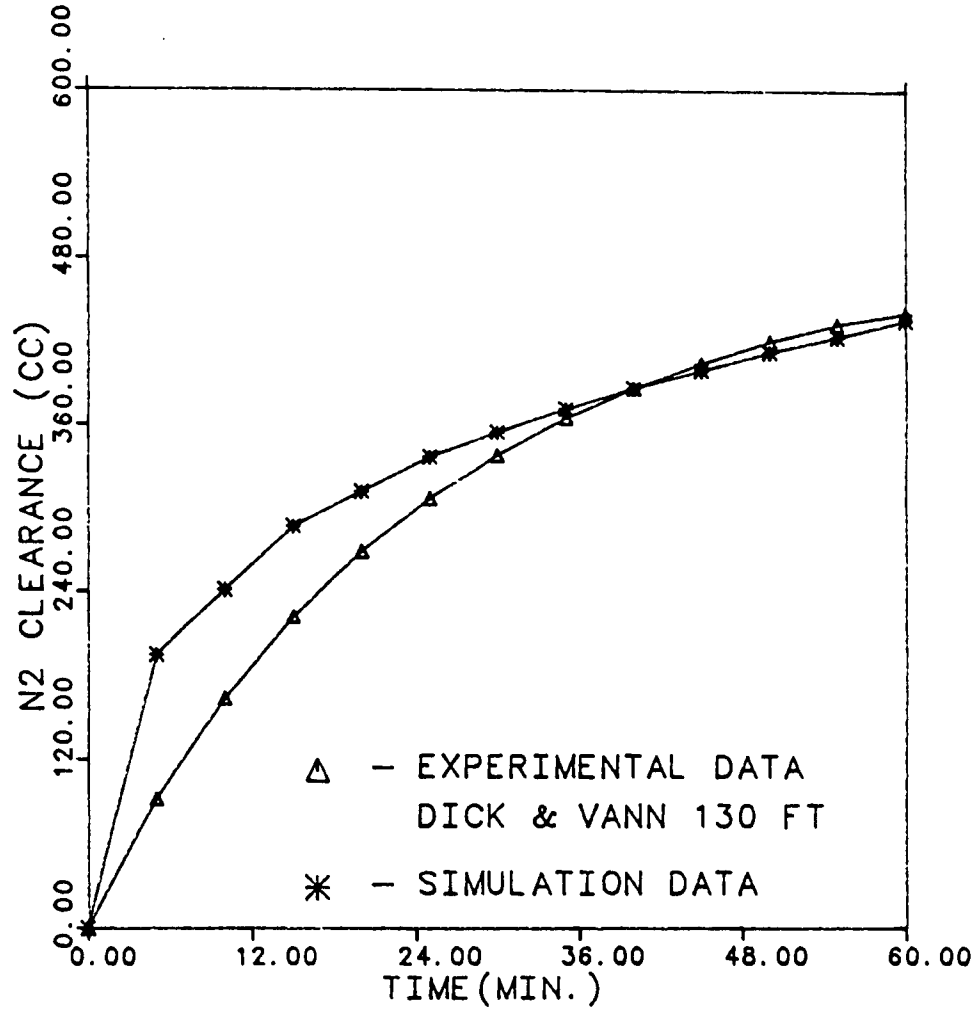

Fig. 13. Computed and measured nitrogen clearances during surface decompression following a bounce dive to 130 fsw for 10 min (9).

corresponding results with bubble formation in Fig. 11, it is clear that extravascular gas is probably present in these dives. Otherwise, the computed amount of nitrogen cleared would be considerably larger than the measured amount. Although the data are not shown in this paper, the effect of suppressing bubble formation was even more pronounced for the 100-ft dive. The reduction in computed rate of clearance at 5 min in all cases is a consequence of extravascular bubble formation.

The other computed curve in Fig. 14 shows the effect of reducing the density of gas nuclei in muscle from 60,000 to 6000/ml. With fewer nuclei present, separation of gas from tissue is inhibited because the interfacial

Table 5
Computed and Measured Values for Three Compressed Air Bounce Dives

Depth, fsw	60	100	130
Bottom time, min	60	25	10
Nitrogen elimination in 60 min, ml at STP			
Measured*	565 (3)[2]**	520 (6)	444 (3)
Computed	437	497	436
Dissolved nitrogen at the end of the bottom interval, ml at STP	2115	2236	1915
Amount of nitrogen in bubbles following 60 min of surface decompression, ml at STP			
In muscle	299	341	188
In fat	59	48	21
Total	359	389	209
Percent in muscle	83	88	90

* Mean values for 1 subject studied at all three depths. ** The number of subjects at each depth is shown in parenthesis.

surface area for transport is lower. Bubbles must grow larger to accept the same total amount of gas when the bubble density is reduced, and the surface area-to-volume ratio for a sphere varies inversely with the radius of a sphere. Furthermore, the asymptotic concentration gradient at the surface of a sphere varies inversely with the radius. Hence, bubble formation occurs more slowly and the inflection point in the clearance curve occurs somewhat later when there are fewer nuclei present, as the curves with bubble formation illustrate in Figs. 11 and 14.

As noted previously, temperature also influences uptake and clearance of inert gas through its effect on perfusion. This is illustrated in Fig. 12 where simulations are shown for environmental temperatures of 25.6°C (78°F) and 30°C (86°F). Although the actual chamber temperature was not recorded, it was believed to have been around 26°C. The enhancement of gas transport provided by higher perfusion largely offsets the inhibiting effect of bubble formation yielding a computed clearance curve that parallels the measured curve during the final 55 min of the experiment, although the markedly higher rate of the first 5 min is still present. These curves suggest that a combination of higher perfusion and more rapid bubble growth would yield computed clearances that agree reasonably well with experimental data for this particular set of dives.

The perfusion rate of active muscle also increases during exercise, and Dick et al. (9) demonstrated that nitrogen clearance is enhanced significantly by exercise at depth. The model exhibited similar behavior, although the computed increase in nitrogen clearance with exercise was generally somewhat

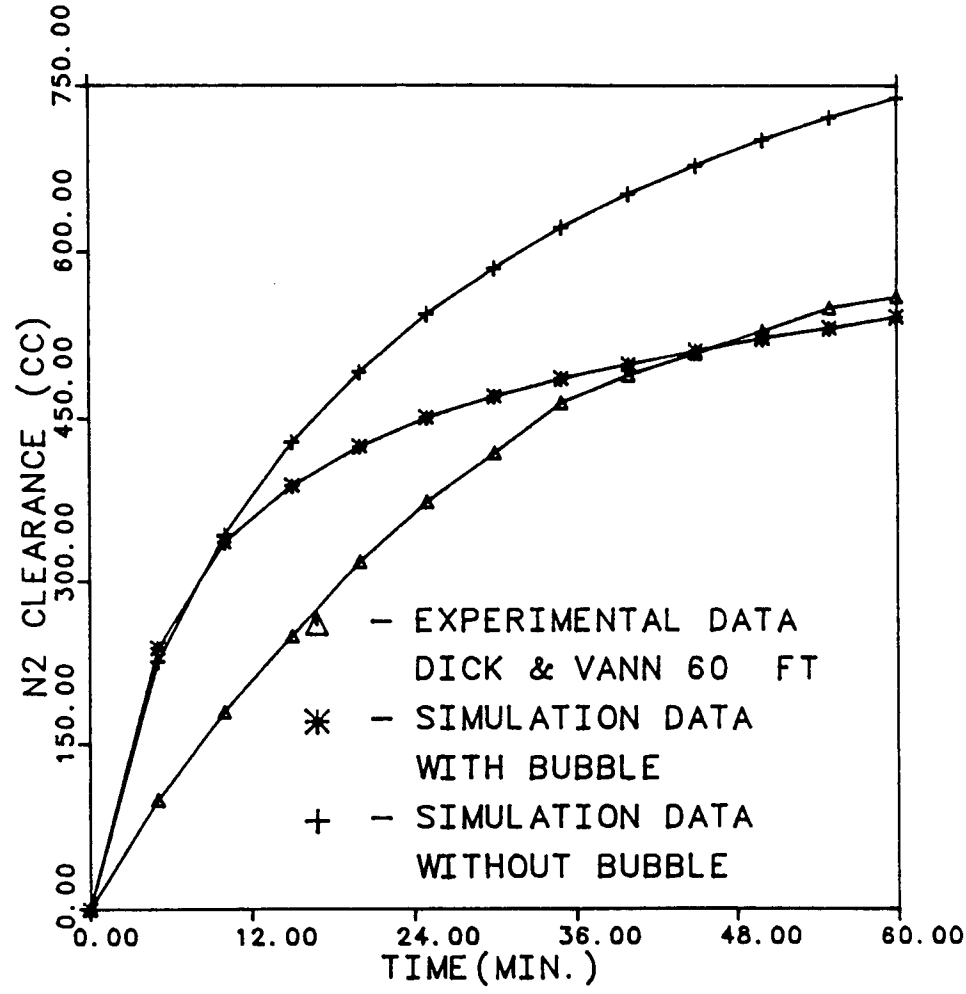

Fig. 14. Computed and measured nitrogen clearances during surface decompression following a bounce dive to 60 fsw for 60 min (6). These simulations illustrate the effect of suppressing extravascular bubble formation either totally, or partially by reducing the density of gas nuclei in muscle from 60,000 to 6000/ml.

smaller than the measured increase. However, the discrepancy could have been due to use of an improper metabolic rate, since the actual rate was not measured. It was characterized as being mild or moderate, but that allows considerable latitude for interpretation.

DISCUSSION

This is believed to be the first quantitative model of inert gas uptake and clearance in man. It is encouraging to see that a single set of parameters exists for which the model describes reasonably well 10 sets of experimental data obtained by four different investigators. These experiments ranged from an isobaric gas switch at 1 atm to a 10-min bounce dive to 130 ft. Of course, reasonable agreement needs to be defined, but since there is considerable variation in nitrogen clearances obtained from replicate experiments using a given subject (9), differences of no more that 10% between computed and measured values should be acceptable.

An important result of this study is that it supports the hypothesis that extravascular bubble formation often occurs during decompression, even though the diver experiences none of the symptoms of decompression sickness. This was established by comparing the results of two simulations—one in which bubble formation occurs when tissue becomes sufficiently supersaturated with dissolved gas, and another in which bubble formation is suppressed. The second simulation always yielded much greater nitrogen clearances than were measured, whereas the first simulation generally produced results in good agreement with measured clearances. Since simulations without bubble formation produced results consistent with measured clearances for isobaric gas switches, one would expect equally good agreement for decompressions if, in fact, there were no bubble formation. It is recommended that allowance for the asymmetry introduced into uptake and clearance curves by bubble formation during decompression be incorporated into any phenomenologic decompression models developed in the future.

This study also provides information about the effect of percent body fat, environmental temperature, and exercise on inert gas uptake and clearance. It was found that nitrogen uptake and clearance increase with increasing body fat, temperature, and level of exercise, although additional systematic studies will be required to define the precise nature of those relationships. Unfortunately, there have been no quantitative experimental studies of those parameters, and theoretic studies of the kind described in this paper are severely limited by a paucity of experimental data. In our opinion, complementary experimental and theoretical studies of those factors would lead to improved understanding of inert gas transport and bubble formation driven by changing environmental pressure.

Although the model was not developed for use in constructing decompression tables, the values of such quantities as maximum bubble diameter and amount of gas separated from solution were reviewed to see whether any of them yield obvious criteria for predicting the onset of decompression sickness. There was reasonable consistency between maximum bubble radii for a series of dives in a given class, such as the 3 safe, no-decompression, bounce dives employed by Dick et al. (9), but bubble size did not seem to be very sensitive to dive parameters such as bottom time at a given depth. For example,

increasing the bottom time at 60 fsw from 60 to 90 min caused only minor increase in the maximum bubble radius.

There was also little consistency across classes. In particular, it appears that different processes must be involved in the occurrence of decompression sickness following a bounce dive and during decompression from saturation. The slow rates of ascent required for decompression from saturation allow ample time for clearance of inert gas from relatively well-perfused tissue in which gas preferentially dissolves during a bounce dive. For that reason, an unperfused tissue element representing ligament or tendon was added to the model, which did indeed introduce a very long time constant.

Bubbles formed in ligament during the 21-h decompression from saturation at 60 fsw, which was observed by Thalmann (10) to cause decompression sickness in 4 of 10 divers, whereas no bubble formation occurred when a safe, 36-h profile was used. However, when the same parameters were tested against the no-stop decompression from saturation at 25.5 fsw found to be marginally safe by Eckenhoff et al. (11), profuse bubble formation occurred in muscle, fat, and ligament. When the surface tension controlling the onset of bubble growth was increased from 12.6 to 18.1 dyn/cm, bubble formation was prevented during decompression for 25.5 fsw, but still occurred during decompression from 29.5 fsw, which was observed by Eckenhoff et al. (11) to cause symptoms. Using a larger surface tension did not totally compromise agreement with the Thalmann study (10), and it is possible that one could find a set of parameters that yield results consistent with a number of decompression studies. However, since that was not the principal purpose of this study, it was not pursued.

It may be useful to speculate about useful modifications that could be made in the model. One possibility is to introduce a mechanism for recruiting additional gas nuclei as the degree of supersaturation increases, which is one of the principal postulates of Yount's varying-permeability model (12). However, when that concept was tested by adding a second set of gas nuclei having a smaller radius than the first set, it was found that only the larger nuclei grew during decompression; the resulting transfer of dissolved gas from tissue to the growing bubbles reduced the gas tension enough to prevent growth of the smaller nuclei. Of course, during rapid decompression, a range of nuclei could grow because bubble growth occurs at a finite rate, presumably with a time constant of order minutes (13). Nevertheless, it is not clear that starting with a range of initial nuclei sizes will greatly alter the rate of inert gas clearance when the dynamics of bubble growth and its effect on residual dissolved gas tension are taken into account. For example, it was shown earlier that reducing the density of nuclei in muscle from 60,000 to 6000/ml caused only a 20% increase in the amount of nitrogen eliminated in a 60-ft, 60-min bounce dive.

Another possible modification is to account for diffusion of inert gas through the skin. Behnke and Willmon (14) commented on the possible importance of that phenomenon in their 1941 paper, and Low's motivation for immersing his subject to the neck was to prevent nitrogen absorption through

the skin (7). The model could be adapted to include diffusive transport of inert gas through the skin and between various tissue elements owing to macroscopic concentration gradients, but that would be a major effort and the resulting program could only be run on a supercomputer.

CONCLUSION

It has been established in this paper that rational mathematical analysis is capable of describing the principal features of inert gas uptake and clearance in man owing to changing concentration of breathing gas. In general, the discrepancy between computed and measured nitrogen clearance for the 7 cases considered was no greater than 20%, and in many cases agreement was excellent. Although experiments involving inert gas exchange during bounce dives do not provide the kind of detailed information necessary to identify specific processes responsible for decompression sickness, they do help to discriminate between various hypotheses concerning gas transport and bubble formation in tissue. They also serve to establish the values of such important parameters as the level of supersaturation necessary for extravascular bubble formation.

Additional detail can be incorporated into the model, but it would only be profitable to do so if additional experiments were performed under well-controlled conditions with careful attention to important parameters that are now known to influence inert gas transport, but have been somewhat neglected in the past. These include percent body fat, the thermal state of the diver, orientation of the diver (upright or supine), and the level of exercise. Since it is known that an individual's level of hydration affects cardiovascular performance (15), that could be an important variable under certain circumstances. Dick et al. (9) also speculated about the possible importance of level of fitness. Hence, there remain many useful experimental studies that need to be undertaken, and if they are done in conjunction with theoretic analyses of the kind described in this paper, our understanding of basic phenomena relating to inert gas transport in man should improve.

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CHANGES IN EEG TOPOGRAPHY INDUCED BY COMPRESSION WITH HYPERBARIC HELIUM-OXYGEN GAS

J. Tatsuno, H. Ohiwa, and K. Ozawa

Electroencephalographs in hyperbaric environments during saturation dives were recorded with relatively few electrodes in early studies (1). This restricted observation to areas around the electrode positions. The development of multichannel recordings reinforced the findings, but recently, topographic studies using two-dimensional isopotential maps, which are calculated by a mathematic interpolation method (2, 3), have been providing more extensive information during compression (4, 5).

To find interrelations among changes in the amplitudes of alpha, beta, and theta activities, correlation mapping is useful. Evidence of changes in alpha and theta activities has been reported previously (5), but the findings in this report describe the beta activity and disclose EEG changes as a whole.

It is possible to evaluate objectively the differences in topographic patterns of two different stages using two sets of coefficient parameters (CP) of the individual maps which are obtained by Unbiased Polynomial Interpolation (UPI). The UPI method was developed in the Department of Physiology of the National Defense Medical College (6). The results of this evaluation using CP add new aspects to the topographic changes.

The purpose of this investigation was to identify EEG characteristics induced by 7 ATA compression, which should be different from high pressure nervous syndrome (HPNS) because of the lower pressure in this experiment. However, these characteristics are important for estimating the mental state of a subject in a hyperbaric environment which influences him physically and psychologically. Clarification of the EEG characteristics induced by higher pressures can be expected by the topography technique described in this report and it may help to define a prodrome of HPNS. The topographic study has the advantage of evaluating all areas of the brain surface where changes might appear.

MATERIAL

The subject was a 44-yr-old, healthy petty officer who volunteered for a saturation dive to 200 ft in a helium-oxygen environment. He had no saturation dive experience but had worked several times as an operation assistant of a hyperbaric chamber. Neurologic examination showed no abnormalities. His preexperiment EEG contained the least artifacts among the subjects evaluated for the experiment.

METHODS

Electroencephalograph recordings were made at a fixed time (2:00 p.m.) but on the compression day it was done immediately after arrival at 7 ATA (11:30 a.m.). The rate of pressure increase was 3 ft/min, but the helium inflation was temporarily interrupted twice.

The EEG was recorded monopolarly (using linked earlobes as reference) from 12 locations: Fp1, Fp2, F7, F8, C3, C4, T5, T6, O1, O2, Fz, and Pz according to the international ten-twenty method (Fig. 1).

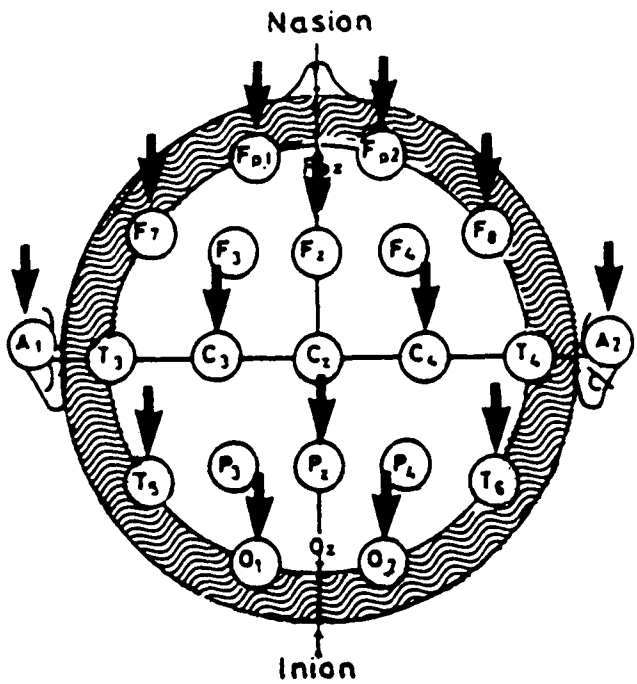

Fig. 1. Electrode position is shown by arrow. The following topographic maps show the area inside the shaded zone. In all maps in the following figures, above is frontal and below is occipital.

The subject lay on a bed with eyes closed for 3 min. An eye-open resting EEG was also recorded for 3 min but the results will be reported in another paper.

Electroencephalograph data were digitized with a 12 bit A/D converter at the sampling rate of 128 Hz. Fast Fourier Transform was made with 0.25 Hz resolution, using 4-s epochs of EEG. The amplitude of A frequency was calculated as an average of the root mean square of the powers of A -0.25, A, A +0.25, and A +0.5 Hz. Epochs contaminated by the artifacts were discarded with the aid of a computer or by visual inspection. Individual isopotential maps were obtained from the selected epochs by UPI (2) which yielded 10 coefficients of the polynomial (CP). Amplitudes of 103 × 103 points in an isopotential map, f(x,y), are expressed in our interpolation as follows:

$$f(x,y) = a_1 + a_2x + a_3y + a_4x^2 + a_5xy + a_6y^2 + a_7x^3 + a_8x^2y + a_9xy^2 + a_{10}y^3.$$

Therefore 10 CP (a_1 - a_{10}) represent one pattern of an isopotential map.

To find a general tendency from the incessantly changing patterns of individual maps, the CPs for a certain period were averaged to get an *average map.*

For investigation of the interrelation between two different frequency bands, correlation coefficients were calculated from the corresponding amplitude values at 103 × 103 points in the isopotential maps, resulting in the production of a *correlation map.* The value of a point in the correlation map should be 1.0, if the amplitudes of the two frequency bands change completely in parallel at the point, and -1.0 if they change completely reversely.

The Mahalanobis distance (Q value) in multidimensional space of the 10 CP was introduced for objective evaluation of the difference of the topographic patterns between the maps recorded on different days. The larger the Q value, the greater the dissimilarity in topographic patterns. The comparison of the Q values becomes inaccurate when the numbers of the 2 groups of data are small. Numbers of the data were sufficiently large in this experiment, ranging from 60 to 75. (The small difference in the numbers was due to the discarded epochs.)

RESULTS

Average Maps

Average maps of beta, beta-1, and beta-2 bands are shown in Fig. 2. They were calculated from the 3-min recordings of resting EEGs with the eyes closed.

In the control EEG recordings, the maximal activity of the beta band (13-25 Hz) was found in the left frontopolar area, which was surrounded by a large value zone and a small amplitude zone spread widely in the left centroparietal area. The topographic pattern of the beta-1 band (13-19 Hz) was

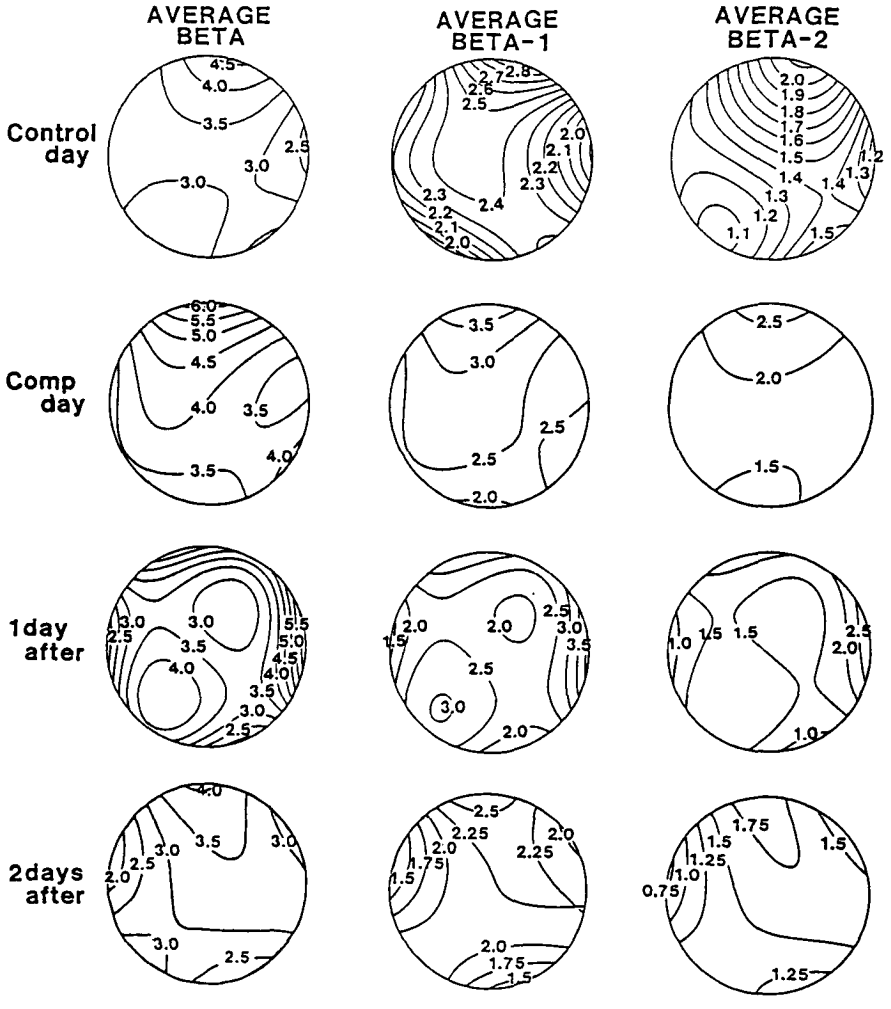

Fig. 2. Average maps of beta band (13-25 Hz), beta-1 band (13-19 Hz), and beta-2 band (19-25 Hz) on control day, compression day, and 1 and 2 d after compression. Numbers on isopotential lines show root mean square amplitude in microvolts. When the lines are crowded, some numbers are skipped.

similar to the pattern of the beta band. The beta-2 band (19-25 Hz) was similar to both the beta-1 and beta bands except that the smallest amplitude zone was observed in parietal midline area.

On the compression day the beta band activity increased remarkably in the frontopolar midline area but there still remained asymmetry (amplitude was smaller in the left hemisphere than in the right and the smallest amplitude area existed in the left parietal area). Beta increase was more prominent in the beta-2 band than the beta-1 band.

The high amplitude of the beta band continued or showed slight additional increases even 1 d after the compression, and the increase was accompanied by a frontopolar symmetrical appearance. The smaller amplitude zone was observed in the left temporal and right occipital areas, which remained the same value or decreased slightly from those of the compression day. Two days after compression the amplitude decreased, especially in the frontal area, with simultaneous disappearance of the symmetry. Amplitude values, as a whole, returned to those of the control day but the pattern was different.

It should be noted that the average topographic maps of the beta-1 and beta-2 bands showed changes similar to those of the beta band. This implies that the beta band can be treated as a whole and does not need to be divided into two bands, such as high and low beta.

Correlation Maps

Topographic changes in the correlation maps of alpha vs. beta, beta-1, and beta-2 bands on control day, compression day, and 1 and 2 d after the compression are shown in Fig. 3.

One of the remarkable features on compression day was the frontopolar symmetrical appearance of the large correlation coefficient values with abrupt decreases toward the central area to zero or minus values. This may be called a *transverse pattern*, whereas on the control day the map had a *longitudinal pattern*, where the large value spread from frontal to occipital areas with a rapid decrease toward the temporal areas on both sides. One day after compression, these patterns disappeared and a high value of 0.7 was found only in a small area of the right anteriotemporal. Two days after compression, correlation values in the whole area diminished and the zone of highest value of 0.4 was found in the left occipital area. The correlation maps of the beta-1 and beta-2 bands were quite similar to the maps of alpha vs. beta bands, and again this implies that the beta activity can be treated as a whole.

The four maps of Fig. 4 are correlation maps of beta vs. theta activity. The maps of control day and compression day were similar to those of beta vs. alpha with almost the same high value of around 0.8 in the frontopolar area, but in the map of compression day the curves were more irregular, and in the occipital area the value dropped to -0.4 (that was the lowest correlation value among the maps). One day after compression, the correlation map showed fairly symmetrical patterns with high correlation values in the frontopolar and occipital areas, but the next day the pattern disappeared completely.

Q Values

Using 10 coefficients of polynomial (CP), Q values between average maps of the beta, beta-1, and beta-2 bands for six combinations (between control day and compression day, between control day and 1 d after, between compression day a 1 d after, etc.) are shown in Table 1.

The largest Q value was found in the distance in the multidimensional space of the beta-1 band between control and compression, calculated from CP

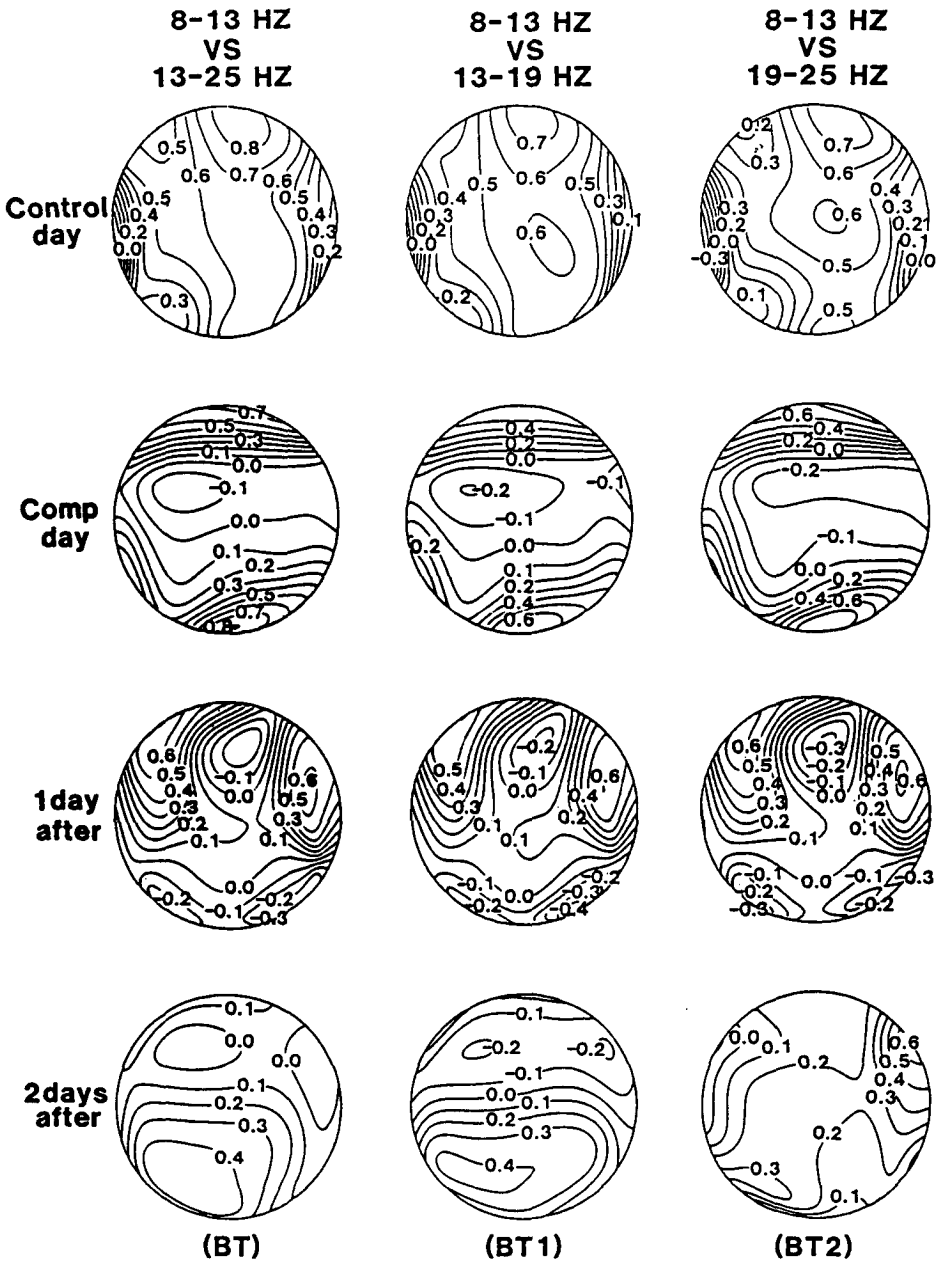

Fig. 3. Correlation maps of alpha vs. beta, alpha vs. beta-1, and alpha vs. beta-2 bands. Numbers on the lines show correlation coefficient values.

of individual maps. The Q values of the beta-1 band were generally the largest of the three bands. The smallest Q value was found in the distance of beta-2

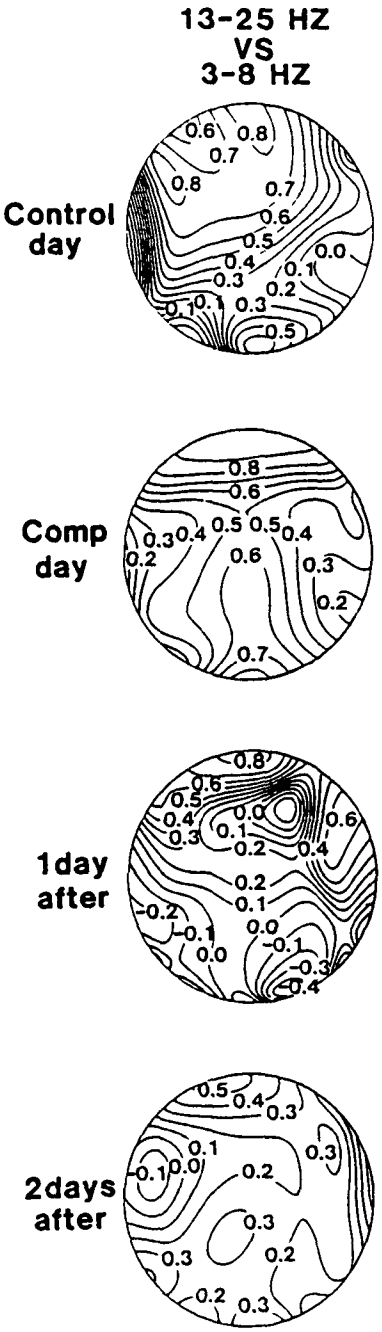

Fig. 4. Correlation maps of beta vs. theta band on control, compression, and 1 and 2 d after compression.

Table 1
Q Values of Beta, Beta-1, and Beta-2 Band in Six Combinations.

	Compression	1 d After Compression	2 d After Compression
Beta Band			
Control	0.971	0.828	0.991
Compression		0.484 (A)	0.977 (B)
1 d After compression			0.531 (C)
Beta-1 Band			
Control	2.380 (A')	2.320 (B')	1.099
Compression		1.771 (C')	1.034
1 d After compression			1.996
Beta-2 Band			
Control	0.679	0.691	0.591
Compression		0.221	0.836
1 d After compression			0.589

maps between compression and 1 d after compression. The Q values were relatively small in the beta-2 band. However, there are small differences between maximum and minimum Q values in the table, indicating that there are slight dissimilarities between topographic patterns. Nonetheless, some discrepancies occur between the Q values and visual comparison of the average maps in Fig. 2. The reason will be referred to in the discussion.

The relation between the three values of *beta band* table should be noted: compression vs. 1 d after compression (A in Table 1), compression vs. 2 d after compression (B), and 1 d vs. 2 d after compression (C). Suppose value A plus value C is almost equal to value B. This result indicates that the same direction of pattern change continued from compression day to 2 d after compression. The same relation was observed in the corresponding place in the *beta-2 band*. The *beta-1 band* table, the Q value of control vs. compression (A') was almost the same as control vs. 1 d after the compression (B'), so the addition of the A' to the Q value of compression vs. 1 d after compression (C') was too large toward B'. In this case the direction of pattern change in A' was considered to be diverted in C'. This case was observed in the corresponding place in the beta and beta-2 bands.

DISCUSSION

As mentioned in the previous report (4), the subject showed no apparent mental abnormality during compression. This was demonstrated by several tests employed before and after EEG recordings. Peculiar emotional behavior such as a fit of laughter was not observed. No tremors, convulsions, or vomiting were observed. The subject also did not report drowsiness during EEG recordings.

There were at least three kinds of EEG changes with different time courses: a) Sudden appearance and quick disappearance on the compression day; b) sudden appearance on compression day and gradual disappearance; and c) delayed appearance 1 or 2 d after compression. Differences in the time courses should correspond to differences in the human mechanisms which were influenced by compression. The first mechanism relates to the acceptance of transient change, such as cessation of gas inflation, or to quickly adapting central nervous system function. Symmetrical frontopolar appearance of alpha amplitude (SFPA) and symmetrical frontopolar pattern in alpha-beta correlation map (SFPP-Cab) belong to this mechanism. The second mechanism relates to a slowly adapting function, in which beta and theta amplitudes increase and symmetrical frontopolar appearance of theta amplitude (SFPT) was included. The third mechanism relates to a slowly developing phenomenon or reactions that occurred on a specific day. Symmetrical frontopolar appearance of beta amplitude (SFPB) and symmetrical frontopolar pattern in beta-theta correlation map (SFPP-Cbt) could be classified in the third category.

From the short appearance of SFPA immediately after the arrival at the target pressure, the pattern seems related to the relief from the psychologic tightening of increasing pressure. SFPT and amplitude increase in beta and alpha as well as in theta bands in the frontopolar region might be triggered by the same mechanism, but they lasted longer than SFPA. Therefore, they may be elicited by the mechanism to try to adapt to the new environment of 7 ATA and the mechanism endures for a long time. Beta increase accompanied by alpha increase was apparently a different phenomenon from the so-called desynchronization. Why SFPB appeared 1 d after compression is difficult to explain, but the existence of different neural mechanisms for SFPT and SFPB was undeniable.

In the recordings on control day, symmetrical frontopolar amplitude augmentation rarely appeared, so we concluded that the pattern is a specific one to a certain brain condition. The distinction of SFPT from Frontal Midline Theta (FMTH) (7, 8) was discussed in the previous report (4), but to summarize, SFPT is not identical with FMTH from the viewpoints of different topographic maximum points (SFPT in Fpz, FMTH in Fz) and of no occurrence of a burst form in the former. SFPT was observed in mental calculations, especially when the job was smoothly carried out (9). Therefore, the pattern may be related to a psychologic condition of concentration with confidence or satisfaction.

Obviously, theta increase in the frontopolar area in this report has no

relation to HPNS, because theta increase in HPNS is accompanied by a decrease in alpha and beta activities (1). It can be concluded from the similarity in topographic patterns of the beta-1 and beta-2 bands to the beta band that the beta band was influenced by compression as a whole. This implies that the effect of hyperbaric gas on spindle mechanism (which produces fast waves of a certain frequency), for example, can be denied.

Among the 10 CP, a_1 (constant term) seemed to be the dominate influence on the Q value, so that large Q values were more easily found in the bias difference (bias = constant) rather than configuration difference such as asymmetry or peak location. Q value and human inspection might give some discrepancy, one of the reasons being that the former deals with individual maps and the latter is the result of averaging them. But scrutiny into the reason and elaboration of the calculation process of Q values must be carried on in the future.

SUMMARY

A 12-channel EEG of a 44-yr-old petty officer was recorded during a saturation dive to 200 ft by inflation using helium and oxygen. The data were investigated by means of two-dimensional maps for average and correlation.

Beta augmentation in the frontopolar area had the same topographic pattern for beta band (13-25 Hz), beta-1 band (13-19 Hz), and beta-2 band (19-25 Hz). Correlation maps of these three bands vs. the alpha band also showed similar topographic patterns. Therefore, it was found that the beta band could be treated as a whole.

Beta augmentation in the frontopolar area was accompanied by slight alpha augmentation on compression day. Therefore, this change was not simply due to desynchronization. The symmetrical frontopolar pattern of the beta band appeared 1 d after compression, whereas the same patterns of alpha and theta bands appeared on compression day. The difference of the time course seemed to indicate different underlying neural mechanisms.

Q values were generally large in the beta-1 band and small in the beta-2 band. In some cases, continuity of the change in multidimensional space was observed.

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DEVELOPMENT OF DECOMPRESSION PROCEDURES FOR UNDERSEA HABITATS: REPETITIVE NO-STOP AND ONE-STOP EXCURSIONS, OXYGEN LIMITS, AND SURFACING PROCEDURES

R. W. Hamilton, D. J. Kenyon, and R. E. Peterson

Techniques developed by undersea research programs of the U.S. National Oceanic and Atmospheric Administration (NOAA) have made it possible for aquanauts to perform vertical excursion dives from undersea habitats. These excursions can cover a useful range of depths and times, and are necessary to make habitat diving practical. They have the advantage of air as the breathing gas, although the divers may live in either air or a nitrox (nitrogen-oxygen) gas atmosphere.

In 1973, the project known an "NOAA OPS" resulted in laboratory-validated procedures for excursing both above and below the depth of the habitat or "storage depth" (1). However, shortly after that report the need for several enhancements had become obvious, specifically the capability to make repetitive excursions and to do longer excursions requiring decompression stops (2). Since then an even more demanding need has surfaced: for an effective but still safe means of managing pulmonary oxygen exposure. Currently the NOAA Diving Manual imposes stringent (but somewhat arbitrary) limitations of exposure to oxygen (3). All of these would lose their operational effectiveness without a reliable and operationally convenient method of performing the final saturation decompression.

This paper reports a new development program for habitat diving performed for NOAA's Office of Undersea Research that includes:

- No-stop descending excursions with repetitive capability.
- Beneficial adjustments for a repetitive excursion after one of less than the maximum allowable duration.
- One-stop excursions that allow longer bottom times.

- Efficient management of oxygen exposures.
- Surfacing techniques, including saturation decompression.
- Procedures for treatment of decompression sickness.

Currently, marine scientists using NOAA procedures can saturate in habitats at depths from 30 to 120 fsw, and are allowed to excurse to depths ranging from the surface to 250 fsw depending on the location of the habitat. (fsw = Feet of sea water. A foot of sea water is defined as 1/33 of a standard atmosphere, and therefore equals 3.0705 kPa. U.S. conventions and contract requirements dictate the use of this unit. 1 fsw = 0.30705 [meters of sea water] where the msw is defined as 1/10 bar or 10^{-4} pascals.) An extensive coverage of the depth range appropriate to each storage depth is possible, but there are limitations based on both decompression and oxygen exposure. A detailed comparison of the capabilities of the new procedures with those currently possible is given elsewhere in this symposium (4).

The new procedures are contained in a contract report prepared for NOAA (5) and are summarized here. A test program (informally labeled REPEX) is in progress that will provide initial, formal, validation of the procedures before controlled introduction into the field.

METHODS AND RESULTS

Calculation Method

The task involved as a first step is the assessment and reevaluation of the current NOAA excursion procedures. Although the excursion diving reported in the literature has not been particularly stressful from a decompression point of view, the reports that are available do not include any decompression problems from use of the NOAA OPS procedures. One reported case of decompression sickness following an excursion was in the workup dives performed at Duke University for the SCORE operation (2). This was an excursion to 300 fsw of 60 min from a saturation storage depth of 60 fsw. It was calculated especially for that program, and used the same method as was used for NOAA OPS, so there was reason for us to take another look at this method.

The method used for NOAA OPS was the classic Haldane-Workman-Schreiner technique (1, 6), versions of which have been used for calculating most of the existing decompression tables, and which has survived a critical assessment (7) in this mode of use. This "Neo-Haldanian" technique has limitations, but it furnishes a superb means for transferring empirical data from yesterday's experience to tomorrow's procedures. It is the basis of most current decompression tables, and of the comprehensive "DCAP" Decompression Computation and Analysis Program we have developed for doing decompression computations (8); DCAP was used for this job. The program keeps track of the gas loading in hypothetical "tissue" compartments, and allows the diver to ascend when the gas loading in each compartment is less than an empirically determined limiting value (M value) for that compartment.

The "matrix" of M values sets the ascent limits, and along with the configuration of half times sets the pattern of the tables generated. The new one was derived from the one used for NOAA OPS, modified to account for the SCORE experience and to correct an aspect considered troublesome since NOAA OPS. Because the NOAA OPS matrix was shaped empirically as a result of the data available, there was an area right in the middle that was quite uneven. From the compartments and depths involved in the SCORE table it appeared that that area should be smoothed and the adjacent M values reduced.

We first adjusted the half times to progress evenly and geometrically through eight compartments from 5 to 640 min; these half times had all been included in the NOAA OPS matrix, but by doing some sample calculations we determined that (in our opinion) the extra intervening ones used by NOAA OPS were neither necessary nor beneficial. The half times are:

NOAA OPS:

5	10	20	40	80	120	160	200	240	320	480	640	720	1000	1240

NOAA Repetitive Excursion Tables (REPEX):

5	10	20	40	80	160	320	640	

An ascent-limiting matrix can be represented by a "base" or surfacing value of the nitrogen gas loading that prevails when it is safe to ascend from 10 fsw, and a "slope" of the change in M value per foot of depth. As a base, we set the 10-fsw values to give times close to those of the USN no-stop tables. We next "smoothed" the uneven section of the NOAA OPS matrix in the conservative direction. There seemed to be a natural break in the M values at 70 fsw, so the matrix was allowed on "expand" from the surface to that point, then to progress without further increases. These changes were directed toward the calculation of excursions that return to a saturation storage depth, not air-nitrox dives from the surface. The new matrix is designated MF0805, and is shown in Table 1. We calculated all the REPEX tables against these new constraints.

The Concept for Calculating Repetitive Excursions

Our first step in calculating repetitive no-stop dives (excursions) was to evaluate what effects the 1st dive may have on the 2nd or subsequent dives, and determine how to allow for this in the calculations. We began with the premise that the 1st dive in a repetitive pair affects the 2nd one in at least three definable ways. The first effect is that the diver's body takes on a gas loading as a result of the 1st dive, and that some of this gas may still be present when the next dive starts.

Other effects have to do with bubbles; bubbles in the body could be affected in two ways, and these could have opposing consequences. In the first place bubbles may be generated or may form as a result of the 1st dive, and

Table 1
Matrix Used for Calculating Repetitive Excursions (0805)

Compartment	1	2	3	4	5	6	7	8
Half Time	5	10	20	40	80	160	320	640
M value at 10 fsw	93	78	65	55	50	47	46	45
Slope	1.60	1.45	1.25	1.20	1.13	1.08	1.02	1.00
M value at 70 fsw	189	165	140	127	118	112	107	105
Slope	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

these could grow following the 2nd dive. On the other hand, gas phase nuclei [which have been shown to be present, persistent, and required for the formation of bubbles (9, 10)] could just as well be eliminated ("crushed"), washed away, or otherwise reduced in number by the 1st dive. Most of the evidence based on ultrasonic bubble detection seemed to support the "reduction" or "elimination" concept (e.g., 11). A look at a number (but not a strict statistical sample) of repetitive dives monitored by Doppler ultrasound at Defence and Civil Institute of Environmental Medicine, Toronto, showed a clear indication of less bubble formation in the 2nd dive. In another example, simulated periods of extravehicular activity (EVA) from a spacecraft performed in sequence showed consistently fewer bubbles in the second EVA as compared with the first (12). We concluded that as far as bubbles are concerned, the "reduction" effect would be more likely to be the dominant one, and any effect of a prior dive on bubbles could, if anything, be beneficial, and that we need not account for bubbles in calculating repetitive dives. Further, any bubble activity will itself be a function of gas loading or gas supersaturation. All this left us comfortable with gas loading as our best computational approach.

Most of the currently available repetitive tables are based on some variation of gas loading (13-15). There is no doubt that additional gas is present, and empirical evidence from such as the U.S. Navy repetitive tables suggests that there is merit to this approach. A further real advantage is that calculation methods for dealing with gas loadings are better established and understood than those dealing with bubble activities.

Deriving a Repetitive Algorithm for Excursions from Saturation

With a matrix in which we had confidence, a clear choice of a concept for calculating repetitive dives, and our established DCAP as a computational tool, we next had to develop a method for performing the special repetitive calculations that would correctly deal with various excursion depths over the entire range from long and shallow to short and deep excursions. The "surface interval" of traditional repetitive diving (here called the "habitat interval") was to be spent at the current habitat storage depth.

We tested numerous sequential combinations of no-stop excursions, and

found that for the range of saturation storage depths and target depths involved the 2nd excursion, or more generally the next one in a sequence, was influenced most prominently by the *order in the sequence* and the *time elapsed since the end of the previous excursion.* For this we presumed that the worst impact on the subsequent dive is by a preceding dive of the same type; this would tend to load the same compartments, and would consequently have the greatest effect.

For a variety of "habitat intervals" and excursion sequences we tested strings of 14 excursions, and included in the tables the allowable no-stop times of the 1st, 2nd, and 14th excursion. These are displayed first according to the order in the sequence, as "first," "second," or "3+." The latter includes the 3rd or any subsequent excursion. Then for each of the latter two categories we calculated excursion times for each of several habitat intervals since the end of the last excursion. Thus the 2nd dive is one following a 1st dive to the same depth for the maximum allowable time. The 3rd and following dives ("3+") use the time calculated for the 14th dive in a sequence, each for the maximum allowable time. Actually, in most cases there were few changes between the 3rd and the 14th excursion calculated.

This pattern required that we establish an interval that would allow a diver to start anew with the 1st dive in a new sequence. Because there could be a 16-h interval between the end of one "normal" 8-h day and the start of the next, we tested 16 h as our "get well" interval. Following each string of 14 identical excursions separated by different habitat intervals we added a 16-h interval and calculated another excursion, and found that in only a few cases of the hundreds calculated was this time shorter than the original 1st dive, and in those it took only 2 or 3 min off the next dive. Because of the operational advantages of a 16-h interval, instead of trying a longer period (the new Canadian tables use 18 h) (16), we reduced the 1st dive time of those combinations by the few minutes needed, and 16 h then allows a new start of a repetitive sequence in all cases.

We calculated a complete set of excursions for each of the three orders in the sequence, and for intervals ranging from 0.5 h to 16 h. A sample of these is shown in Table 2.

Submaximal Excursions

In a repetitive sequence each dive is "penalized" by the effects of the dive before it. In these tables this penalty is implemented as a reduction of the allowable excursion time to a given excursion depth. If the preceding dive does not last as long as it could, then the penalty to the next one can be reduced. A method of making this interpolation is included as part of the table set. It is based on three values: the fraction of the allowable time used by the submaximal excursion, the time (from the tables) that would be allowed if the preceding submaximal dive had not taken place at all, and the time (from the tables) that would be allowed if the submaximal dive had used the full time allowed. The shortest of these times is increased by the fraction of unused

Table 2
Sample No-Stop Excursions from 60-64 fsw

No-stop Excursions from 60-64 fsw 85 Aug D55RO0.K12; .K13

		Allowable time (min) at each excursion depth (fsw)												
No.	Interval, h	65	70	75	80	85	90	95	100	105	110	115	120	125
1st	> 16									480	480	420	282	199
2nd	8-16									480	480	397	281	198
2nd	4- 8									480	477	327	250	186
2nd	2- 4			...All 480...						480	382	244	191	159
2nd	1- 2									480	314	182	135	121
2nd	0.5- 1									480	271	114	93	83
3+	8-16									480	480	397	281	198
3+	4- 8									480	409	290	224	183
3+	2- 4									426	240	167	129	104
3+	1- 2									244	132	90	72	63
3+	0.5- 1									132	69	59	43	34

No.	Interval, h	130	135	140	145	150	155	160	170	180	190	200	220	240
1st	> 16	159	119	94	79	69	56	47	37	31	26	23	16	11
2nd	8-16	157	119	94	79	69	56	47	37	31	26	23	16	11
2nd	4- 8	147	118	94	79	68	56	47	37	31	26	23	16	11
2nd	2- 4	123	103	88	74	64	56	47	37	30	26	23	16	11
2nd	1- 2	91	78	69	61	53	47	42	34	28	24	21	16	11
2nd	0.5- 1	60	54	50	45	38	35	32	27	23	19	17	14	11
3+	8-16	157	119	94	79	69	56	47	37	31	26	23	16	11
3+	4- 8	147	118	94	79	68	56	47	37	31	26	23	16	11
3+	2- 4	88	77	71	63	57	52	47	37	30	26	23	16	11
3+	1- 2	52	45	39	35	31	28	26	22	19	17	15	13	11
3+	0.5- 1	28	24	21	18	16	15	14	12	13	11	10	08	07

time in the submaximal dive. The logic is simple enough, but making the calculations is somewhat difficult and there are several places to make an error. Fortunately, a common-sense look at the calculation will spot a serious error; the biggest problem is our experience so far seems to be looking up the excursion times on the tables. A worksheet, a program in BASIC, and a template for Lotus 1-2-3 may make things simpler, but this calculation points up strongly how much the whole operation could be improved if the entire excursion package could be put into an on-site computer.

"One-Stop" Excursions

A diver can spend a longer time at the bottom if he or she can make appropriate decompression stops on the way back to the habitat. Given the calculation setup described above it is a relatively simple and straightforward task to calculate a table for excursions from a given storage depth. But when all the available storage depths, bottom depths, and bottom times are considered it becomes a formidable task that would perhaps defeat its own usefulness by its volume and weight. Further, numerous stops in the water might be difficult to manage operationally. We therefore took the approach that a relatively simple dive pattern with decompression stops at a single stop depth might be a more useful approach. This could be implemented by having a "way station" at a depth 10 to 20 fsw deeper than the habitat as a stop station for all excursions that need stops. This would give the divers a definite stop depth, some protection from cold and current, easier communications, extra gas, and perhaps other advantages. Even without a way station the idea of a single stop depth for all excursions offers some advantages.

Work at the U.S. Naval Experimental Diving Unit has shown that in at least one type of conventional diving it is acceptable to take the 10-fsw stop at 20 fsw (17). This seemed entirely reasonable for return to the habitat, so we planned the one-stop excursions to use a stop between 10 and 20 fsw deeper than the habitat, and calculated them for 15 fsw. We chose a specific set of bottom times ranging between 30 and 240 min, then calculated the stop time at the one-stop depth. We selected only those excursions with required stop times of less than about 60 min.

In this process we noted that a number of additional excursions would be possible within these limitations if we could stop for 1 or 2 min at a stop depth 10 fsw deeper than the "one-stop" depth. Reasoning that an extra 2-min stop at that point would be beneficial even if not required, we added the requirement that all one-stop dives have a 2-min stop 10 fsw deeper than the main stop depth. This causes a bit of momentary confusion with terminology, but is physiologically sound and gives a greatly increased operational capability to the overall set of procedures.

Because some of these dives allow for extremely long excursions, we felt it best to limit them somewhat. Only two intervals are allowed, between 2 and 16 h, and over 16 h; thus at least 2 h must elapse before a one-stop excursion. No more than 4 one-stop dives can be done without a 16-h break, and each is considered as a "3+" excursion in determining the sequence; the latter point means that if the one-stop dive is the 1st one of the day then the 2nd one has to come from the 3+ table. Also, the submaximal procedure is not allowed with the one-stop tables. A sample set of one-stop tables is shown in Table 3.

General

There were other considerations. For example, we had to allow for a storage depth not falling evenly on a 5-fsw increment. Intermediate storage

Table 3
Sample One-Stop Excursions from 75-79 fsw

One-stop excursions from 75-79 fsw 85 Aug D584O0.K19

Interval > 16 h

Excursion depths (fsw) with bottom and stop times, min

145	150	155	160	170	180	190	200	220	240
187 0	151 0	111 0	90 0	66 0	46 0	36 0	30 0	23 0	16 0
210 5	180 9	120 2	120 6	90 8	60 4	45 3	45 6	29 5	16 2
240 10	210 15	150 9	150 17	120 20	90 15	60 10	60 15		
	240 20	180 18	180 26	150 33	120 33	90 26			
		210 24	210 36	180 45					

Interval 2-16 h

Excursion depths (fsw) with bottom and stop times, min

145	150	155	160	170	180	190	200	220	240
100 0	85 0	76 0	69 0	55 0	45 0	36 0	30 0	22 0	16 0
150 31	120 27	120 42	90 17	90 50	60 9	45 3	45 8	29 5	16 2
180 50	150 50		120 58			60 32			

depths are to be adjusted in the most conservative way; how to do this may not always be obvious, and is different for ascending and descending excursions. Because of this and the nature of the tables, we chose to display them in terms of the habitat depth, putting all tables for use from a given storage depth—which will usually be fixed for a complete mission—on a single pair of pages. This includes oxygen exposure doses, the oxygen window range, ascending excursion limits (the same as those in the NOAA manual), all no-stop and one-stop excursion limits, and the saturation decompression profile for that depth.

As general limits, in addition to a maximum of 14 no-stop or 4 one-stop dives without a break, divers are limited to no more than 12 h of diving time outside the oxygen window each 24-h day.

Oxygen Window and Ascending Excursions

During interdive intervals a diver is allowed to excurse into the water as long as the excursions do not result in any increase in inert gas loading. When the habitat is filled with a near-normoxic mix (a relatively low Po_2 of 0.3 to 0.35 atm is recommended where pulmonary oxygen toxicity is an issue) the diver excursing on air may have quite a useful range without having to consider decompression. This ranges from +4 fsw (deeper than the habitat) at 30 fsw to +27 fsw at 110 fsw storage. These are considered as excursions within the "oxygen window"; the allowable unrestricted range depends on the

inspired Po_2.

New ascending excursion tables were not calculated as part of this project. The existing ones from NOAA OPS have been shown to be adequate. A minimum of 24 h at storage depth or in the oxygen window is recommended before making an ascending excursion. For convenience, the allowable ascending excursions from each storage depth are displayed on the same pair of pages as the other tables.

Oxygen Exposure Limits

To make these longer excursion times usable it is necessary to have longer allowable times for exposure to oxygen than those given in the current NOAA Diving Manual (3), which are based on a set of Navy limits that do not segregate the pulmonary and CNS toxicity levels. We have prepared a new set of limits that uses the U.S. Navy limits for CNS toxicity, but considers pulmonary toxicity separately.

Any excursion that involves exposure at a Po_2 of greater than 1.5 atm is felt to be in the CNS risk zone and is time limited without regard to decompression. With air as the breathing gas this level of 1.5 atm is reached just beyond 200 fsw. We interpreted the U.S. Navy limits to allow a maximum of 29 min at 220 fsw and 16 min at 240 fsw, and no tables in the set have been allowed to exceed these limits.

For pulmonary limits we are basing our proposed limit on mostly unpublished commercial and laboratory experience. To implement it we used Lambertsen's well-known Unit or Cumulative Pulmonary Toxicity Dose (UPTD or CPTD) (18), considering the limits for a daily dose (per 24 h) rather than for a specific dive. The validity of the CPTD has been questioned because there is no provision in the formula for "recovery," but used on a daily dose basis the concept works quite well.

The limits we propose apply to all diving at a Po_2 of 1.5 atm or less. They cover two situations, the continuous operation where divers are exposed up to the CPTD limit every day for a week or more, and the less regular or intermittent operation. For the continuous, routine operation we propose a daily (24 h) CPTD limit that is based on the mission duration, with the daily dose decreasing for longer missions. This would be about 380 U/d for a diver working 7 d straight. For an occasional daily exposure the dose may reach 850 U, but only if the diver receives a minimal dose on the days before and after this higher exposure and does not exceed the weekly total. For missions longer than about 10 d, a lower average daily dose of 300 U/d is specified in the REPEX procedures.

Pulmonary oxygen toxicity develops gradually, can be stopped by limiting the oxygen exposure, is completely reversible, and is highly influenced by individual sensitivity (18, 19). We are therefore presenting what we feel are "operational maximums" that are physiologically acceptable for the kind of exposures contemplated for use in scientific habitat diving. Individual divers, operational organizations, and even NOAA's Diving Office may choose to

reduce these limits on the basis of their own experience.

Surfacing

Surfacing from habitat operations involves both saturation decompression and recovery of the divers to the surface. The method of choice for getting the divers to the surface is with a closed diving bell system, with decompression carried out in a surface decompression chamber under topside control. Other choices include decompression in place in the habitat and transfer to the surface either in a closed bell at 1 atm pressure or by swimming up after reaching surface pressure. Variations on these and some other techniques are also available for contingency or emergency use.

Saturation decompression profiles have been prepared for all depths to 120 fsw; these are covered in another report (20). A sample table is shown in Table 4.

Treatment of Decompression Sickness in Habitat Diving

Special procedures are furnished for treatment of decompression sickness that may occur after an excursion or during saturation decompression in a habitat. As an essential item we recommend that the habitat be pressurized so that recompression can be initiated immediately. Treatment in the habitat comprises the usual characteristics of compression in defined steps, breathing of treatment mix (or oxygen if in the right range) at each step, continuing compression and cycles of treatment mix until relief, and decompressing either back to the habitat or depth of onset with an appropriately slow table. There is a provision for returning to diving if the situation warrants.

DISCUSSION

A test program is in progress involving 3, week-long saturations, each packed with as many excursions as can be done, with all intervals kept to a precise minimum and all excursions to the full table value. Each saturation team is 4 divers, with 1 female team and a wide range of ages represented to emulate the scientific diver population. No-stop, one-stop, and submaximal excursions are being tested, as well as daily oxygen doses and oxygen-window habitat intervals. This is by no means enough testing to develop new procedures, but it should be enough to validate a reliable set of tables and procedures for initial field use.

Preliminary results indicate that all aspects of the new procedures will be found to be physiologically acceptable.

One point needs to be made about the general approach. It has been possible to fit all these decompression requirements into a few tables (actually there are 36 pages of tables, plus the instructions) that can be used for field operations. However, putting these tables together has required a number of groupings, many intervals, and more than a few approximations and assumptions. Using the tables, and especially the calculation of the time allowed in

Table 4
Sample Saturation Decompression Table From 75-79 msw

Saturation decompression from storage at 75-79 fsw

Selecting precursory starting depth:

Max excn last 36 h	100	105	110	115	120	125	130	135	140	145	150	≥150
Starting depth to use	80	85	90	95	100	105	110	115	120	125	130	130

Precursory Table:

Depth, fsw	Time To Go	Stop Time	RRat min/ft	Gas mix	PO_2, atm	CPTD Stop
130	3085	45	9	air	1.04	48
125	3040	45	9	air	1.01	45
120	2995	45	9	air	0.97	43
115	2950	45	9	air	0.94	41
110	2905	45	9	air	0.91	38
105	2860	60	12	air	0.88	48
100	2800	60	12	air	0.85	44
95	2740	60	12	air	0.81	41
90	2680	60	12	air	0.78	37
85	2620	60	12	air	0.75	34
80	2560	95	19	air	0.72	48

Precursory	10.3 h	CPTD	467
Main	1 d + 17.1 h	CPTD	181
Total	2 d + 3.4 h	CPTD	648

Main Table: k = 4.4131

Depth, fsw	Time To Go	Stop Time	RRat Min/ft	Gas mix	PO_2, atm	CPTD stop
75	2465	100	20	air	0.69	44
70	2365	105	21	air	0.66	40
65	2260	110	22	air	0.62	34
60	2150	120	24	air	0.59	29
55	2030	125	25	air	0.56	22
50	1905	130	26	air	0.53	12
45	1775	140	28	air	0.50	0
40	1635	150	30	air	0.46	0
35	1485	160	32	air	0.43	0
30	1325	175	35	air	0.40	0
25	1150	185	37	air	0.37	0
20	965	205	41	air	0.34	0
15	760	225	45	air	0.31	0
10	535	250	50	air	0.27	0
5	285	285	57	air	0.24	0

the excursion following a submaximal one, can result in errors. There are some operational limitations that could be improved with better methods of displaying the known data. All this can best be done by means of an on-site computer. This could begin with being able to calculate upcoming excursions, but it will soon grow to include data monitoring, emergency management, and real-time control. Small "pico" computers are available in 1986 that can perform all the calculations needed for these dives, in a package the size of a large notebook. They are tolerant of pressure and can be made fire safe. We strongly recommend that this be implemented in future habitat diving operations.

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COMPUTATIONAL MODEL VS. STANDARD TABLES FOR DECOMPRESSION IN COMMERCIAL DIVING

W. Sterk

Standard decompression tables, such an U.S. Navy or Royal Navy tables, are often not suited for use in commercial diving. It is often not recognized that these tables have been developed for navy use, and often such tables are considered "official" and interpreted as "law" (1, 2). Sometimes these tables are not safe because of extreme conditions of cold water, hard work, and daily diving. In that case, the diving supervisor usually adapts the table, based on his own skill and experience. Several such adaptations have been in use in the North Sea. To avoid controversy over established decompression tables, Norwegian tables for air and nitrox diving, based on both U.S. Navy and Royal Navy tables, were developed in 1980. These tables yielded a much lower rate of bends incidence (3). However, the tables also have been adapted to meet safety requirements for dives with heavy work, and so on (4). These Norwegian tables are a set of tables that cannot be used under every possible operational circumstance, and must be modified by the dive supervisor for special conditions.

Since 1975, we have used a modified neo-Haldanian computational model to calculate a set of tables for any special job, depending on the specific operational circumstances to be anticipated. This approach is found to be more versatile than a book of "standard" tables, which can only be used for standard diving operations. When dive and decompression circumstances differ significantly from our previous experience, a limited series of test runs are done before operational use to get the divers acquainted with the specific procedures and to eliminate errors in decompression. It is realized that a large number of trials are necessary to decide whether a decompression procedure can be considered safe (5, 6). Unfortunately, economic reasons prohibit extensive testing most of the time. Even many "official" tables are based on a

(sometimes surprisingly) low number of trials (5-8). Apart from the number of trials, the number of participants seems crucial because divers tend to become acclimatized in any series of decompression tests (7).

To overcome these problems, we developed a protocol for collecting actual dive and decompression data from all dives made on our schedules. These data are fed into a computer database and used to readjust the model and the tables where necessary, as well as to control proper use of the tables. This procedure stimulates an intensive interaction between diving practice and theory. Over the years, the computational model gained in reliability for the prediction of safe decompression for all kinds of diving procedures. In 1985, for instance, a grand total of 6534 dive and decompression procedures have been reported in this way, involving air, nitrox, and trimix bounce dives, with only one case of decompression sickness.

The flexibility of using a reliable computational model instead of standard tables is demonstrated from data collected during an air diving job in the Far East, where the use of existing tables was not possible.

MATERIALS AND METHODS

Early in 1985, an inspection and cleaning job was needed in the air diving range offshore, near Bombay, India. The structure to be worked on had a large fire flare on top, which, because of the extreme heat, made it impossible for the diving vessel to come nearer than 150 m of the diving site. This also made it impossible for the divers to surface at the site. The flare could not be put out because to do so would force several oil drilling rigs to close down.

The solution to this problem was found by attaching to the structure a wet bell as a diving platform at a fixed depth of 6 m, with an umbilical to the ship for the supply of diving gases (Fig. 1). Transport of the divers to and from the bell was done by underwater scooters. In the bell, the divers changed scuba for surface-supplied Kirby Morgan gear. They wore ordinary dry unisuits all the time, permitting diving times of many hours as the water temperature was around 25°C. Usually the diving team consisted of 2 divers, 1 acting as standby diver and the other doing the job, hereafter called the work diver.

This situation required alternative decompression procedures that standard tables do not provide. It was established that 15 min would be sufficient for the divers to reach the bell and change gear. To ensure safety, schedules were developed that allowed for 20 min travel to or stay inside the bell before diving time started. The standby diver stayed in or around the bell and was not allowed to dive deeper than 15 m, except for emergencies. He normally stayed within no-stop diving time.

Decompression took place by short, in-water stops on air and the use of oxygen inside the bell, after which the divers were free to stay in the bell or to go back to the ship. The decompression schedules had to be conservative because no serious decompression sickness could be allowed to occur in divers

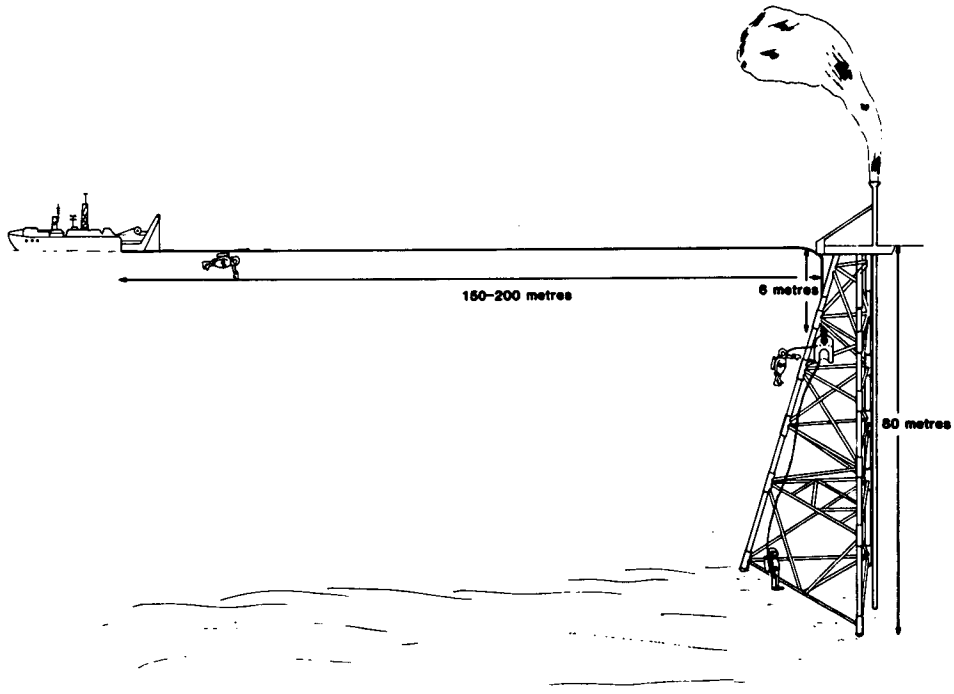


Fig. 1. Schematic drawing of the situation at the diving site offshore, near Bombay.

remote from the ship. For example, part of the decompression table for a maximum diving depth of 35 m is shown in Table 1.

Tables were calculated with 2.5-m depth intervals and 10-min time intervals for depths shallower than 30 m, or 5-min time intervals for depths equal to or in excess of 30 m. Normally, the interval between dives was to be at least 12 h, but tables for repetitive diving were also provided.

The diving team consisted of 10 experienced divers, including the diving supervisor. They were instructed to record all diving profiles as accurately as possible, from the moment of leaving the ship until return. This resulted in extensive diving logs which were fed to our computer for analysis.

RESULTS

On this job, 351 air dives were made by the 10 divers, including the diving supervisor who made 3 dives, within 47 d, to depths ranging from 6 to 55 m. No cases of decompression sickness occurred during this period of nearly daily diving. The distribution of the dives according to the depth range is shown in Fig. 2, and the distribution of actual diving depth and time in Fig. 3. As could be expected the longest dives were made at the shallower depths, because of the lower decompression "penalty." Most of these long and shallow dives were standby dives. In total, 178 of such dives were reported; only 15 of

Table 1
Sample Decompression Table for a Maximum Diving Depth of 35 m*

	Stop Depth in Meters						
	In Water			In Wet Bell			
Diving Time, min	15	12	9	6 O_2	6 Air	6 O_2	Total Decompression Time, min
35	0	2	5	25	10	1	45.9
40	0	3	7	25	10	3	50.9
45	0	3	9	25	10	6	55.9
50	0	5	11	25	10	10	63.9
55	0	6	15	25	10	14	72.9
60	0	8	17	25	10	19	81.9

* Diving with air. Maximum 20 min for travel to and stay inside bell, after which diving time starts. Ascent speed is 10 m/min. Ascent time not included in stop time. Surface interval is at least 12 h.

them required decompression.

Although not especially instructed to do so, the divers acted in turn as standby or work diver. When looking at the diving activities over the entire period, this resulted in alternately deeper work dives and shallower standby dives, as shown in the diving profile of diver SU in Fig. 4. In total, only 26 repetitive dives were made, 6 of them requiring decompression, 3 by standby divers and 3 by work divers. Despite the request for repetitive tables, the use of them was clearly avoided as much as possible.

Careful examination of the diving logs revealed that although the divers tried hard to keep good records of their diving activities, the standby divers moved in and out of the bell so many times that no reliable profiles could be obtained from these dives. The remaining 163 work dives, however, were well documented. Fifteen of these dives were within no-stop diving time. When we also subtract the incidental repetitive dives, 145 work dives with decompression remain useful for analysis.

The average dive and decompression profile for these dives is shown in Fig. 5. The average time to travel from the ship to the bell and change diving gear was 14 min; well within the design of the tables that allowed 20 min. The average table profile, as indicated by the *heavy solid line*, is somewhat deeper and longer than the dive profile, but not as much as standard tables would have prescribed. The average profile demonstrates the procedure nicely, but gives no real information about the use of the tables.

The procedure for using the tables during the 145 work dives is displayed

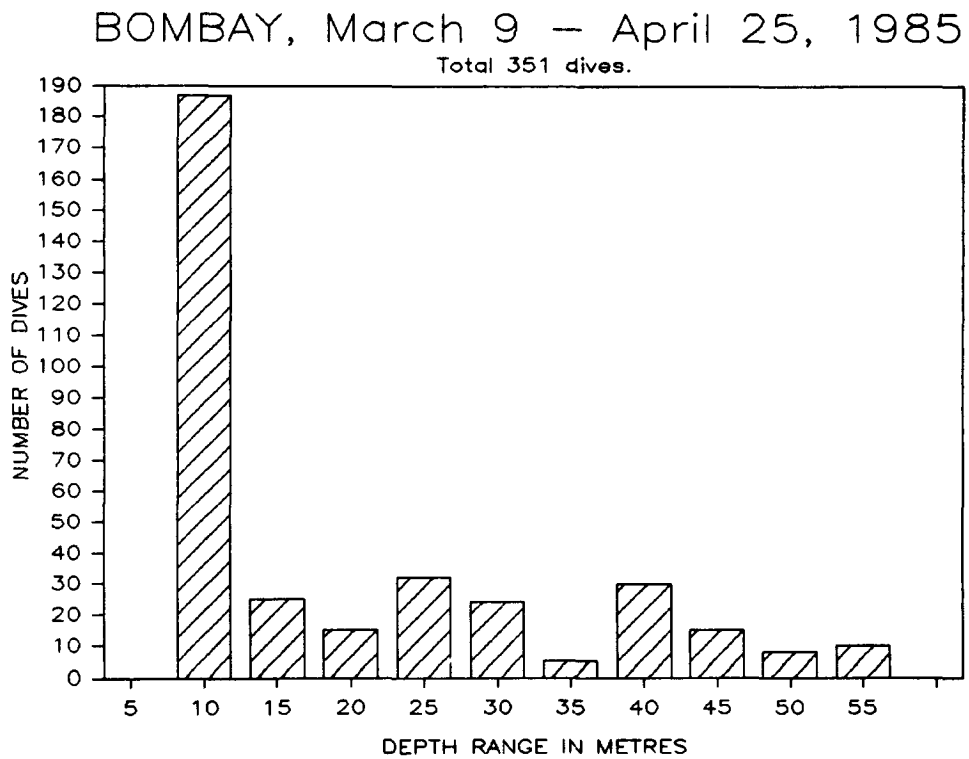
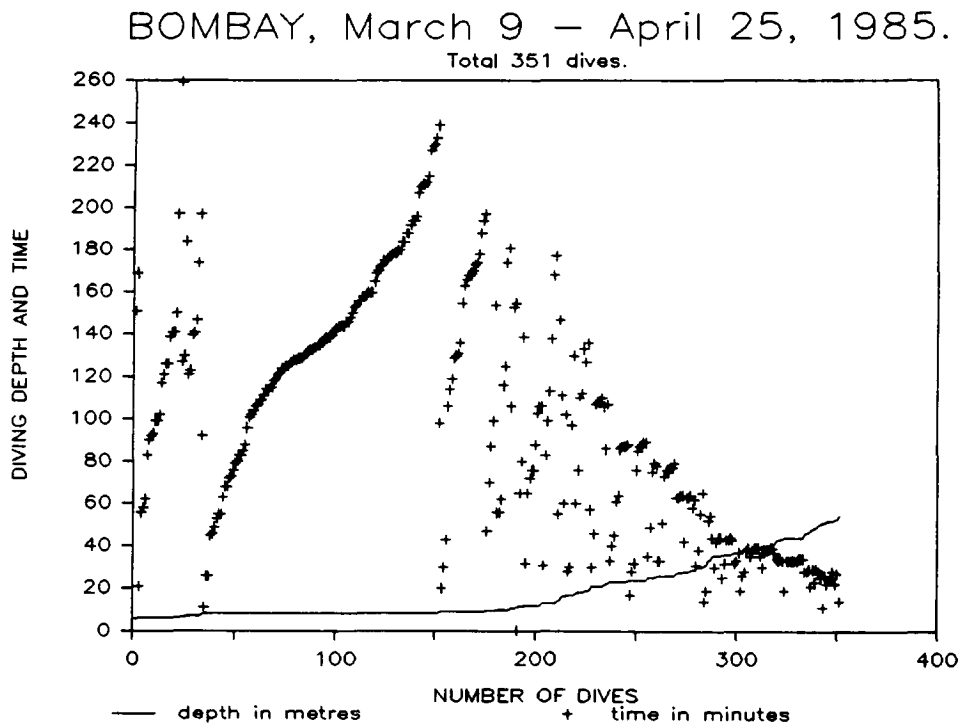


Fig. 2. Distribution according to range of diving depths for all reported dives. Most of the dives in the range between 5 and 10 m are made by standby divers in or around the wet bell diving platform.

in Fig. 6. The upper graph shows which parts of the tables were used. On any depth between 12.5 and 55 m, some dives were made. There is a wide scatter of diving times, with some clusters between 80 and 110 min for dives shallower than 30 m and some between 30 and 65 min for the deeper dives. The *lower graph* in Fig. 6 shows the deviation of actual diving depth and time from the selected table depth and time. The *squares* represent table depth minus diving depth, the *crosses* diving time minus table time. The boundaries of time and depth steps in the tables (10 or 5 min and 2.5 m, respectively) are also indicated. Although our computational model was developed to produce tables to be used "up to time and depth," in 7 dives the boundaries are exceeded, meaning that a choice has been made for the next deeper depth or longer time, without a clear need. In fact, all data in this *lower graph* should have



been on the zero line to test the actual performance of our computational model. As can be seen, there is not a single case where depth as well as time of dive and table coincide, although some dives are close.

As demonstrated in Table 2, the least difference between data of dive and table is present in the deepest dives. This is due to table design and the awareness of the divers that decompression "penalty" increases rapidly in this depth range. When we consider dive profiles within 1 m and 2 min of the table profile close enough to serve as test for our computational model, this appears to be the case in only 27 dives.

DISCUSSION

The advantage of using a computational model instead of standard tables for commercial diving is clearly demonstrated. In fact, this job could not have been done with unmodified standard tables. In that respect, it would be more useful for the diving industry if research institutes provided reliable computer programs to solve decompression problems instead of a book of tables. In this job, in which 351 dives were made by 10 divers within 47 d without a single case of decompression sickness, the conclusion can be drawn that the procedures originating from our computational model are very safe. However, analysis of the collected data shows that such a conclusion would be

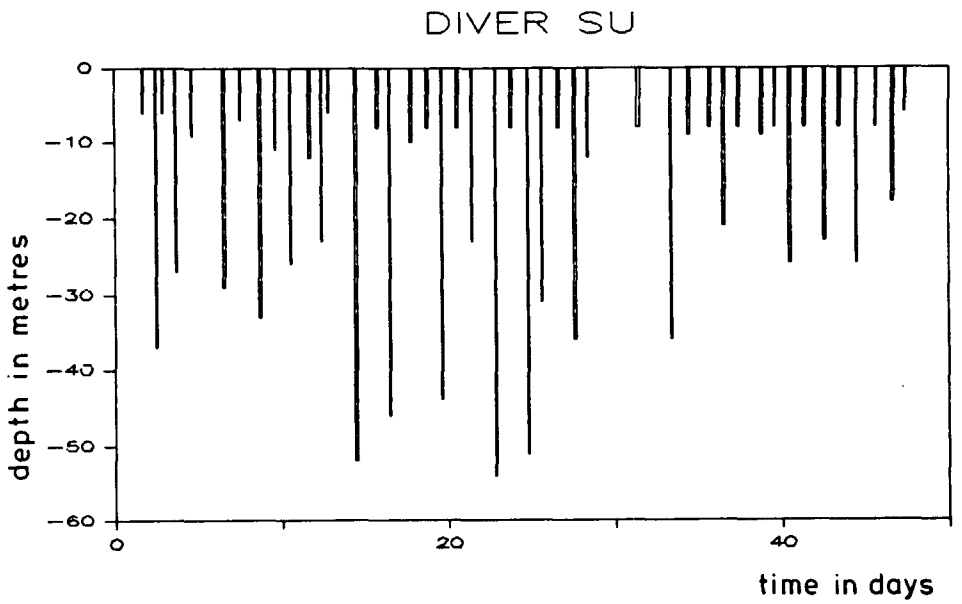


Fig. 4. Dive profile of diver SU over the entire 47 d. The profiles of the other divers show a similar pattern of alternating deep and shallow dives.

Table 2
Distribution of 145 Work Dives According to Table Selection

Table Time Minus Diving Time, min	Table Depth Minus Diving Depth, m					
	67 Dives With Table Depth Shallower Than 30 m			78 Dives With Table Depth Equal to or Deeper Than 30 m		
	< = 2.5	< = 1	< = 0.5	< = 2.5	< = 1	< = 0.5
< = 10	64	21	7			
< = 5	44	16	6	74	29	5
< = 2	15	5	1	52	22	2
< = 1	8	3	1	20	10	1

premature. First, more than half of the reported dives were made within no-stop diving time. Second, for the dives requiring staged decompression, diving up to table depth and time was done in a very limited number. Other factors possibly contributing to safety are making alternately a deep and a shallow dive over the entire period and avoiding repetitive diving as much as possible. Furthermore, the high water temperature could have been favorable. Analysis of a very large number of dives by Parker (9) suggests, in contrast to prevailing thought, that dives conducted in warm conditions have a higher incidence of accidents.

Analysis of our data demonstrates once more that statements about the safety of decompression tables, based on accident incidence within even a large number of dives, are a least questionable. Impressive figures of dives made in the U.S. Navy, for instance, collected from Diving Log-Accident/Injury Reports, show a very low accident and decompression sickness incidence (10, 11). This suggests that the U.S. Navy decompression tables are very safe. Reports from use of these tables in the North Sea by commercial divers, however, show a different picture (3, 12). This is usually attributed to unfavorable conditions such as cold water, rapid ascent, heavy work, and repetitive diving. It could well be, however, that the way the tables are used

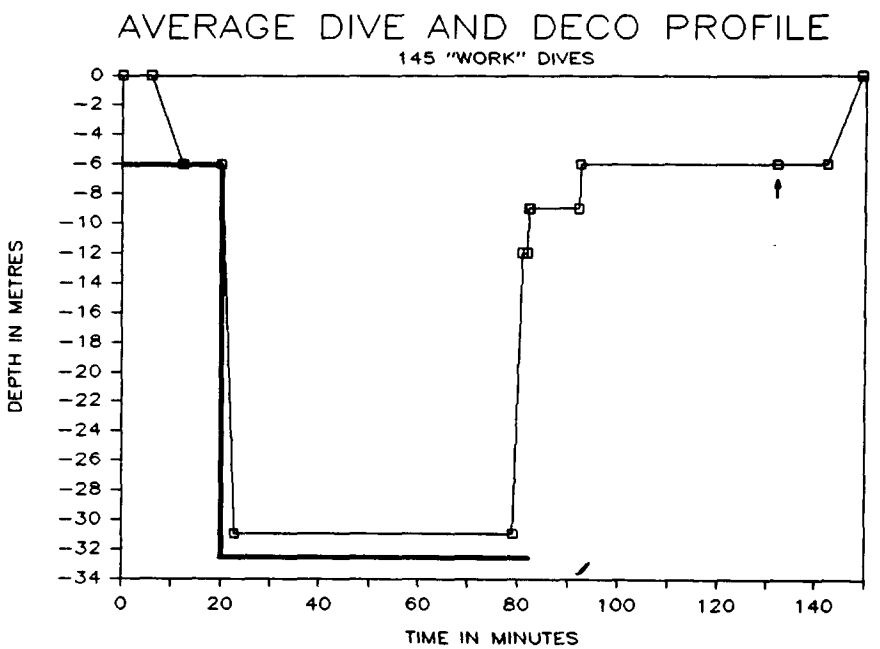


Fig. 5. Average dive and decompression profile of 145 work dives. Squares represent recordings of depth and time from the extensive diving logs. Arrow indicates end of decompression in the wet bell. Heavy line represents average table profile.

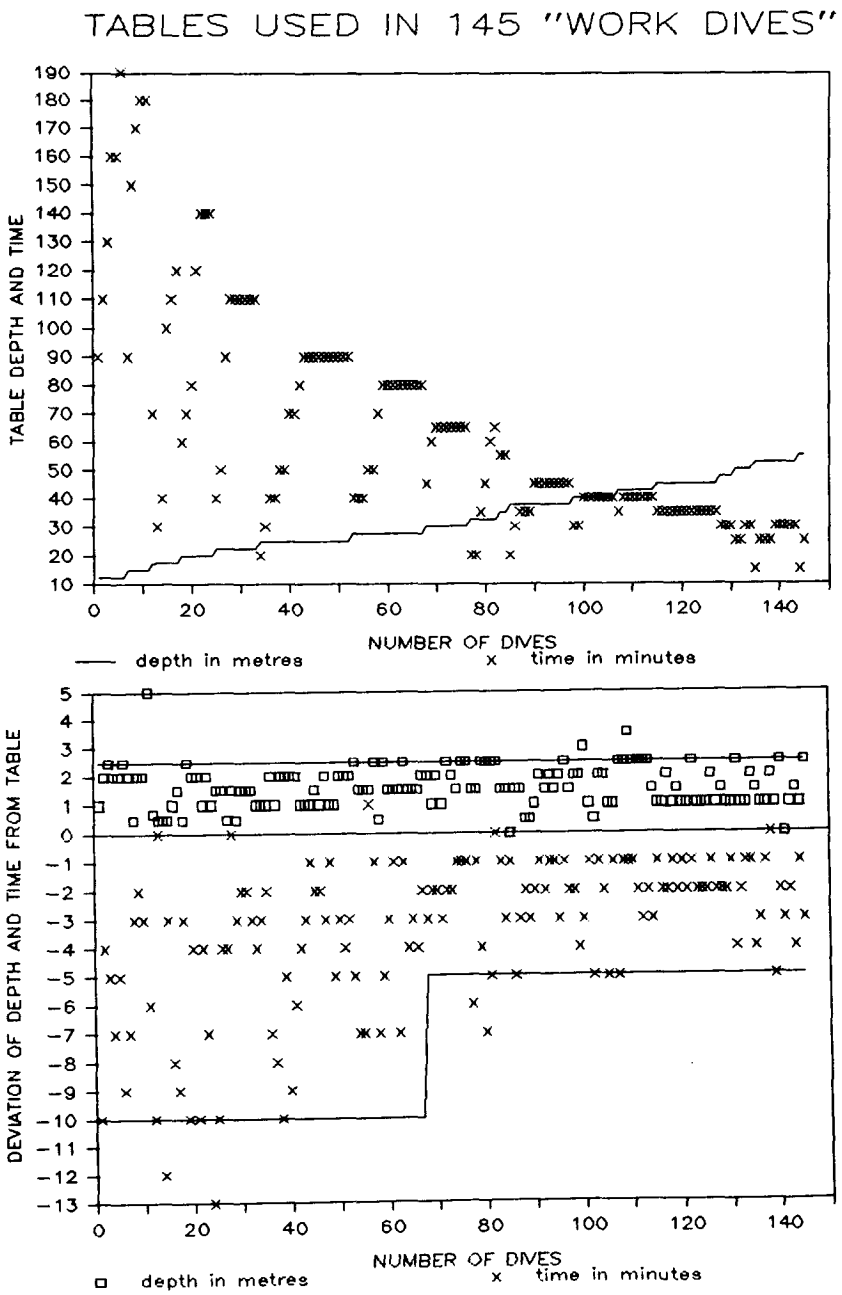


Fig. 6. Top shows which part of the tables were used. Bottom depicts deviation of actual diving depth and time from table depth and time. Data outside boundaries of table depth and time steps (2.5 m, 10 or 5 min) indicate selection of next deeper depth or longer time.

contributes to this controversial outcome. While looking for decompression sickness incidence of the U.S. Navy tables, Berghage et al. (13) found that in over 16,000 dives half of the schedules were not used at all. For schedules used at least 50 times, the incidence ranged from 0 to 4.8%.

Diving logbooks, at least those in use in the North Sea, only indicate which table is used, and not the exact details of the decompression with respect to such factors as selected table depth and time. Furthermore, the information about actual dive profiles is limited to records of diving time and the maximum depth reached. Even in the present data, where thorough bookkeeping is performed, it seemed impossible to obtain accurate data on all profiles, particularly those of the standby divers. Here, the use of a dive profile recorder, as demonstrated by Nashimoto et al. (14), could be beneficial, although this is not feasible for every commercial diver. Such a device, however, would put an end to concern about the accuracy with which diving data are provided.

The displayed inaccuracy of data obtained from operational diving does not mean that the collection of such data is not important. At present there is more operational than experimental diving being performed. Even the testing of tables, considered to be "official" is based on a very limited number of trials. Much knowledge and experience is lost when we do not try to collect data "in the field." First, we should decide which data should be collected; second, find a way to accurately collect the data; and, finally, determine how to analyze it.

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BREATH-BY-BREATH ANALYSIS BY MASS SPECTROMETRY OF ALVEOLAR INERT GAS EXCHANGE IN MAN AT NORMOBARIC PRESSURE

R. Araki, Y. Gotoh, and I. Nashimoto

There has been widespread agreement that formation of gas bubbles within the body plays an important role in the occurrence of decompression sickness. From this viewpoint, underwater physiologists have been concerned with intake and elimination of inert gas in the human body. The characteristics of alveolar inert gas exchange during and after hyperbaric exposures have been examined in man for nitrogen (1-6), helium (2, 4, 7), and argon (7).

Mass spectrometry has been used extensively to measure the partial pressures of respiratory gases in studies of respiratory physiology. In recent years, the use and application of mass spectrometry in hyperbaric research has also become popular. By using this technique, several authors have attempted to measure decompression-induced inert gas elimination (5, 7, 8). However, the methodologic problems due to the transport delay and the dynamic response of conventional mass spectrometry are still matters to be solved for more accurate and precise analysis.

This study was designed to establish the precise measurement of alveolar inert gas exchange by means of a computer-assisted data correction method. We evaluated the usefulness and limitations of our computer-based measurement system applicable to the analysis of nitrogen intake and elimination in human subjects during and after hyperbaric exposures. Emphasis will be given to corrections for the response and delay of the mass spectrometer. The correction for the electrical drift of the output signal will also be discussed. Furthermore, to determine the amount of nitrogen in the tissue compartment, we attempted to estimate a nitrogen washout pattern in human subjects during pure oxygen inhalation at normobaric pressure. The details are reported in this paper.

MATERIALS AND METHODS

Healthy male subjects were examined in this study. Partial pressure of nitrogen in the expired gas was continuously monitored with a medical mass spectrometer (model Medspect II, Chemetron Medical Products, St. Louis, MO), of which detector and analog computing circuits were slightly modified to obtain more stable and precise signals. Flow rate of the expired gas was measured by a pneumotachometer (model 9104, San-ei Sokki, Japan). Temperature of the expired gas was measured with an electric thermometer with a thermister placed inside the pneumotachometer head. Temperature and humidity of room air were measured before each experiment. Humidity of the expired gas was assumed to be 100%, and the volume of the expired nitrogen was represented as STPD.

Data Acquisition System

Each output of the mass spectrometer and the pneumotachometer was appropriately amplified and then digitized by an A-D converter (model ANALOG-PRO, Canopus Electronics Ltd., Japan). The digitized data were then transferred at high speed to an external RAM (1 megabyte capacity, I/O Data, Japan) through an 8086-based personal computer (model PC-9801M2, NEC, Japan). The sampling frequency was chosen at 50 Hz because the preceding frequency analysis confirmed that no frequency component higher than 25 Hz was included in the signal. The data were stored on the floppy disks and then processed. An 8086 assembler and C compiler were used to write programs for data acquisition and calculation. All the data were recorded simultaneously on a multipen recorder. Figure 1 summarizes the experimental setup used in this study.

Corrections for the Response Time and Delay of Mass Spectrometer

Since the output signal of the mass spectrometer has characteristic delay in time and response, appropriate data corrections were required to obtain precise data. In this study, we used a first-order correction (9) instead of second- or third-order corrections (10). The dynamic response and delay of the mass spectrometer were measured according to the method of Noguchi et al. (9). The time constant of the first-order response and the transport delay were typically determined to be 50 and 185 ms, respectively. The transfer function between input and output signals of a mass spectrometer can be written finally as

$$x(t) = y(t+L) + T dy/dt(t+L) \qquad (1)$$

where t is the elapsed time; x(t) and y(t) are the input and output signals, respectively; L in the transport delay; and T is the time constant. The signals of the mass spectrometer were corrected according to Eq. *1*.

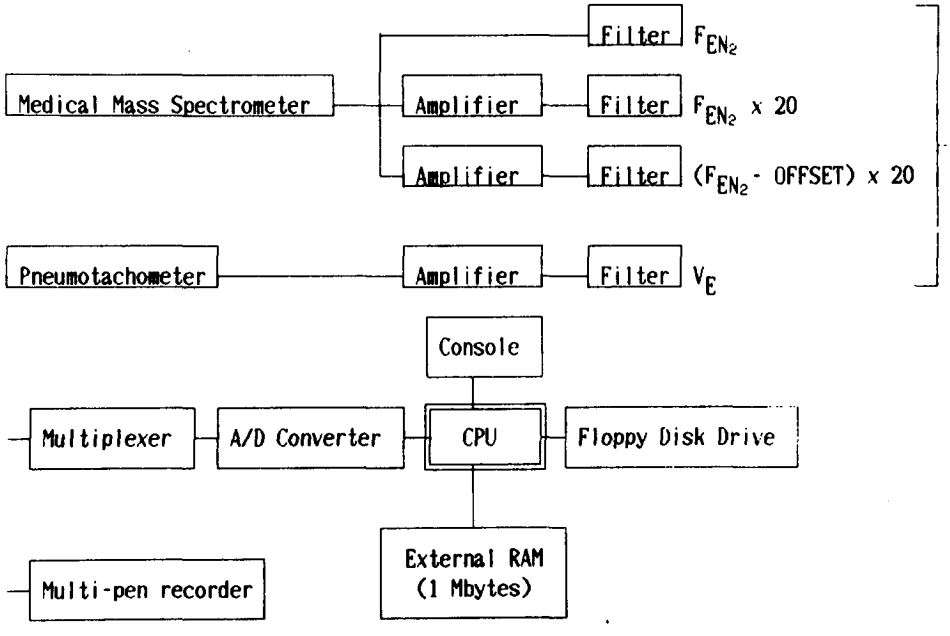

Fig 1. Block diagram of the experimental setup. Details are described in the text. CPU; 8086-based personal computer (model PC-9801M2, NEC). Filter; anti-alias filter (cut-off frequency 25 Hz).

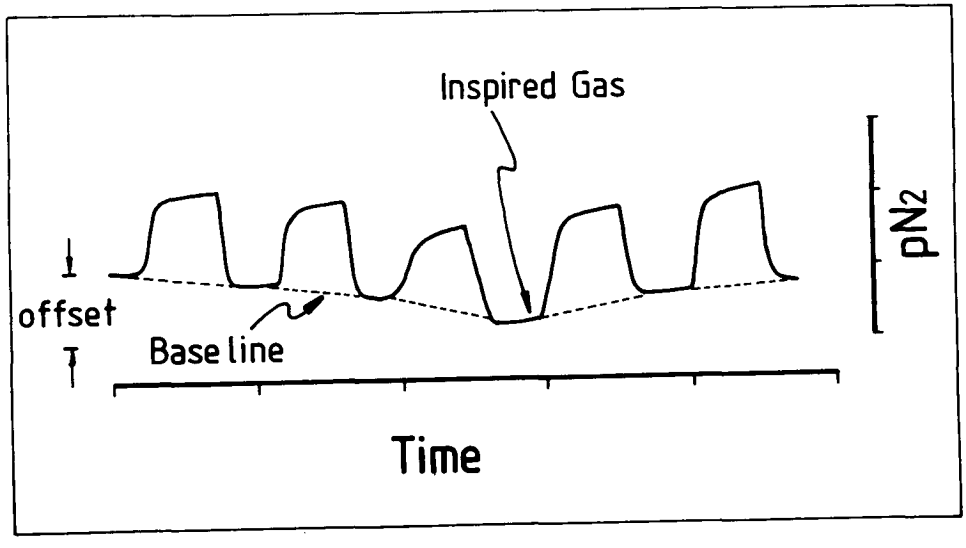

Fig. 2. Schematic representation of the baseline correction. The lines connecting the lowest points of each inspiration period are regarded as "true" baselines.

Since the partial pressure of nitrogen in the expired gas during pure oxygen inhalation is considered to be extremely low, correction for the electrical drift of the baseline was also performed. Figure 2 illustrates the method for the baseline correction used in this study. The lowest points of each inhalation period were connected with lines in turn, and then the lines were regarded as "true" baseline. The offset value can also be subtracted from the signal by this correction, even if the initial baseline is not adjusted at zero.

RESULTS AND DISCUSSION

Since the data collecting system was equipped with 1 megabyte of external RAM, the system was capable of performing more than 160 min of continuous digital data recording. The output signals of the mass spectrometer were confirmed not to be affected by increases in pressure up to 6 ATA.

Figure 3 shows the effect of corrections for the dynamic response of the mass spectrometer. Response of the mass spectrometer to a step change in partial pressure of nitrogen was recorded and corrected with various time constants (Fig. 3 *A*). The original trace without correction was also superimposed. A semilogarithmic plot of the response of the mass spectrometer is shown in Fig. 3 *B*. From the slope of the plot, the theoretical value of time constant was determined to be 50.01 ms. This value agreed with the results shown in Fig. 3 *A*. Second- and third-order corrections (10) did not give satisfactory results in our system because of deterioration of signal-to-noise ratio which arises from the differentiation process included in the second- and third-order correction formulas. Thus we concluded that appropriate corrections can be achieved by the first-order correction, and that simultaneous measurement of flow rate of the expired gas is required to determine the transport delay in time.

Breath-by-breath patterns of flow rate and partial pressure of nitrogen of the expired gas are shown in Fig. 4. These data were obtained during 0 to 10 s (*top*), 60 to 70 s (*middle*), and 1200 to 1210 s (*bottom*) after the onset of pure oxygen inhalation, respectively. In this figure, the baseline correction was not performed. At the initial stage, the amplitude of fluctuation in nitrogen pressure was over 10% and diminished to approximately 2% after 60 s (Fig. 4, *middle*). Twenty minutes of pure oxygen inhalation caused diminution in the amplitude to no more than 0.05%, which was comparable with the electrical drift of the baseline during the measurement. In addition, the offset value was more than twofold greater than the amplitude of the fluctuation (Fig. 4, *bottom*). Thus we concluded that the baseline correction is requisite for the prolonged breath-by-breath analysis.

Figure 5 shows curves for nitrogen washout pattern during pure oxygen inhalation at 1 ATA in man. Inspired gas was switched from normal air to pure oxygen, and then partial pressure of nitrogen in the expired gas and the flow rate were continuously recorded. The curves for nitrogen washout were calculated by the digital multiplication and integration of the flow rate and the

partial pressure of nitrogen after appropriate data corrections. *Trace A* represents total volume of the expired nitrogen with elapsed time. *Trace B* and *C* represent nitrogen volume expired for each 5 s. Ninety percent of nitrogen was expired within the first 7 min. The rate of nitrogen washout fell below 24 ml/min after 15 min of the switching. These data indicated that the partial pressure of nitrogen in the expired gas fell lower than 1% of the initial level after 15 min of pure oxygen inhalation.

In summary, a) care should be taken for the appropriate correction of signals, b) measurement of the flow rate was required as well as that of

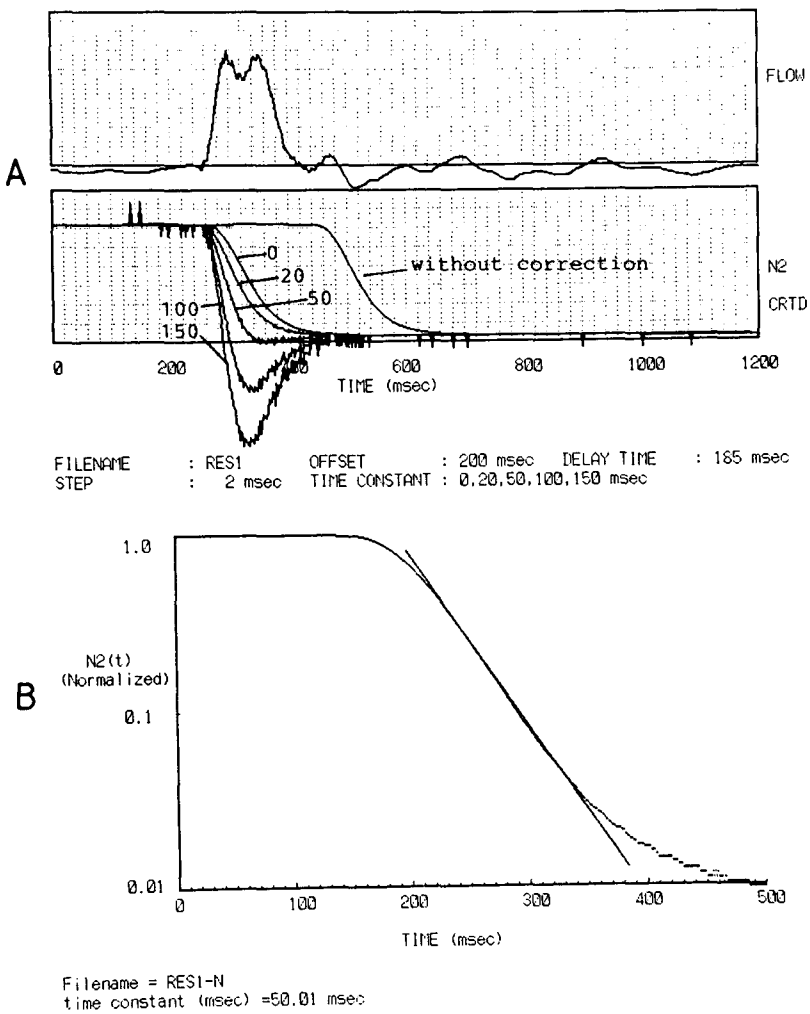

Fig. 3. A, corrections for the dynamic response of the mass spectrometer. Individual time constants used for the corrections are shown in the figure. B, semilogarithmic plot of partial pressure of nitrogen. The time constant was estimated to be 50.01 ms from the slope of the plot.

partial pressure of nitrogen for accurate and precise compartment analysis by breath-by-breath measurement, and c) the amount of nitrogen in the tissue compartment was extremely low as compared with that in the alveolar compartment.

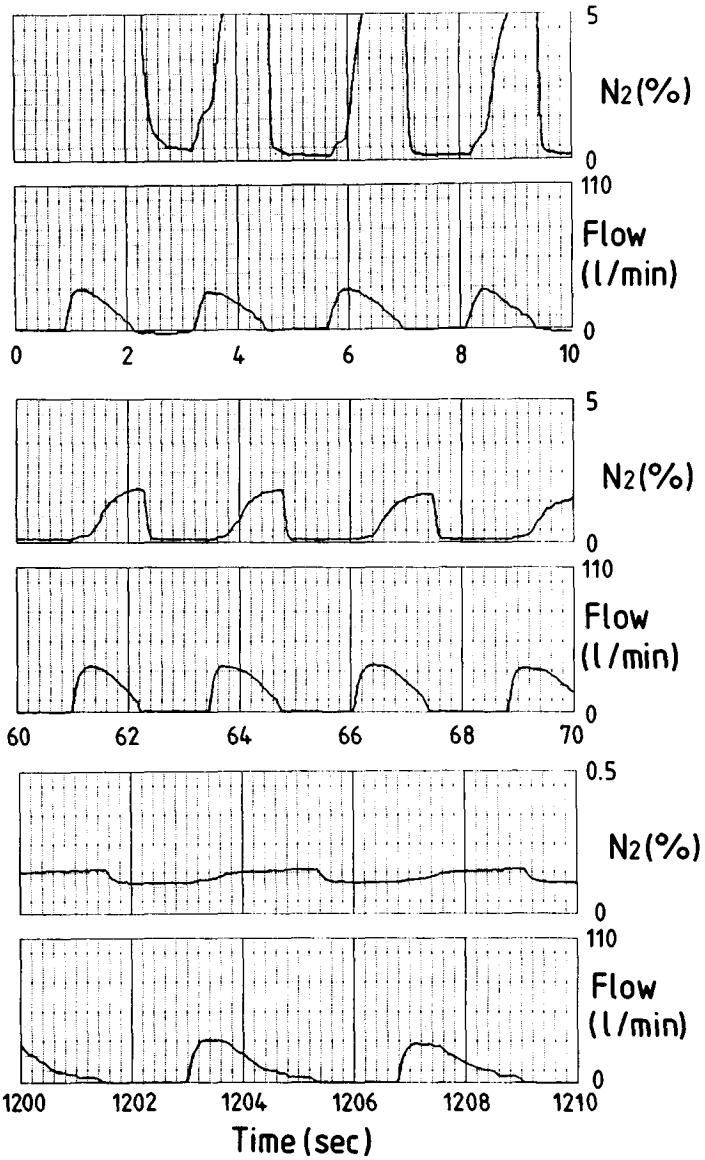

Fig. 4. Breath-by-breath patterns of nitrogen concentration and flow rate of the expired gas. These data were measured during 0 to 10 s (top), 60 to 70 s (middle), and 1200 to 1210 s (bottom) after the onset of pure oxygen inhalation, respectively.

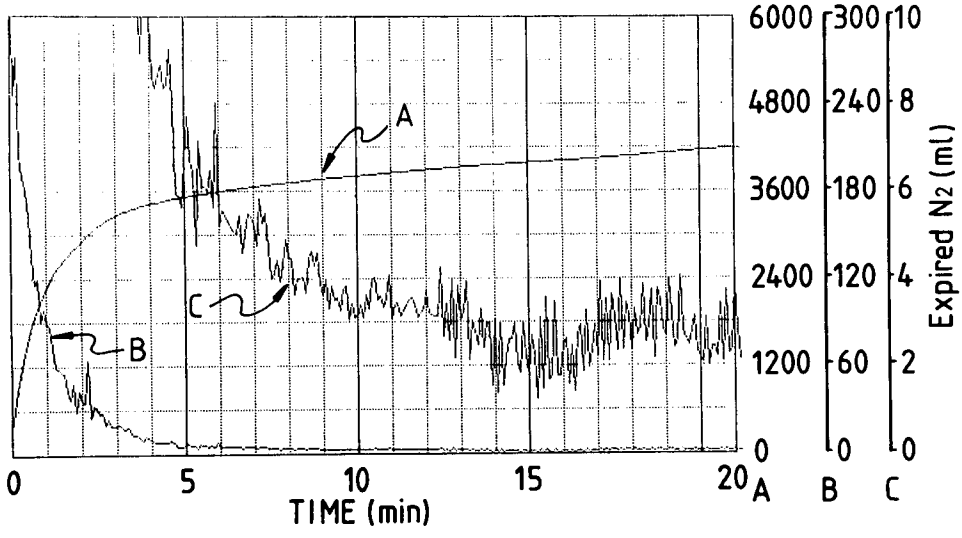

Fig. 5. Typical curves for nitrogen washout during pure oxygen inhalation at 1 ATA. A, total expired nitrogen with time; B and C, expired nitrogen for each 5 s. Trace C represents magnification of Trace B ×30.

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AN APPRAISAL OF DIVE PROFILES IN SPORT DIVING IN RELATION TO THE RISK OF DECOMPRESSION SICKNESS

K. Kobayashi, Y. Gotoh, and I. Nashimoto

After World War II, sport diving with open-circuit scuba became popular, and with the spread of sport diving, the number of sport divers suffering from decompression sickness has increased considerably. Thus, many studies have addressed decompression in sport diving (1, 2), but few have studied the dive profiles and the risks of decompression sickness resulting from technical difficulties. We have investigated the relationship between actual dive profiles and the risk of decompression sickness in harbor and shellfish divers (3) using a dive profile recording system that we developed. The purpose of the present investigation is to appraise the dive profile in sport diving in relation to the risk of decompression sickness from the viewpoint of decompression threshold, Doppler bubble score, and incidence of bends.

METHODS

The investigations were performed on sport divers in off-shore Okinawa and around the Izu Islands from May 1983 to December 1985. All of them had dived as a recreation and used open-circuit scuba.

The dive profiles were taken with a dive profile recording and analyzing system (DPRAS) which consists of a diving memory recorder (DMR), an interface, and a personal computer (PC; NEC, PC-9801) or a handheld personal computer (HPC; EPSON, HC-20) (Fig. 1).

The dive depth data were recorded in the DMR which the divers carried during their dives. After the final dive of a day, the DMRs were taken off and connected to an HPC through the interface on a diving boat or from shore. The recorded DMR data were transferred to HPC and stored permanently on magnetic tape. The stored data on the tape were transferred to a PC and

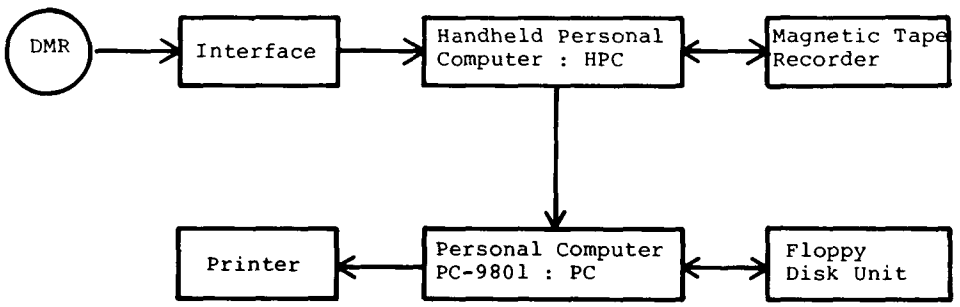

Fig. 1. Block-diagram of DPRAS.

stored on floppy disks. The data were then read out to reproduce dive profiles and processed to obtain theoretical tissue nitrogen pressure (TNP) and decompression threshold (DTH), according to Hempleman's assumption (4).

The maximum depth and the bottom time of the dives were calculated from the dive profiles. They were used to construct the decompression schedules according to the British Sub-Aqua Club (BSAC) tables (5).

Venous gas emboli (VGE) were examined to appraise the risk of decompression sickness. The monitoring was done after the diver surfaced from the final dive of the day, using an ultrasonic Doppler bubble detector (IAPM model 1032G). VGE was graded according to Spencer's classification (6).

RESULTS

Eighty daily dive profiles were obtained from 37 divers, including 4 women. Their ages ranged from 23 to 59 yr (mean 35.8 yr). Maximum depth of individual dives ranged from 10 to 50 msw and the bottom time 8 to 107 min.

Figure 2 shows an example of the daily dive profiles of a sport diver with changes in TNP and DTH due to dives. Frequent ascents and descents were always observed. Actual decompression schedules did not exceed the theoretical DTH in any of the 80 daily dives except 1.

Decompression schedules for the dives shown in Fig. 2 were calculated based on BSAC tables (Fig. 3). In these dives, the stage decompression was required in the 2nd and 3rd dives according to the BSAC tables, but none was done. Had the BSAC tables been used, 69 out of 80 dives should have undergone decompression stops.

Venous gas emboli were monitored after surfacing from the final dive of a day in 34 daily dives, and appeared in 19 of 34. Table 1 shows the number of dives in a day and VGE rate. VGE were found to appear after surfacing from the 3rd or later dive of a day.

Of 34 daily dives, 30 exceeded the maximum diving time and/or decompression schedules of the BSAC tables, whereas 4 dives were done within the range of the BSAC schedules. VGE rate was 19/30 (63%) in the former (non-

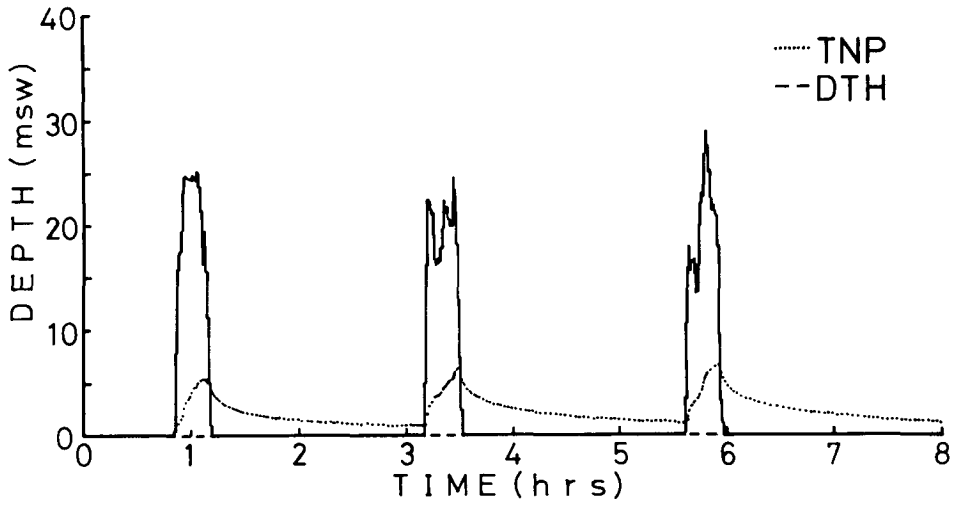

Fig. 2. A daily dive profile with TNP and DTH in a sport diver.

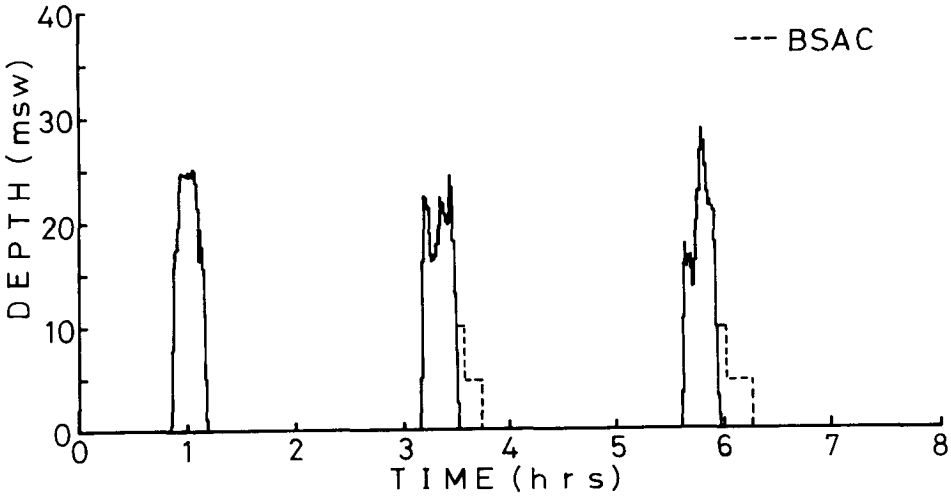

Fig. 3. Calculated dive schedules based on BSAC tables. 1st dive: no-decompression; 2nd dive: 10 msw, 5 min and 5 msw, 10 min; 3rd dive: 10 msw, 5 min and 5 msw, 15 min.

BSAC dives) but 0/4 (0%) in the latter (BSAC dives). Table 2 shows the difference of VGE rate which between two groups is statistically significant ($P< 0.05$, $\chi^2 = 5.74$).

Figure 4 shows the dive profile of the bent diver. According to his dive profiles, he seemed to dive too deep and too long. His decompression profiles obviously exceeded theoretical DTH. Before developing bends symptoms, he had VGE of grade IV.

Table 1
Relationship Between the Number of Dives in a Day and VGE Rate

No. of Dives	1	2	3	4	5	Average
VGE Rate	0/4 (0%)	0/2 (0%)	6/10 (60%)	8/12 (67%)	5/6 (83%)	19/34 (56%)

Table 2
Appearance of VGE in Divers Whose Diving Profiles Were Within or Over the Range of BSAC Schedules

VGE	Over BSAC	Within BSAC	Total
Appearance	19	0	19
Nonappearance	11	4	15
Total	30	4	34

$\chi^2 = 5.74$, $P < 0.05$.

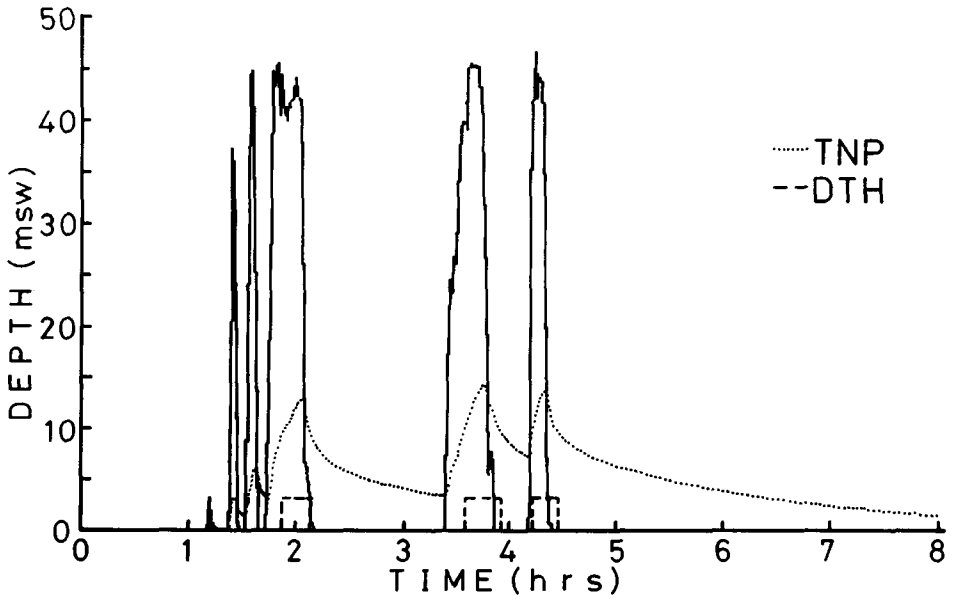

Fig. 4. A daily dive profile with TNP and DTH in a bent diver.

DISCUSSION

As we found in our previous investigations on harbor or shellfish diving (3), divers do not use the diving tables but follow their instincts. On the other hand, empirical decompression profiles of divers who went up and down frequently underwater did not exceed DTH, except once. This may be related to low incidence of decompression sickness.

Venous gas emboli rate was higher in non-BSAC dives than in BSAC dives ($P < 0.05$). The result supports the view that BSAC tables are effective in preventing decompression sickness. VGE did not appear after the surfacing from the 1st or 2nd dive of a day, but did appear after surfacing from the 3rd or later dives. From these data it seems that in sport diving repetitive dives of 3 or more times a day increase the risk of decompression sickness.

High-grade VGE were found in a bent diver whose decompression profile exceeded DTH. It has been reported that a correspondence can be determined between the level of detected bubbles and the incidence of decompression sickness (7). These findings suggest that the appearance of VGE and DTH can be used to appraise dive profiles with reference to the risk of decompression sickness, although VGE did not appear to be of diagnostic value for decompression sickness (8).

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THE EFFECT OF LONG HYPERBARIC EXPOSURE ON ERYTHROCYTE METABOLIC ENZYMES IN DEEP SEA DIVERS

J. A. Paciorek

Hyperbaric exposure to depths of 200 m induces permanent morphologic changes in the older erythrocyte fractions and a concomitant decrease in cell number, hematocrit (Hct), and hemoglobin (Hb) concentration. An investigation of the activity of some glycolytic and pentose phosphate pathway (PPP) enzymes and their role in the acceleration of red cell aging is reported here.

Mature erythrocytes are enucleated and have lost most intracellular structures, such as riboses and mitochondria, which means that lost or damaged proteins cannot be resynthesized nor can the cell use oxidative phosphorylation as a source of energy. With no capacity to reproduce protein molecules, the red cell, when exposed to the effects of aberrant or dysfunctional enzymes, becomes damaged. Cellular energy requirements are met by the anaerobic glycolytic pathway and supplemented by the additional provision of reducing power by the PPP. As glucose enters freely into the red cell by facilitative diffusion a deficiency of the molecule per se will never be a cause of glycolysis or PPP failure. A constant supply of adenine nucleotides and inorganic phosphates are also needed for energy production, the nucleotides are recycled within the cell and inorganic phosphates are generally present in excess of requirement. The rate-limiting step in glucose catabolism by red cells is hexokinase (1). The activity of hexokinase is low under optimum conditions and very susceptible to product inhibition by glucose-6-phosphate (G-6-P), which itself is affected by the inhibition of phosphofructokinase when there is a decrease in cellular pH. Control and utilization of G-6-P in the PPP is by enzyme glucose-6-phosphate dehydrogenase (G-6-PD) to reduce NADP to form NADPH. G-6-P metabolism will affect the glutathione cycle by its requirement for NADPH reducing power to combat oxidative stress within the membrane. During an increased rate of glycolysis dihydroxyacetone, phosphate

accumulates because glyceraldehyde phosphate dehydrogenase becomes rate limiting secondary to an increased ratio of reduced NADH to oxidized NAD (2). NADPH is necessary for the reduction of methemoglobin. Formation of diphosphoglycerate (DPG), a modifier of hemoglobin, is by the Rapoport-Lubering shunt of the glycolysis path. A decrease in DPG indicates a defect in the enzymes above the shunt and an increase below the shunt by pyruvate kinase (PK) inactivation.

This paper reports on measurements of DPG and the enzymes glutathione reductase (GR), G-6-PD, and PK in 5 dives deeper than 300 m involving 18 divers and in which an average 10% loss of hemoglobin concentration was measured at the postdive physical examinations and within 1 wk of completing the dive.

MATERIALS AND METHODS

Subject Selection and Blood Sampling Protocol

Blood was collected from 30 healthy young men selected to take part in deep diving trials in land-based chambers in the United Kingdom and Norway. Eighteen subjects were finally chosen to take part in these dives to an average depth of 446 m. Ten of the remaining subjects acted as surface controls and donated blood at approximately 5-d intervals during the pre- and postdive periods. During the NUTEC 500-m dive, 3 control subjects gave blood throughout the dive and these samples were used as external and daily quality controls of assays, in addition to the usual predive baseline data of each diver. Routine hematology and serum chemistry monitoring were made at the dive site or at the university laboratories on at least 3 predive samples, 3 dive samples, and at a postdive physical.

Enzyme Measurement

Blood for enzyme assay was collected into heparin, and during the dive it was decompressed in a small pressure vessel at between 1 and 2 m/min. Serum samples were collected into EDTA. Samples for G-6-PD assay were made according to Beutler et al. (3) and enzyme activity assay performed as recommended by World Health Organization special committee (4). Assay of PK was as described by Dacie and Lewis (5). DPG end reduced glutathione were measured using Sigma diagnostic kits (355-UV, Sigma Chemical Co., St. Louis, MO). Morphology studies using light and scanning electron microscopy were made on all samples using red cells collected into EDTA after the maximum decompression times (7). The formation of many abnormal cells during the in vitro decompression procedures of blood samples collected at depth in the 500-m dive at NUTEC resulted in a glutaraldehyde treatment of cells used in morphology studies directly after venesection and before decompression (7, 8).

In Vitro Pressure Trial Using Hyperbaric O_2 and N_2 at 5.0 MPa and in Vivo Pressure Stressed Cells

Postdive red blood cell samples were collected into heparin from the divers and divided. Half of each sample was repressurized in vitro by oxygen or nitrogen to 5.0 MPa. All the samples were then centrifuged, and packed cells separated on a density gradient by centrifugation (9). The activity of PK in the stratified red cell fractions was assayed and compared to the surface controls PK assays.

RESULTS

Cell Counts and Morphology Changes

The data in Table 1 support previous reports of a decrement in red cell numbers and hemoglobin concentration at the end of a long saturation dive. An adjustment in these parameters, allowing for the increase in Hct due to hemoconcentration during the bottom phase, also revealed a decrease of Hb and cell counts in early decompression in some divers. Reticulocyte counts were lowered during the use of a breathing gas with a high Po_2 but the reticulocyte counts were at the predive level of 1 to 2.2% after the divers had returned to surface. Cell morphology changes occurred in all the divers with the appearance of acanthocyte cells in some divers following compression. Echinocytes numbers, present at a normal level of 1% in peripheral circulation, were increased but <10% and persisted during the decompression phase. Monitoring of these abnormal cells has shown that several factors predispose

Table 1
Pattern of Enzyme Activity and Red Blood Cell Indices in 18 Men During 5 Diving Trials to a Mean Depth of 446 m

Parameter	Predive	Middive	Postdive	Reference Range and Units
Hb	163 ± 6	155 ± 3	150 ± 4	149 ± 17 g/liter
Hct	46 ± 2	50 ± 2	44 ± 2	44 ± 4 %
G-6-PD	4.1 ± 0.5	3.3 ± 0.4	2.8 ± 0.4	4.0 ± 0.9 IU/g Hb
PK	15.7 ± 0.9	16.8 ± 0.3	16.9 ± 0.7	15.0 ± 2.0 eu/g Hb*
Cell counts:				
Erythrocyte	4.5 ± 0.2	4.0 ± 0.3	3.6 ± 0.3	4.7 ± 0.7 × 10^{12}/liter
Reticulocyte	1.3 ± 0.5	0.3 ± 0.2	2.2 ± 0.1	1.1 ± 0.9 % at Het45%
Abnormal cell morphology	2.0 ± 1.0	9.0 ± 2.0	11.0 ± 2.0	<2.0 %
Cell pH	7.3 ± 0.0	7.1 ± 0.1	7.3 ± 0.05	7.35 ± 0.1 units**

* eu = engine units. ** Based on measurements after decompression.

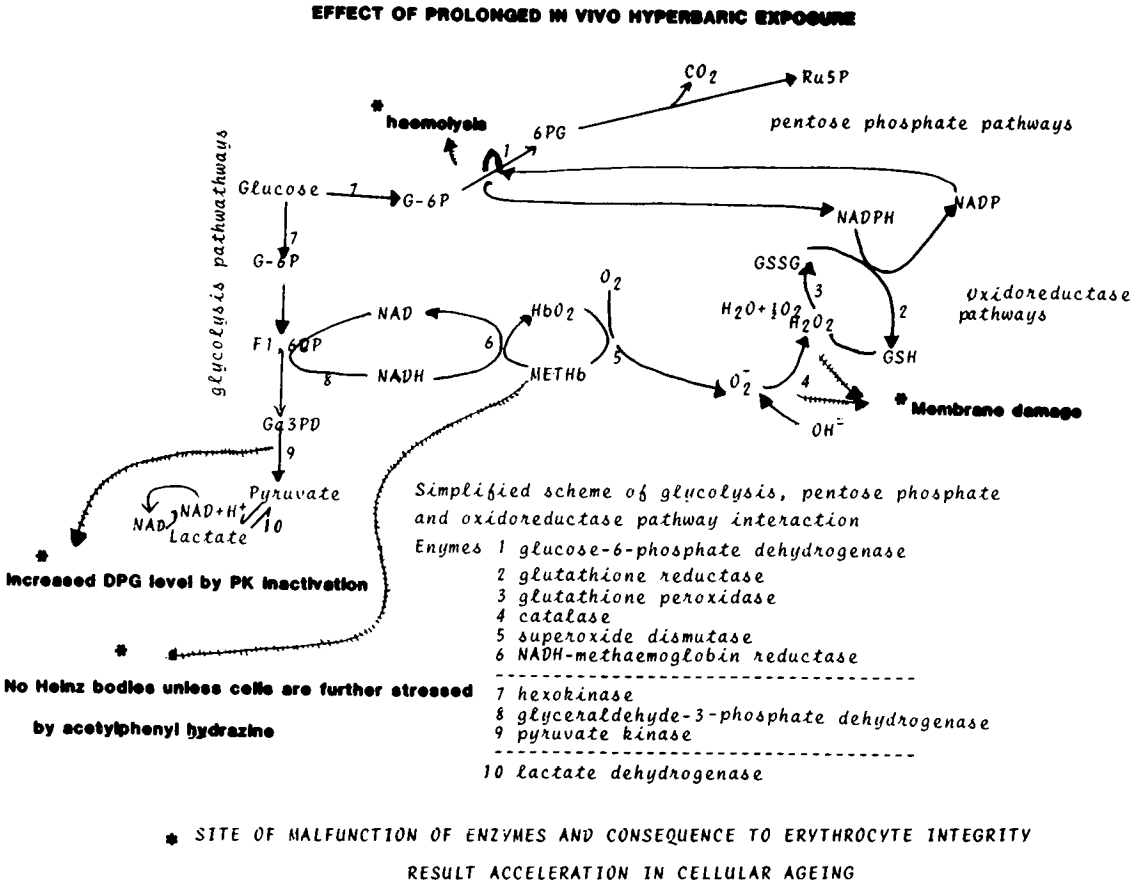

Fig. 1. Changes in metabolic enzymes and the effect on the erythrocyte population of a diver continually exposed to a hyperbaric environment at 3.1 MPa or greater for more than 20 d.

the cell to become an irreversibly nondiscoid form and are listed in Fig. 1. The decrease in cell pH was insufficient to cause the formation of stomatocytes as previously reported by in vitro manipulation of cell suspension media (8).

Enzymology

Erythrocytes G-6-PD are naturally decreased during cell aging and are often used as a marker of aging. A fall in G-6-PD activity occurred in the densest cell fractions (10) and closely followed the formation and appearance of nondiscocytes (11). Abnormality in the PPP, caused by a decrease in G-6-PD, in the denser cell fractions resulted in a lowered production of reduced glutathione (Table 2) which may then cause an oxidative denaturation of Hb. Reduced glutathione is needed by the cell for the maintenance of sulfydryl groups in the cell and membrane as well as an acceptor for H_2O_2. The production of G-6-P was maintained and the presence of only a 0.2 U decrease in intracellular pH did not affect the hexokinase step in glycolysis.

Table 2
Glutathione Reductase Deficiency Screening Test Expressed as Time in Minutes to Oxidize NADPH With Loss of Fluorescence in Long Wave UV Light (320-420 nm), in 8 Divers During 2 Diving Trials to a Mean Depth of 520 m.

	Predive	Middive	Postdive	Reference time, min
Divers	20 ± 5	39 ± 4	25 ± 4	20
Controls	21 ± 3	24 ± 3	23 ± 2	20

The concentration of phosphorylated intermediates of the glycolytic pathway is increased in cells with defective PK. Table 3 shows a small increase in DPG level in divers' blood samples, both during and after the dive, and were found only in samples with a lowered PK activity. An increase in cellular DPG would facilitate a decrease in oxygen affinity which would be beneficial in an anemic subject.

Table 3
Measurement of DPG in Erythrocytes of 12 Men During 3 Dives to a Mean Depth 487 m. Those Samples Collected at Depth were Decompressed at 2 m/min or Rapidly to Surface Within 1 min. Samples were Extracted in TCA and Assayed in Duplicate.

	Predive	Middive	Postdive	Reference Range and Units
Divers	13.0 ± 2.0	15.2 ± 3.0	14.6 ± 2.5	12.2 ± 3.7 μmol/g Hb
Controls	12.8 ± 3.0	13.1 ± 2.0	13.0 ± 2.0	12.2 ± 3.7 μmol/g Hb

Table 4 shows that the level of PK activity falls with an increase in cell density both in the erythrocytes of divers and surface controls. In vitro repressurization of the divers' erythrocytes clearly demonstrated that exposure to hyperbaric O_2 or N_2 had no effect on PK activity in the two younger cell fractions but was reduced in the oldest fractions.

Table 4
Comparison of PK Activity (ref. 15.0 + 2.0 eu/g Hb) in Whole Blood Samples after In Vivo Pressure Exposure in 12 Divers, in Surface Controls (Nonpressurized), and Repressurized Blood using Hyperbaric Nitrogen and Oxygen in a Small In Vitro Pressure System

			In Vitro Hyperbaric Trials at 5.0 MPa and Cells in **	
Separated Cell Fraction	In Vivo Trial Men at 475 m*	Surface Controls Men at Surface	Oxygen	Nitrogen
Top	17.0 ± 0.1	16.3 ± 0.3	16.4 ± 0.8	15.9 ± 0.6
Middle	15.8 ± 0.5	15.9 ± 0.5	16.0 ± 0.2	15.2 ± 0.5
Bottom	15.3 ± 0.3	14.9 ± 0.5	13.5†± 0.1	13.4†± 0.1

* Mean values of 3 samples per diver in the bottom phase of 2 dives. ** In vitro pressure trial was conducted at 37 ° C for 60 min at max 5.0 MPa: redecompression and before enzyme assay was 30 min at 25 ° C. †Denotes hemolysis in samples but potassium remained within 0.3 mmol/liter of starting values.

DISCUSSION

Divers exposed to high pressure for several days can develop various erythrocytic morphologies, of which acanthocytes and later echinocytes have been reported (8-10). Patients with PK deficiency often have both these types of "prickle cell" and associated hemolytic anemia (12).

The L-type pyruvate kinase in man, present in red blood cells and liver, is thought to undergo sequential transformation in which the subunits L-type are synthesized as the active precursor L′ and are then proteolytically degraded into L. In the liver the homotetramer L_4 is formed but in reticulocytes and erythrocytes cell maturation results in the L'_2 L_2 form. In vitro proteolytic tryptic incubation results in L'_4. Sprengers et al. (13) proposed that PK defects are not only caused by structural or regulatory gene changes but by defects in postsynthetic modification mechanisms. These authors, however, could not conclude whether their patient's new PK variant arose from increased breakdown or reduced enzyme synthesis. Diver's red cells have normal PK activity before the dive. Long-term pressure exposure cannot alter gene

expression in the existing cell population, and the reduction in enzyme activity must be in a postsynthesis modification of precursor L′ conversion to L'_2 L_2.

If G-6-PD reduction results (10) in less NADPH, decrement in reduced glutathione, or more membrane perturbation the cells integrity has been compromised. Impaired shape recovery and membrane permeability will facilitate higher intracellular calcium, which will enhance proteolytic degradation. The red cells' total precursor PK subunits may then be converted to L'_2 L_2. Pyruvate kinase also catalyzes one of the two ATP-producing reactions. PK also contributes to the control of NADH levels via pyruvate and is necessary in obtaining the steady-state level of 2,3-bisphosphoglycerate. Both ATP and 2,3-bisphosphoglycerate are important in regulating the rate of O_2 release at tissue level because of the preferential binding to deoxygenated rather than oxygenated hemoglobin (14). Other glycolytic enzymes tested in these series of assays have not demonstrated marked alterations in activity, and PK dysfunction represents one consequence of a hyperbaric-accelerated erythrocyte senescence. Further work on the indirect evidence that human erythrocyte and L-type PKs are regulated in vivo by phosphorylation-dephosphorylation (15) is now being assessed using an in vivo, hyperbarically stressed cell's PK.

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PRESSURE-INDUCED CHANGES IN BLOOD CELL RIGIDITY: A MECHANISM FOR CAUSING ASEPTIC BONE NECROSIS

M. R. Cross and J. K. Pimlott

Aseptic necrosis of bone afflicts divers and tunnel workers. The incidence varies considerably in different groups but in the North Sea oilfield diving population the total incidence is just below 5%. Within a population the incidence of the disease varies as a function of experience, age, depth of maximum dive, and history of decompression sickness. It may also be related to stature or weight (1). The highest incidence is seen in divers who have dived to the greatest depths and who have a history of decompression sickness (1); those who have dived to 200 m or more have an incidence of lesions greater than 15%. In a group of 54 men who have dived deeper than 300 m (1), 22.2% had evidence of aseptic bone necrosis, the majority having lesions in the shafts of the long bones.

In divers who willfully disregard acceptable decompression and exposure practices the incidence may be much higher. Wade et al. (2) studied 20 Hawaiian diving fishermen and found that lesions were present in 13 divers (65%), and similar experiences have been reported with other groups. Although the problem of aseptic bone necrosis has concerned the diving industry for some time, little progress has been achieved in finding either the cause or the prevention of the disease. Undoubtedly, a correlation exists between the occurrence of aseptic bone necrosis and previous decompression sickness (1) but this correlation is not good enough to prove a direct cause and effect between the 2 disease conditions. That decompression plays some part is evidenced by the reduction in the incidence of the disease in the diving and tunneling population as procedures improve, but clearly this is not the whole story. One of the original explanations for the cause of aseptic bone necrosis was the "bubble theory." Adherents of this theory believe that blood flow to bone marrow is obstructed by intravascular bubbles, caused by decompressing

too fast. Although this theory is extremely difficult to disprove, lack of conclusive evidence does not prove its correctness. Many alternative concepts have been advanced to explain the etiology of aseptic bone necrosis, including:

- Swelling of bone marrow fat cells as a result of oxygen causing an increased resistance to bone blood flow and a redistribution of blood away from the medulla (3).
- Inert gas osmosis causing fluid shifts with ischemic consequences (4).
- Hematologic factors, such as platelet aggregates and microthrombi (5).

Despite the number of hypotheses, no single factor has been identified as the definitive causal mechanism. In an attempt to elucidate more clearly the possible cause of aseptic bone necrosis and to decide which of the many hypotheses are most valid we posed a fundamental question: Is bone necrosis similar to that seen in diving also seen in any other disease process and, if so, what is the mechanism of its causation?

One condition that produces patterns of aseptic bone necrosis similar to that seen in diving is sickle cell anemia. The causes of bone necrosis in sickle cell anemia are well established, and the primary deficit seems to be a loss of the normal ability of the red cell to deform when it passes through the capillaries of the body. The capillaries of bone marrow are some of the smallest in the body (5), followed closely by those of the spleen (6). In sickle cell disease the rigid red blood cells cannot pass the capillaries and hence "log jam," causing local obstruction to flow and ischemia of the tissue downstream of the obstructed area. The stiffness of the red cells in sickle cell anemia is caused by crystallization of the abnormal hemoglobin under hypoxic conditions when it becomes desaturated (7).

The similarity between the changes in the bones of patients suffering from sickle cell disease and those with typical lesions of caisson disease was first noted just after World War II (8), and often since that time (9). Patients with sickle cell disease suffer from bone infarction at the time of sickling crisis, often provoked by hypoxia. Local bone pain is noted in these cases; there is generalized illness associated with a hemolytic anemia, and cerebral damage is a recognized feature. The triad of pain in the joints, bone infarcts, and long-term cerebral damage makes the sickle cell patient sound remarkably like a saturation diver!

Sickle cell patients suffer from many bone disorders (10), and young children have a different pattern of disease from adults, with cortical infarction of the diaphyses. In older children and adults, cortical infarction is much rarer; however, medullary infarction is relatively common and retrospective surveys have demonstrated this condition in approximately 21% of adults suffering from sickle cell disease (10). Sickle cell patients have shown that a large number of lesions in this group heal without scarring (11). We have obtained similar results with scintigraphy of divers where approximately 33% of scan-positive lesions turned x-ray positive, 33% persisted as scan

positive only, and the remainder eventually disappeared.

Bone marrow seems to be particularly sensitive to infarction, both in sickle cell disease and in diving. This vulnerability suggests that the microcirculation at this site may have special characteristics. The microanatomy is complex (12) and it has been shown that the bone capillaries that connect with the marrow sinuses are exceptionally small (13).

The pathophysiologic link between medullary microanatomy and bone infarction may lie in the characteristics of the red blood cell itself. Red blood cells are of the order of 8.5 μm in diameter, and these small capillaries are approximately 5 μm (10). To pass through these capillaries, deformation of the red cells is necessary and in sickle cell disease this ability is lost (11). It has been observed (14) that "log jamming" of sickle cells leads to necrosis of tissues in sickle cell disease, not only in bone marrow but also in the spleen and probably in the brain (13). Bone marrow infarction in sickle cell disease is associated with swelling and pain over the afflicted site, which is a prominent feature of the disease. Kenny et al. (11) showed that a decrease in the deformability of red cells preceded these crises. It may be possible to carry the sickle cell-diving hypothesis too far; what is intended is that the sickle cell model should be used as an illustration that bubbles *need* not be the only mechanism for the production of divers' ills. Other mechanisms may also cause the same syndromes, although bubbling could remain the intravascular trigger.

METHOD

We studied the effect of compression and decompression on a whole blood and red cell preparation in vitro to see if a raised environmental pressure would induce sufficient changes in the deformability of red cells to cause bone necrosis if similar changes happened in vivo. Kenny et al. (11) demonstrated that during sickle cell crises with bone infarction a doubling of the index of filtration (IF) of red cells can be demonstrated. To measure the IF, red cells are suspended in a suitable buffer and passed through a filter whose pores are 5 μm in diameter. The time taken for a fixed volume of the red cell suspension to pass through the filter is noted and compared with the time taken for an identical volume of the buffer used to suspend the cells. Stiffer cells take longer to pass through the filter, hence, the higher the IF, the more rigid the cells. Following the observations of Kenny et al. (11), we assume that increases of 200 to 300% would constitute a risk of bone necrosis formation if they occurred in vivo.

The rheologic behavior of blood and hence its filterability depends on a number of factors.

a) Temperature. This as of minor importance except that all observations should be made at the same temperature; in our laboratory an ambient temperature has been maintained at 18 to 22°C.

b) Concentration of the various cellular elements. Those studied to date using membrane filtration have used relatively low concentrations of cellular

elements in suspension. In accordance with the techniques described by Stuart et al. (15) we have standardized a hematocrit value of 7%.

c) The nature of the suspending medium and its viscosity. To date we have found it advantageous to mix the red cells suspended in their parent *plasma* with the phosphate buffer of Hendry (16) in the ratio of 1.5 ml blood:8.5 ml plasma. Given that 0.7 ml of blood contains 0.75 ml of plasma and the normal plasma albumin level in 40 g/liter, our final mix contains 6 g/liter albumen, which is very close to the 0.5% recommended as being adequate to prevent cell crenation. Since we use the donor subjects' own plasma for suspension of the cells, at least in part, all chemical elements of the blood will be present in the final suspension. Fibrinogen will be sufficiently diluted to make little contribution to the viscosity of the buffer.

d) The presence of cellular aggregates. These may be the product of the method of collection of blood samples, the particular anticoagulant used, or the result of the effects of pressure on the cellular elements of the blood, causing release of substances which finally result in aggregation. In our studies we have measured the filterability of the blood both as is, i.e., with aggregates, and also after filtration through Immugard wool (c), which results in the removal of particles greater than unicellular masses and white blood cells. Observations made without filtration reflect the filterability of whole blood including aggregates; studies after filtration reflect the mean change in the deformability of the mass of the erythrocytes.

The Filtrometer

We have built a small and simple filtrometer for blood filtration studies which is modeled on the hemorrheometer available commercially. The modifications were made by us so that the equipment could be used safely in the chamber. It is therefore totally mechanical and has note of the electronics associated with the commercial machine. Figure 1 is a diagram of the equipment which consists of a base (*1*) with a 5-μm nucleopore (c) filter on top (*2*). Over this is clamped the upright apparatus, which consists of a long Perspex tube (*3*) which leads into an expanded portion (*4*) into which an injection of the blood cell suspension can be made. The whole equipment is held together by a clamp (*5*).

In use, the apparatus is assembled with the membrane filter in place, and the blood sample is introduced through the port (*p*) until the level of the suspension rises above the top mark. The tap (*t*) is turned while the observer notes the time taken for the blood suspension meniscus to fall from the first mark (*i*) to the second mark (*ii*).

Before the passage of the blood cell suspension, the time required for a sample of the suspensory buffer to pass through the same filter is noted, and from this value the IF may be determined. There are many objections to this method, but we believe that most are overcome by this experimental protocol. The principle objection to our method is that we do not completely remove all white cells from the preparation. However, we assume that the white cell

count is the same in the control as in the experimental tubes, and therefore this systematic error need not concern us.

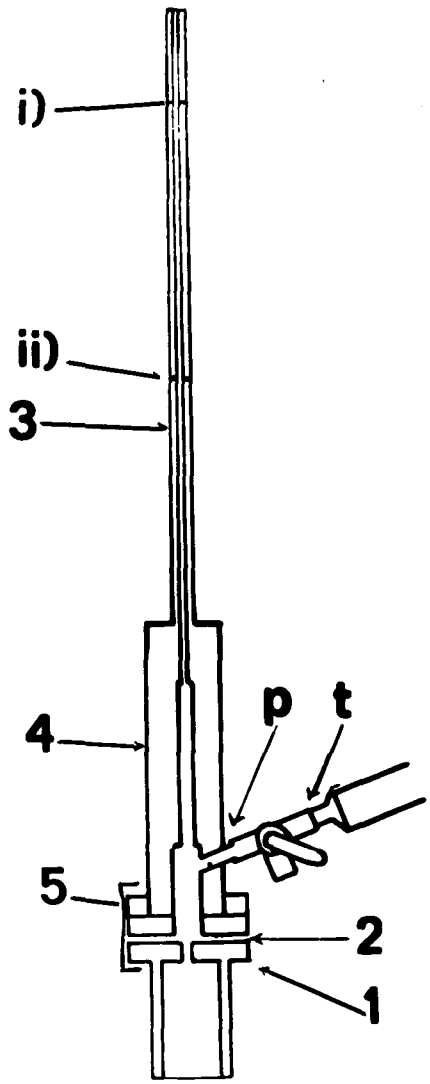

Fig. 1. Diagram of the filtrometer, i = upper level mark; ii = lower level mark; 1 = base; 2 = filter; 3 = Perspex tube; 4 = expanded portion; 5 = clamp; p = port; and t = tap.

The result depends on the hematocrit but not on the ambient temperature. In our work with the apparatus we have used hematocrit values of 7%, which is very similar to the value used and recommended by Hanss (17). Considerable controversy exists as to the ideal buffer for the suspension of the blood cells.

We have used the phosphate buffer recommended by Hendry (16). In fact we keep the cells in their own plasma until the last moment, at which point we add 8.5 ml of Hendry buffer to the 1.5 ml of blood, thus producing a suspension of the required hematocrit.

Because we wish to maintain the viability of the red cell preparation for 6 h, we have not made a preparation of washed red cells suspended in buffer, as recommended by Stuart and Kenny (18), and we leave the cells suspended in their own plasma for the duration of the experiment.

Protocol

Blood was taken from volunteers using a wide-bore needle and minimum hemostasis. The subjects, all divers, were asked not to dive for 24 h before the experiment. After sampling, the blood was placed in plastic tubes, with lithium-heparin as anticoagulant, and transferred immediately to a roller. A portion of blood (50 ml) was then filtered through Immugard wool (c) to remove red cell aggregates and to reduce the white blood cell count to a low level. Our studies show that after filtration the white cell count was less than 2000 cells/mm^2. One and a half milliliter portions of the blood were then pipetted into plastic tubes and the tubes placed under pressure in a chamber. During the exposure to pressure, the tubes were mixed constantly by a rotating roller.

Red cell filterability was measured as follows: eight and a half milliliters of phosphate buffer was added to the tube to make the volume up to 10 ml. Given that the starting hematocrit was approximately 0.48, this gives a final suspension of cells consisting of approximately 7% red cells. The suspension of red cells was injected into an apparatus so that the suspension was forced by gravity through a polycarbonate membrane in the form of a sieve with (Nucleopore(c)) pores 5 μm in diameter. The filtration pressure was 10 cmH_2O at the start of the filtration, and the volume of the system was 6.5 ml. The time taken for the first 0.5 ml of red cell suspension to pass through the filter was noted and compared with a control time obtained in exactly the same manner for the buffer alone. Control observation were made for each filter.

We followed the practice of Stuart in cleaning the membranes in an ultrasonic bath after use. Using our method, 0.5 ml of buffer takes 1.2 s to pass through the filters. We discarded any membrane in which the control time exceeded 1.5 s.

Sensitivity of the method may be checked by testing a blood sample which has been altered so as to contain an abnormal population of cells. These may be fixed either with heat or chemicals. Using our filtrometer, a four-to fivefold increase in the measured IF may be observed after adding 4% glutaraldehyde-fixed cells to the preparation; this represents adequate sensitivity for clinical purposes.

Normal Values

Before studying the effect of hyperbaric pressure on cells, we confirmed that whole blood kept on a mechanical roller would not undergo a significant change in its IF for the time exposures planned. Figure 2 shows the results of a typical pair of calibration experiments in which the IF of blood samples was measured at intervals of 18 h (study 1) and 10 h (study 2); the latter study involved 2 samples. It can be seen that for at least 8 h, fresh blood anticoagulated with lithium-heparin does not undergo deterioration if kept mechanically agitated.

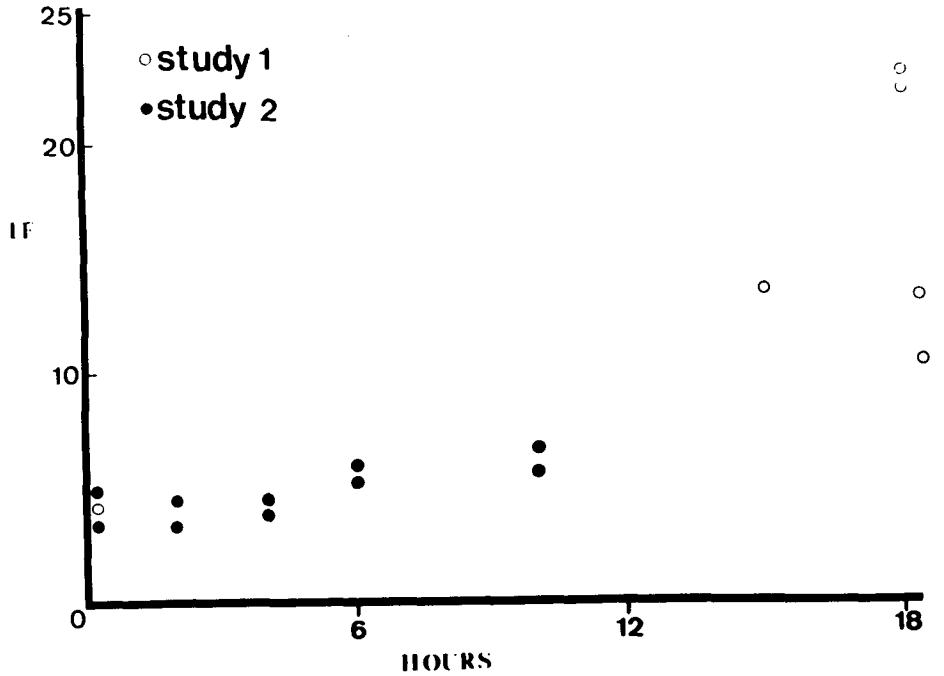

Fig. 2. Change in index of filtration with time. Two experiments in which the IF of whole blood was measured over a period of time while it was kept agitated on the roller; the blood was anticoagulated with lithium-heparin. No significant deterioration occurs in the 6- to 8-h periods used in this study.

Normal value for IF in our laboratory is 5.18 ± 1.9 SD for blood samples examined immediately after being drawn. It is our practice to determine a second control value at the end of the experiment, 6 to 7 h after sampling. This postcontrol value is 5.29 ± 1.9 SD, showing that cells kept in their own plasma do not greatly stiffen over the time scale.

Effects of Compression and Decompression

Our initial studies have been confined to air dives at depths of 15 m (2.5

ATA) and 30 m (4.0 ATA) with pressure exposures of 4 and 6 h. Observations have been made at the following time intervals:

- *Immediately before the blood surfaced.* This observation is made with the apparatus in the chamber. Of necessity, presurfacing observations made in the chamber at 30 m are limited because there is a risk of inducing "bends" in the experimenters.
- *Immediately upon surfacing.* Blood samples are decompressed from pressure in the medical transfer lock of the chamber. Immediately upon arrival at the surface, 8.5 ml of buffer is added and, after 8 min rolling to mix the sample, the observation is made.
- *At intervals thereafter.* It is our practice to make observations at 15-, 30-, and 60-min intervals after the blood surfaces.

As indicated above, observations are made on the undived blood samples before the experiment (control) and at the very end (postcontrol).

RESULTS

Experiments were also conducted to determine the effect of hematocrit on the IF. Figure 3 shows the change in IF with hematocrit for one experiment. The dependency of IF on hematocrit means it is extremely important to check the hematocrit of the sample whenever a measurement is made.

In the first experiment we determined the change in IF of whole blood after a 4-h exposure to 2.5 ATA on air. Measurements were made on samples of blood both before and after filtration through Immugard wool (c). The results of this experiment are presented in Fig. 4.

It can be seen that the IF of the red cell suspension (filtered blood) does not greatly change, whereas the IF of whole blood increases considerably in a progressive manner while in the chamber and even more so after surfacing. The Nucleopore (c) filters, after filtration, were cleared with dilute acetic acid and stained with brilliant cresyl blue. Although many platelet aggregates were seen, the principle cell observed blocking pores was the polymorph leukocyte, suggesting that the pressure-induced decrease in filterability of the blood is primarily a white cell phenomenon.

Effect of 4.0 ATA Air as Compared with 2.5 ATA

We conducted studies to compare the IF change induced by exposure to 4.0 ATA air with that produced by 2.5 ATA. Observations were made under pressure after 4 and 6 h and upon surfacing. The results are presented in Table 1.

DISCUSSION

Although in sickle cell disease stiffened red blood cells are identified as the agent causing the local marrow ischemia, the ability to block small capillaries is not a monopoly of red cells. Under different conditions, all the

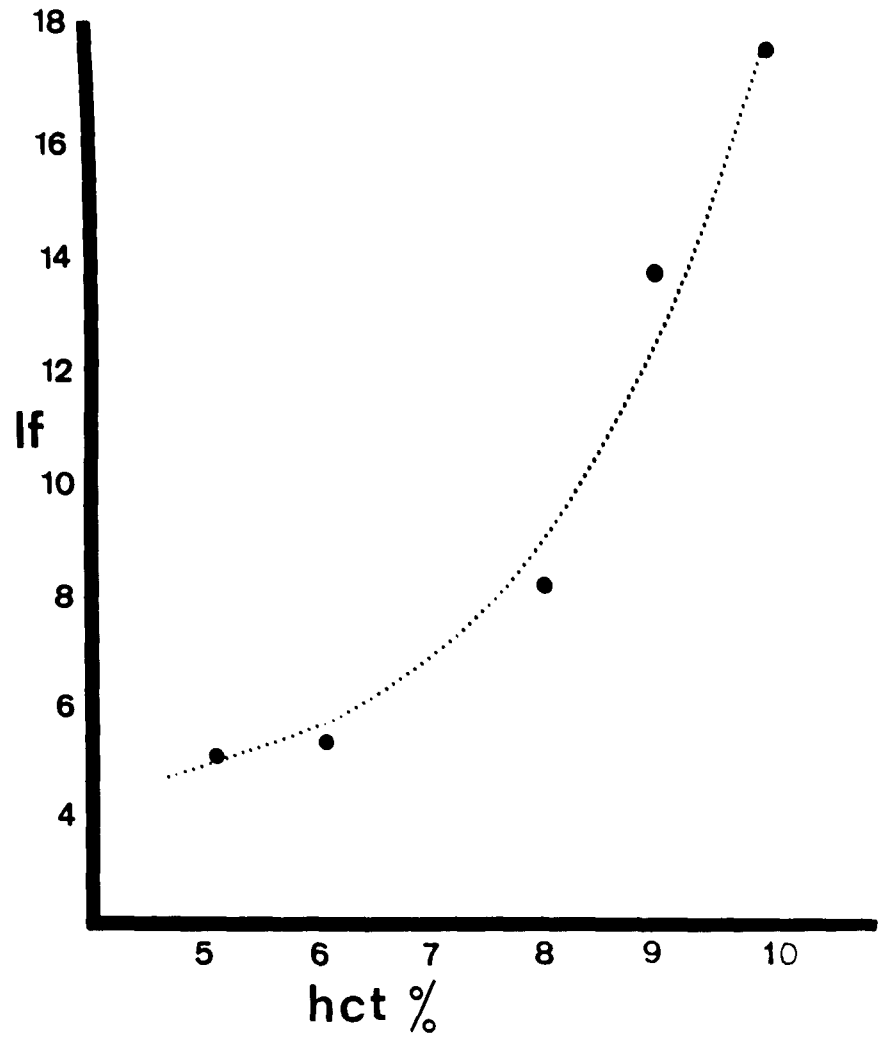

Fig. 3. Effect on observed index of filtration of hematocrit. Sample of blood was taken and the IF determined at different hematocrit levels in the range which might be used experimentally. It is important to ensure that exactly the same hematocrit preparation is used throughout an experiment.

cellular elements of the blood have been demonstrated to cause capillary obstruction in different disease states, and even the liquid medium of the blood plasma can, if its viscosity is sufficiently altered, be responsible for local perturbations of blood flow. In this study we have shown that a considerable decrease in whole bleed filterability occurs with both pressure exposure and decompression.

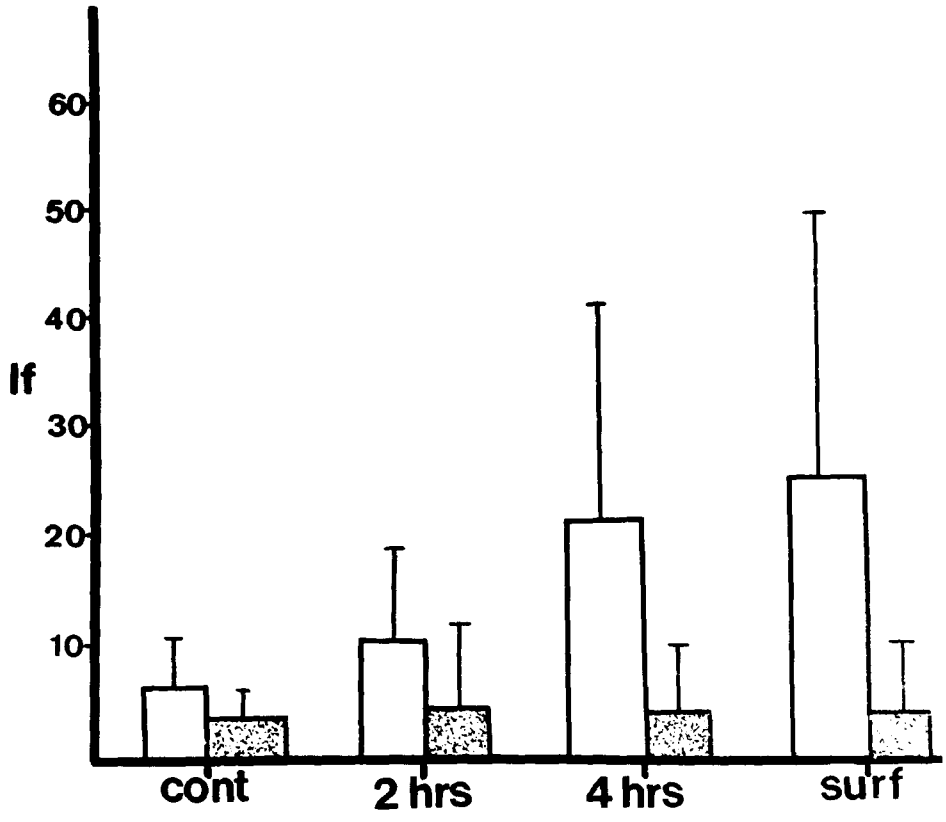

Fig. 4. Change in IF with exposure time and immediately after decompression from 2.5 ATA air. Blood samples were taken and exposed to increased (2.5 ATA) ambient pressure in the chamber. Observations were made at 2 and 4 h under pressure and immediately after decompression. This figure shows the IF change of whole blood (open bars) and a pure red cell suspension (stippled bars). The increase in IF is attributable to changes in the cellular elements of blood other than erythrocytes.

The extent of the decrease in filterability is linked to the pressure, but we have been unable to separate this from the effect of the inert gas solution. Hyperbaric oxygen has already been shown to cause a lowering of the measured filterability of blood (19), and in a limited series of unpublished observations we have determined that hyperbaric oxygen can to some extent reverse the increase in IF that we report. Our light microscopic observations of the filters after use show that granulocytes are the predominant cell blocking the pores. We hypothesize that the primary effect of elevated pressure is on the granulocyte, perhaps in causing activation. This may then be followed by platelet activation and aggregation.

Table 1
IF Changes at 40 ATA Compared with 2.5 ATA

	Air 2.5 ATA		Air 4.0 ATA	
	4 h, ($n = 10$)	6 h, ($n = 10$)	4 h, ($n = 7$)	6 h, ($n = 9$)
Control	4.8 ± 0.53	4.4 ± 0.52	4.3 ± 0.2	5.1 ± 0.8
End exposure	8.2 ± 1.5	6.0 ± 0.66	8.4 ± 2.0*	9.3 ± 3.7*
Immediately post-decompression	8.0 ± 1.8*	6.4 ± 0.72**	7.8 ± 1.0**	14.8 ± 3.8*
60 min post-surfacing	5.9 ± 0.72	5.2 ± 0.67*	6.2 ± 0.8*	8.7 ± 1.5*

* P 0.025; ** P 0.0125.

In another study (20), we showed that the greatest correlation between the onset of decompression sickness in our subjects after experimental profiles was with the *prediving* granulocyte count. This is similar to the observations made in victims of cerebrovascular disease in Japan (21). In a series of observations on a cohort of Japanese Prentice et al. (21) showed that a high white blood cell count obtained at a random measurement 2 yr before the year of the study correlated well with the subsequent development of cerebral infarction, but not with cerebral hemorrhage. They considered that a likely explanation was excess production of complement C5a, causing granulocyte clumping and subsequent microvascular obstruction.

Complement elevations are seen after deeper air diving exposures (22). Granulocytes as well as red cells and platelets can be induced to aggregate intravascularly and form masses that may block blood vessels (23). This phenomenon was first observed in 1968 in relation to the blood of patients undergoing renal dialysis, when passage of the blood through the dialyzer coils resulted in activation of leukocytes and their subsequent removal from the circulation, causing leukopenia (24). These authors wondered whether leukocyte aggregation might be responsible for the increased incidence of cardiac arrhythmia and other circulatory disturbances associated with early hemodialysis. The hypothesis that the pulmonary fibrosis-calcinosis syndrome, which develops in many patients given long-term hemodialysis treatments, may result from the microvasculature being plugged by leukocytes has never been proven, although leukocyte aggregation has been postulated as a mechanism for the causation of embolic microvascular disease. There is evidence that the sudden blindness of acute pancreatitis may be the result of leukocyte embolism of the retina (25). Bone necrosis may also be found in patients suffering from chronic pancreatitis (26). Interestingly, agents that inhibit platelet activation and aggregation do not necessarily interfere with the same phenomenon in leukocytes; however, high doses of steroids do inhibit white cell aggregation, but paradoxically cause aseptic necrosis of bone by a different mechanism (27).

Inasmuch as the ratio of leukocytes to red cells in normal blood is only 1:700, they are not generally recognized as contributing greatly to blood viscosity. However, leukocytes are generally larger than red cells and have a nucleus. They have been observed plugging the nutrient vessels under normal conditions in animals (28) and in humans (29).

In a review article, Lowe (30) stated: "Whole blood filtration using filters of 5-micron pore diameter measures pore plugging by erythrocytes and leucocytes in equal measure since the former are 700 more times less frequent but 700 times more likely to plug pores."

Capillaries plugged by leukocytes are a recognized complication of the hyperleukocytic leukemias, and in these conditions both bone and splenic infarction is a recognized feature (31). It has also been shown that after hypoperfusion under experimental conditions, normal white cells can be seen plugging mesenteric capillaries in an animal model.

Bagge and Branemark (29) showed that normal white cells behave quite differently from red blood cells in that they are capable of deforming as viscoelastic bodies, but the viscous component is much slower than that of red blood cells. They showed that lymphocytes were more resistant to deformation than polymorph leukocytes (PMNG), and observed that even in normal circulation, leukocytes can temporarily block capillary blood flow, although ultimately they concluded: "It is concluded that the influence of WBCs on the distribution of blood in the microcirculation is small under normal flow conditions. However, the behaviour of the WBCs in the normal microcirculation indicates that the WBCs may be an important hindrance to the blood flow in situations of disturbed haemodynamics."

The small size of platelets makes them unlikely candidates to plug capillaries in their natural state. However, if shear stresses are applied, spontaneous aggregation to form large clumps of activated cells is a natural consequence, particularly if other provocative conditions exist. Platelet aggregates are not only responsible for the physical obstruction of blood vessels but by their very presence can cause local alterations in blood flow, which predispose to vascular wall trauma and to atherosclerosis. Platelets are more likely to aggregate spontaneously if other formed elements of whole blood are present. This may be due to the physical presence of the other formed elements of the blood or may be the consequence of leukotriene release from damaged granulocytes, thromboxane release as the result of surface stimulation (32), or ADP from damaged erythrocytes (33).

We conclude that exposures to even modest pressure can cause a decrease in the filterability of blood, occasioned largely by an increase in the stiffness of the white cells. We do not know whether this is a direct physicochemical effect on the white cell membrane or the consequence of some form of activation. However, the magnitude of the changes observed could, under certain circumstances, be responsible for causing pathologic changes in humans. A study is presently being undertaken to determine whether the changes observed in vitro are also to be found in vivo.

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PULMONARY FUNCTION DURING COLD GAS BREATHING AT DEPTHS TO 305 M

J. R. Clarke, M. E. Bradley, and E. T. Flynn

The U.S. Navy has adopted limits for minimum inspired gas temperature for mixed-gas divers. Originally, Braithwaite (1) recommended that a minimum temperature of 13°C be maintained at 305 m, and this recommendation was adopted by the U.S. Navy Diving Manual, 1975 edition. More recently, however, Piantadosi (2) recommended a higher temperature, 22°C, which was included in Change 1, revision 1, U.S. Navy Diving Manual, 1982. Both of these temperature limits were designed to limit drops in core temperature to no more than 2°C on a 4-h mission.

However, cold gas inhalation can precipitate airway obstruction induced by copious secretions (3, 4). While experience suggests that the recommended inspired gas temperatures prevent the catastrophic consequences of cold gas breathing, it is not known if there are more subtle effects. For example, the inhalation of subfreezing air is used as a provocative test for asthma; asthmatics may increase their airway resistance by 100% or more following cold gas exposure (5-7). However, even normal subjects can react to this cold gas challenge with 9 to 10% reductions in forced expiratory flow rates (8). Asymptomatic smokers and ex-smokers show an even greater reactivity to cold gas inhalation (9). Since the respiratory heat loss at 31.3 ATA with 13°C gas is about 4 times greater than that resulting from provocative testing at 1 ATA, altered pulmonary function might result, affecting the safety of Navy divers. Our intent then was to search for subclinical effects of cold gas breathing during exercise at simulated depths to 305 m.

METHODS

Four U.S. Navy saturation divers were subjects on a simulated 305-m dive

at the hyperbaric facility of the Naval Medical Research Institute (Fig. 1). Two were moderate-to-light smokers and two were nonsmokers. Pulmonary function tests were performed before and after bicycle exercise at 16.2 and 23.7 ATA during the compression phase, and during 4 d at 31.3 ATA. A partial sequence of pulmonary function tests was performed during decompression at 24.0 and 18.0 ATA without exercise.

The two pulmonary function tests were the interrupter measurement of respiratory resistance (10, 11) and the forced vital capacity maneuver. The interrupter measurements were made during relaxed tidal breathing. From the forced exhalation were obtained measurements of forced vital capacity (FVC), peak expiratory flow rate (PEFR), volume exhaled within the first second (FEV_1), $FEV_1/FVC\%$ flow rate when 50% of the FVC has been exhaled (FEF_{50}), and mean flow existing between the first and last quartiles of the forced vital capacity (FEF_{25-75}).

The protocol was as follows: Before exercise, the subject breathed an $He-O_2$ mixture with an O_2 of 0.35 ATA supplied from a heat exchanger located within the chamber (Fig. 2). Gas was supplied at 1 of 3 test temperatures (described below) at a constant flow rate which exceeded the ventilatory demands of the diver, the excess being dumped into the chamber complex. The diver's expirate was directed into a Wedge spirometer having first passed through an airway interrupter. This latter device interrupted air flow twice a second for approximately 0.1 s, allowing mouth pressure to equilibrate with alveolar pressure. The resulting rise in mouth pressure during expiration was (ΔPm). The flow which resulted from that alveolar pressure was determined from flow measured by the spirometer immediately preceding interruption ($\Delta \dot{V}$). Respiratory resistance was then $\Delta Pm/\Delta \dot{V}$. Approximately 1 min of recording was obtained during relaxed tidal breathing. Mouth pressure was measured by a Validyne transducer and transducer amplifier (Validyne Engineering Corp., Northridge, CA). Flow and pressure signals were recorded on a digital oscilloscope (Nicolet Scientific Corp, Northvale, NJ). After the resistance measurements, three FVCs were performed into the same spirometer through a second port. For the approximately 3-min duration of the FVC maneuvers, the diver subjects inhaled chamber gas at ambient temperature (31-33°C at 31.3 ATA).

Upon completion of the preceding control measurements, the divers began bicycle exercise at a moderate work rate of 1 W/kg while breathing gas at one of the following temperatures; at 31.3 ATA, 11.9 ± 1.2°C (mean ± SD, approximating the Braithwaite limit of 13.3°C), 22 ± 0.3°C (the Piantadosi limit), or chamber gas at 32.2 ± 0.8°C. At 16.2 ATA inspired gas temperature averaged 23.3 ± 0.8°C, and at 23.7 ATA, 13.2 ± 0.8°C. During decompression at 24.0 and 18.0 ATA, chamber gas at approximately 31°C was breathed.

Once every 10 min during the exercise period, expired gas was collected to measure minute ventilation, which was typically 30 liter·min^{-1}. Following 30 min of exercise the diver left the bicycle and breathed gas from the interrupter circuit at the same test temperature. Continuous airway cooling was

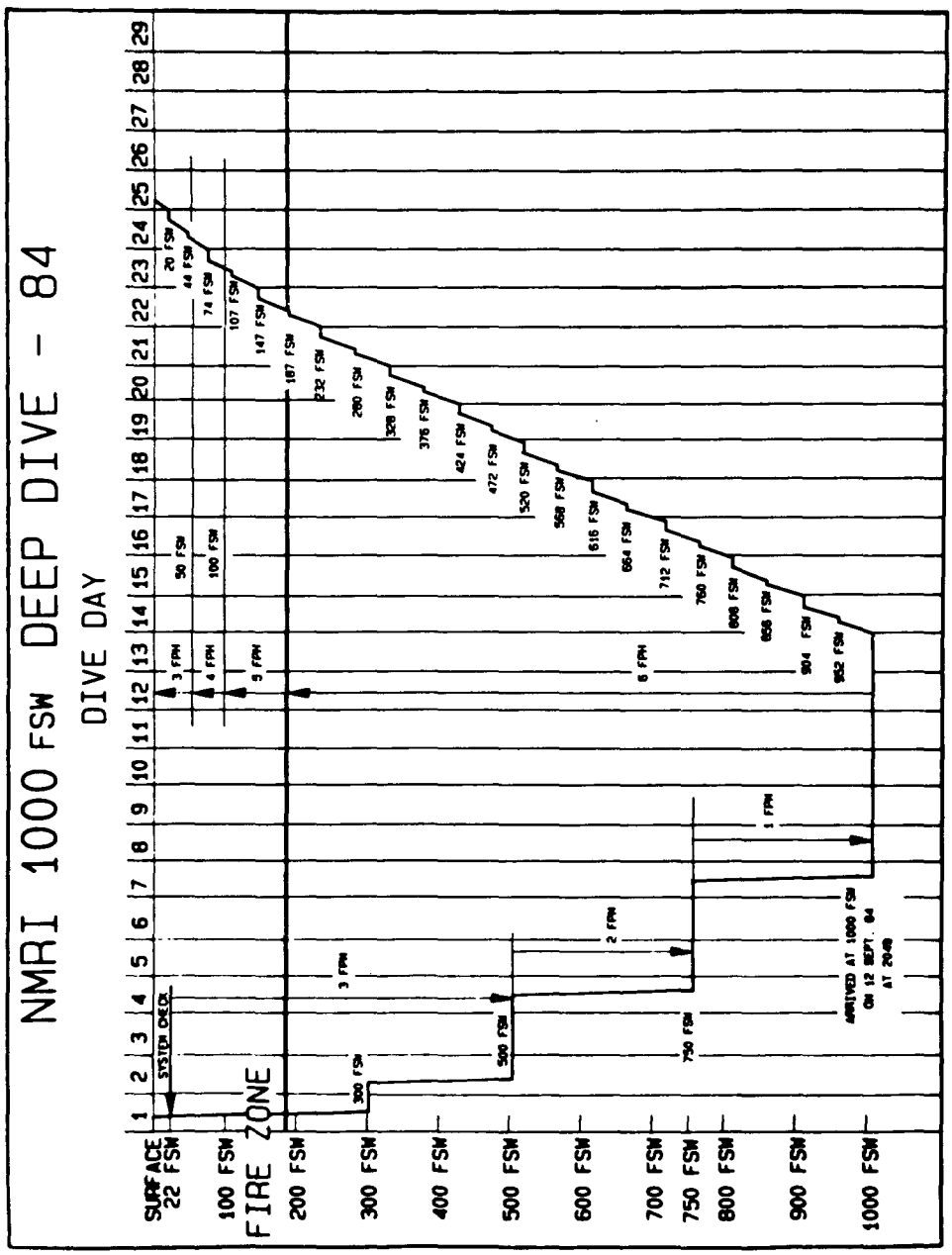

Fig. 1. Dive profile.

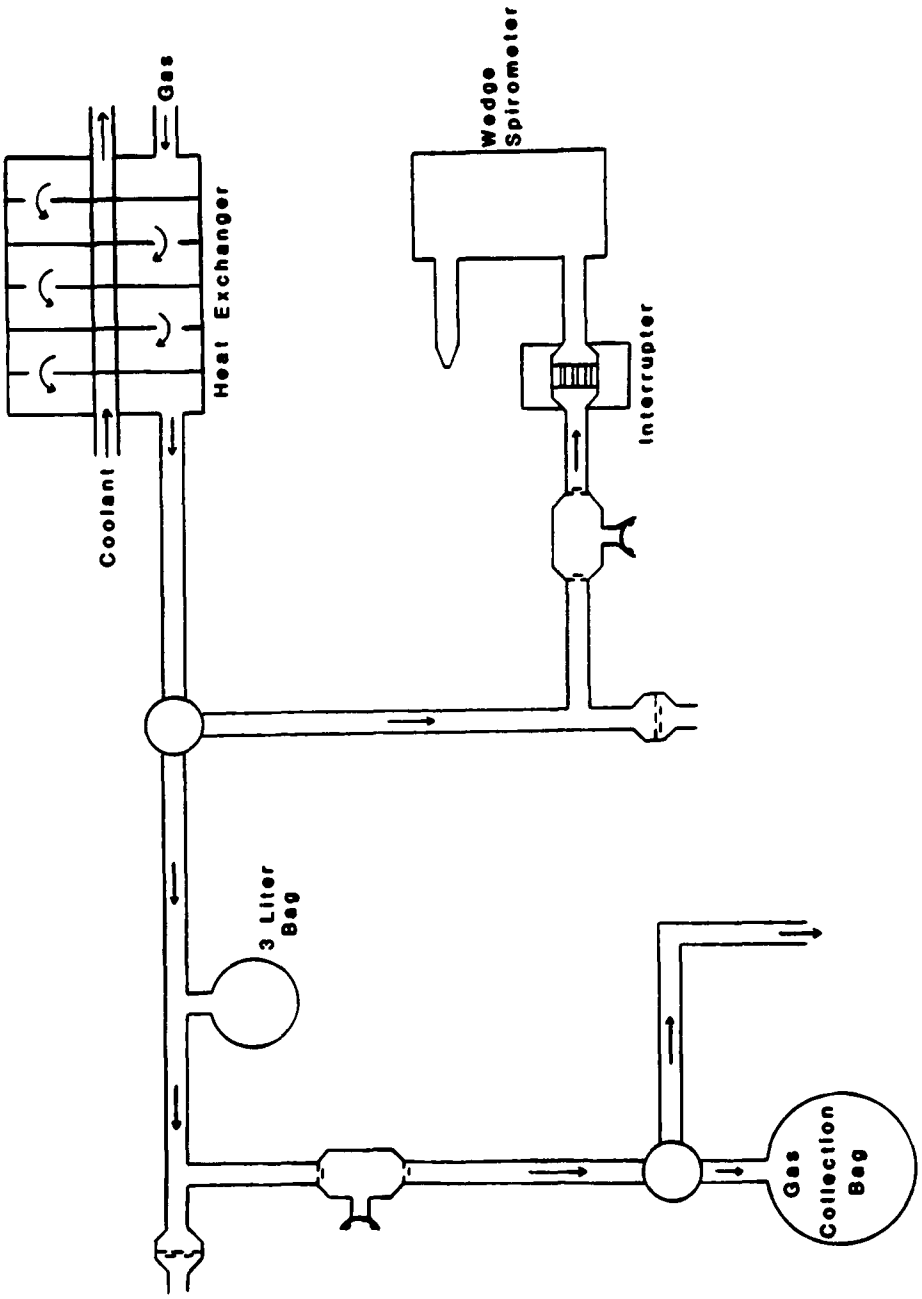

Fig. 2. Experimental set-up inside the hyperbaric chamber. Gas from the heat exchanger, upper right corner is delivered to a mouthpiece and interrupter circuit (lower right) or separate mouthpiece supplying the diver on the bicycle (left). The 3-liter bag served as an inspiratory reservoir.

impossible during the approximately 10-s transition from bike to interrupter. At the end of 5 min of quiet breathing of conditioned gas, the diver's expired breath was diverted into the spirometer and resistance measurements were repeated. Following this, 3 FVCs were performed, with the diver breathing chamber gas at ambient temperature.

All studies were performed in the afternoon. Each morning included exercise and cardiovascular testing as part of another study. The present studies began immediately after lunch and continued throughout the afternoon. Divers were always run in the same order to minimize the effects of circadian rhythms.

The resistance of the interrupter equipment was measured at 1 ATA and 20°C during a 6.5 liter•min^{-1} flow of air and 80% SF_6-20% O_2. Resistance was 1.4 cm H_2O•liter^{-1}•s^{-1} in air and 2.0 cmH_2O•liter^{-1}•s^{-1} in SF_6-O_2. These resistances were used to estimate equipment resistance as a function of depth in the following manner: gas density was calculated as 3.08 g•liter^{-1} at 16.2 ATA, as 4.33 g•liter^{-1} at 23.7 ATA, and 5.58 g•liter^{-1} at 31.3 ATA. Equipment resistance was assumed to depend linearly on gas density. Using the resistances measured in air and SF_6 to establish that regression, the equipment resistance was estimated to be 1.7 cmH_2O•liter^{-1}•s^{-1} at 16.2 ATA, 1.9 cmH_2O•liter^{-1}-•s^{-1} at 23.7 ATA, and 2.2 cmH_2O•liter^{-1}•s^{-1} at 31.3 ATA, assuming a resting tidal ventilation of 6.5 liter/min.

Equipment resistance was subtracted from measured resistances to yield values for respiratory resistance at each depth. At 31.3 ATA the changes in expired gas temperature resulting from altered inspiratory gas temperatures were estimated to result in no more than a 2% change in expired gas density. The density-dependent resistance of equipment in the expiratory stream was therefore assumed constant regardless of inspired gas temperature.

All pulmonary function data were expressed as a mean value for each diver on each day, both pre- and postexercise. The interrupter measurements, for example, represented the mean of 10 to 12 individual measurements of expiratory resistance. The FVC measurements were expressed as the mean of typically 3 forced expiratory maneuvers. All spirometric values were corrected to BTPS conditions. Statistical comparisons between pre- and postexercise pulmonary function tests were by the Student's t test ($P \leq 0.05$).

The heat exchanger used a 50:50 mixture of propylene glycol and water chilled by a Neslab ULT-80 Chiller unit (Neslab Instruments, Inc., Portsmouth, NH). The coolant flowed through a central finned tube with the inspired gas flowing in the opposite direction over the fins. When required, the gas entering the heat exchanger was prechilled by passage through a coil immersed in the chilled chamber wetpot. The flow rate and temperature of the gas leaving the heat exchanger was constant. Inspired gas temperature was measured by YSI (Yellow Springs Instruments, Yellow Springs, OH) thermistors.

Respiratory heat loss (RHL) was calculated by the method of Nuckols (12). His equations were modified to allow for the temperature dependence of the latent heat of vaporization of H_2O (4), and to allow the calculation of expired

gas temperature from inspired gas temperature using the equation published by Piantadosi (2), $T_{expired} = 25.4 + 0.28 \cdot T_{inspired}$. Inspired gas, derived from the helium banks, was assumed to be dry.

RESULTS

There was no consistent change in postexercise resistance at 31.3 ATA following warm gas breathing (32°C) (Table 1, Fig. 3). On the other hand, pooled resistance values decreased significantly from preexercise values of 4.3 ± 0.6 $cmH_2O \cdot liter^{-1} \cdot s^{-1}$ to postexercise values of 2.2 ± 0.3 $cmH_2O \cdot liter^{-1} \cdot s^{-1}$ at 22°C. At 12°C, the respective resistances were 4.0 ± 0.6 and 2.0 ± 0.2 $cmH_2O \cdot liter^{-1} \cdot s^{-1}$ (Fig. 4), respectively. This represents a mean drop in resistance of 49% following exercise with cool gas (≤ 22°C). The preexercise values of respiratory resistance were not significantly different from day to day.

Table 1
Respiratory Resistance

	Diver 1				Diver 2			
Day (°C)	1(12)	2(12)	3(22)	4(32)	1(12)	2(12)	3(22)	4(32)
Pre	5.4	3.0	2.6	4.3	6.3	3.6	5.6	3.8
Post	1.4	1.9	2.7	4.5	2.1	3.0	2.0	5.3
	Diver 3				Diver 4			
Day (°C)	1(12)	2(12)	3(22)	4(32)	1(12)	2(12)	3(22)	4(32)
Pre	4.8	4.3	4.1	9.1	0.9	3.3	4.8	5.6
Post	1.7	2.7	1.3	5.5	2.2	1.1	2.6	4.0

Gas temperature is given in parentheses next to the dive day at 305 m.

Cold gas (12 °C) was applied on Days 1 and 2. Day 3 was 22 °C gas, and Day 4 was 32 °C gas breathing.

Pre = preexercise resistance ($cmH_2O \cdot liter^{-1} \cdot s^{-1}$).

Post = resistance 5-10 min postexercise.

Mean respiratory resistance dropped numerically but not significantly during exercise and cold gas breathing at 16.2 ATA (from 6.1 ± 0.6 $cmH_2O \cdot liter^{-1} \cdot s^{-1}$ to 5.0 ± 0.4 $cmH_2O \cdot liter^{-1} \cdot s^{-1}$). The postexercise data at 23.7 ATA were incomplete for technical reasons.

Preexercise respiratory resistances rose during compression from 3.4 ± 0.2 $cmH_2O \cdot liter^{-1} \cdot s^{-1}$ at the surface to 6.4 ± 1.2 $cmH_2O \cdot liter^{-1} \cdot s^{-1}$ at 16.2 ATA and 7.7 ± 0.4 $cmH_2O \cdot liter^{-1} \cdot s^{-1}$ at 23.7 ATA. During 4 d at 31.3 ATA, the preexercise resistance dropped to an average of 4.5 ± 0.5 $cmH_2O \cdot liter^{-1} \cdot s^{-1}$.

During decompression at 18 ATA, pooled preexercise resistances averaged 4.3 ± 0.3 $cmH_2O \cdot liter^{-1} \cdot s^{-1}$.

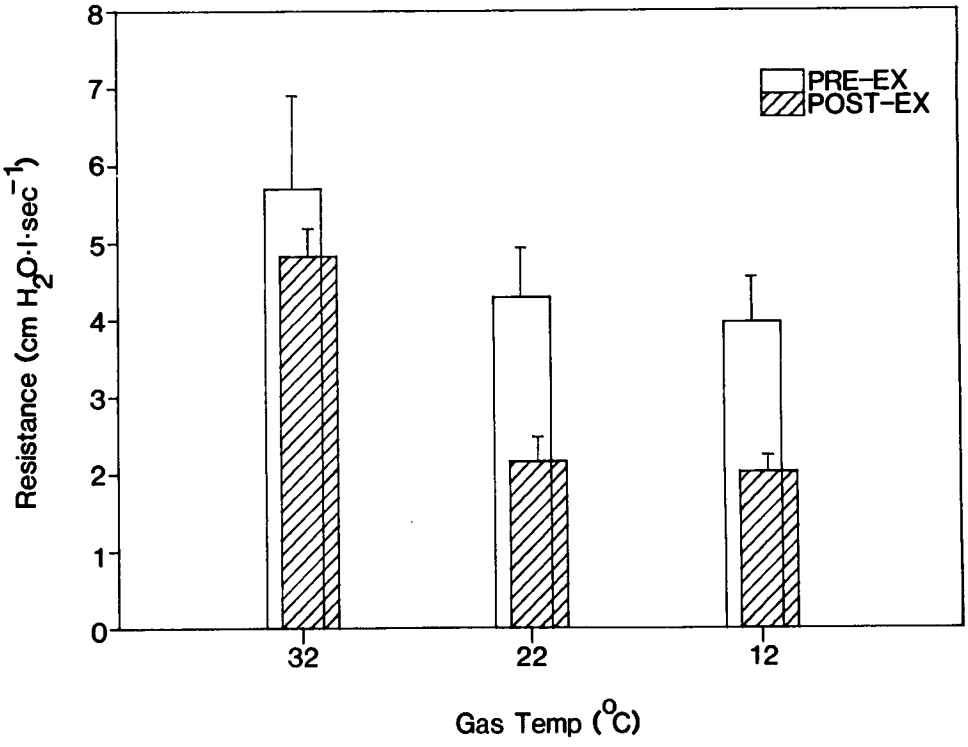

Fig. 3. Pooled respiratory resistance measured at 305 m during the inhalation of various temperature gases. Pre- and postexercise values are given, mean ± SE.

Neither exercise nor gas temperature exerted an influence on the measurements obtained from the forced expiratory maneuvers (Table 2). Flow rates (PEFR, FEV_1, FEF_{50}) did show the expected decline with increase in depth.

Respiratory heat loss was calculated to be 229, 159, and 80 W for an average minute ventilation of 30 $liter \cdot min^{-1}$ and inspired temperatures of 12, 22, and 32°C, respectively. By the end of the 30-min exercise period, rectal temperature fell an average of 0.40 ± 0.06°C (mean ± SE) during exposure to 12°C gas, fell 0.15 ± 0.09°C with 22°C gas, and rose 0.3 ± 0.05°C with 32°C gas by the end of the 30-min exercise period. A correlation between RHL and T_{rectal} was highly linear.

DISCUSSION

In contrast to expectations, exercise ventilation with cool gas at a

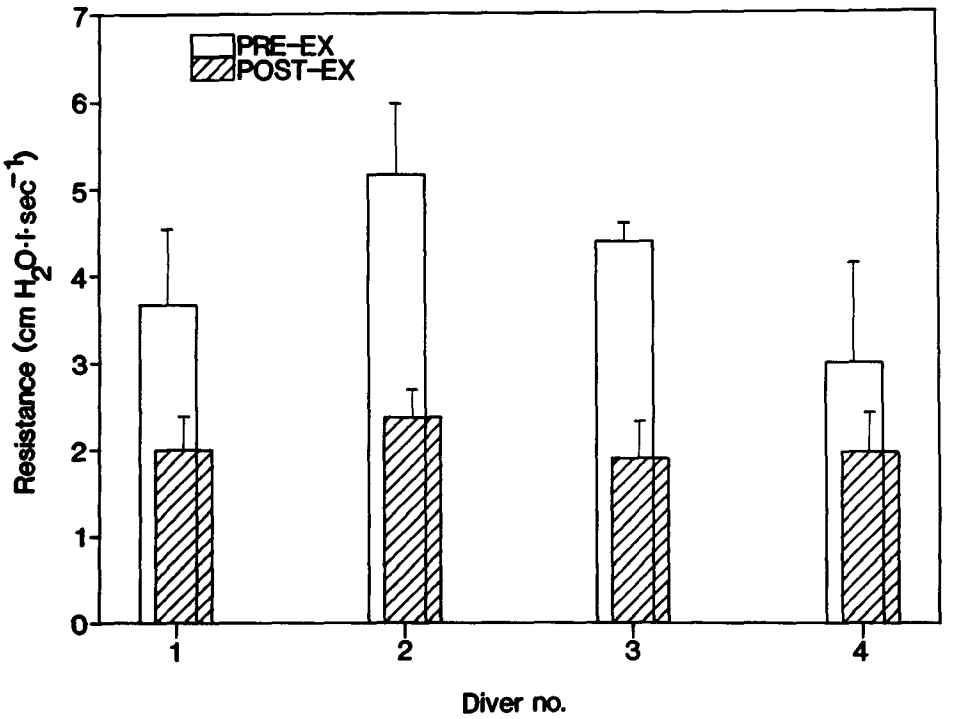

Fig. 4. Respiratory resistance in individual divers at 305 m during cool gas breathing. The data from 22 and 12 ° C exposures are combined. Pre- and postexercise values are given, mean ± SE.

simulated depth of 305 m induced a considerable decrease in respiratory resistance. Both the Braithwaite (1) and Piantadosi (2) limits for inspired gas temperature were equally effective in reducing resistance.

Several mechanisms for decreasing respiratory resistance exist, one being relaxation of large airway smooth muscle with resultant bronchodilatation. Some evidence suggests that bronchodilatation may be induced by a direct effect of airway cooling on bronchial smooth muscle (13). Isolated tracheal segments from the guinea pig relax following cooling from 37°C to 26-21°C. In some cases the relaxation was preceded by a transient contraction. This bronchodilatory effect of airway cooling appears to be modulated by the activity of the Na/K pump, and by prostaglandin and leukotriene synthesis.

Bronchomotor tone is believed to be neurally mediated, and indeed neural receptors sensitive to inspiratory cooling have been identified on the vocal cords of dogs (14). It is not known, however, if similar receptors exist in man, or whether in some conditions they might induce bronchodilatation.

An alternative means of decreasing respiratory resistance is by increasing lung volume, thereby increasing airway diameter. FRC was not measured in this study and thus a change in lung volume in response to cold gas cannot be

Table 2
Pulmonary Function Values From the FVC Maneuver

	FVC	PEFR	FEV_1	FEV_1/FVC	FEF_{50}	FEF_{25-75}
16.2 ATA Preex	6.00 ± 0.32	5.48 ± 0.07	4.12 ± 0.06	0.72 ± 0.03	3.72 ± 0.09	3.28 ± 0.19
16.2 ATA Postex	6.40 ± 0.33	5.62 ± 0.23	4.22 ± 0.11	0.67 ± 0.04	4.02 ± 0.25	3.30 ± 0.16
23.7 ATA Preex	5.68 ± 0.35	5.11 ± 0.33	3.84 ± 0.02	0.71 ± 0.04	3.57 ± 0.05	3.36 ± 0.19
23.7 ATA Postex	6.00 ± 0.10	4.72 ± 0.13	3.68 ± 0.04	0.65 ± 0.01	3.40 ± 0.07	3.01 ± 0.06
31.3 ATA Preex	5.99 ± 0.10	4.58 ± 0.13	3.74 ± 0.04	0.63 ± 0.01	3.16 ± 0.07	2.74 ± 0.06
31.3 ATA Postex	6.03 ± 0.14	4.36 ± 0.16	3.68 ± 0.05	0.62 ± 0.02	3.14 ± 0.08	2.81 ± 0.07

FVC = forced vital capacity (l, BTPS); PEFR = peak expiratory flow rate ($liter \cdot s^{-1}$).
FEV_1 = expired volume after 1 s (liter), $FEV_1/VC\%$ = FEV_1 expressed as percent of FVC.
FEF_{50} = flow rate at the point where 50% of the vital capacity has been exhaled ($liter \cdot s^{-1}$).
FEF_{25-75} = average flow rate during the second and third quartiles of the forced vital capacity ($liter \cdot s^{-1}$).

ruled out. There are, however, no known mechanisms by which FRC would be affected by inspired gas temperature.

Cold-induced bronchoconstriction is the usually reported effect of very low temperature at 1 ATA with moderate respiratory heat loss sustained during eucapneic hyperventilation. The current study is the only one to date measuring resistances during moderate temperature exposures but with relatively high respiratory heat loss due to exercise. The differing stimuli may account for the different results of the two types of studies.

During warm gas breathing, an exercise-induced bronchodilatation might have been expected due to the action of circulating catecholamines. Warren and Dalton (15) found plasma epinephrine levels averaging 1.89 nmol·liter^{-1} in normal subjects following moderate bicycle exercise. When epinephrine was infused at rest to provide the same plasma levels, airway resistance decreased by 24%. In the present study, however, exercise ventilation with 32°C gas resulted in resistance reductions in only 2 of the 4 divers (Table 1). We might hypothesize that the combined stimuli of exercise and respiratory heat loss under hyperbaric conditions may have provoked a more vigorous release of both epinephrine and norepinephrine (16, 17), resulting in our observed 49% decrease in respiratory resistance.

Mean preexercise values of respiratory resistance on the present 305-m dive followed a pattern similar to that on a previous dive to 457 m (10). In the present case, resistance rose during compression from 3.4 $cmH_2O \cdot liter^{-1} \cdot s^{-1}$ at the surface to 6.4 $cmH_2O \cdot liter^{-1} \cdot s^{-1}$ at 16.2 ATA, and 7.7 $cmH_2O \cdot liter^{-1} \cdot s^{-1}$ at 23.7 ATA. However, in all 4 divers the respiratory resistance at 31.3 ATA was surprisingly low, equal to or less than the resistance at 16.2 ATA during compression. Following the initial reduction in resistance measured 24 h after reaching maximal depth, no further change in preexercise resistance was noted during the ensuing 4 d of testing. The same pattern of increase in expiratory resistance during compression followed by a decrease at the storage depth was seen in the individual data from 4 of the 6 divers on the previously mentioned 457-m dive, although the resistance reduction was not as dramatic as in the current study.

Increases in respiratory flow rates after the cessation of compression have been reported by Hamilton et al. (18), Dougherty and Schaefer, (19), and Schaefer et al. (20). It is conceivable that this repeated finding of apparent airway relaxation represents a compensation for high gas density breathing by chronic bronchodilatation once depth stabilizes. Alternatively, compression may lead to an elevated resistance due to a subtle action of the high pressure nervous syndrome (HPNS) on bronchial smooth muscle. During prolonged stays at depth HPNS usually subsides, as might bronchoconstriction. In none of the above-mentioned dives was HPNS noticeable. However, the usual tests of HPNS were not performed and thus the degree of HPNS involvement is not known. In future dives, specific evidence of airway relaxation should be sought by examining the time course of resistance changes throughout a dive.

In contrast to the interrupter measurements, forced expiratory flows

decreased steadily with increases in depth, as has been frequently reported. There was no sign of an increase in forced expiratory flow rates after a prolonged stay on the bottom. It is reasonable to assume, however, that if mild bronchoconstriction were present during compression it would be immediately reversed by the inflation to maximal lung capacity required by the forced expiratory maneuver (21).

The absence of detectable effects of cold and exercise on forced expiratory parameters can be explained by a presumed drop in plasma catecholamine levels by the time these measurements were made, approximately 15 min after the cessation of exercise. Furthermore, warm gas (32°C) was breathed for technical reasons before and during these maneuvers, potentially reversing the effects of cold gas breathing.

Discrepancies between resistance and flow rate measurements commonly occur during the use of bronchodilator drugs in normal subjects. These drugs reproducibly reduce airways resistance in all populations, but in normal subjects they have unpredictable effects on flow-volume curves. Bronchodilatation operates through smooth muscle relaxation, which can decrease the stiffness of large airways resulting in increased dynamic compression during forced expirations, thereby reducing flows. Only in asthmatics are resistance measurements and maximal flows following bronchodilatation therapy reliably correlated.

In conclusion, although it has been documented elsewhere that severe cold stress can induce an immediate adverse reaction of the airways, moderate respiratory heat loss, on the order of 230 W, does not appear to compromise pulmonary function at depths to 305 m. In fact, respiratory resistance was improved under these conditions. We might hypothesize that catecholamines were involved in this response. Alternatively, airway cooling may have exerted a direct bronchodilatory effect on airway smooth muscle. The lability of airways to HPNS is another topic requiring further study.

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SESSION 6: HYDROSTATIC EFFECTS AND SATURATION DIVING

9th International Symposium on Underwater and Hyperbaric Physiology
Undersea and Hyperbaric Medical Society, Bethesda, MD, 1987

FIRST HUMAN HYDROGEN SATURATION DIVE AT 450 MSW: HYDRA V

B. Gardette, X. Fructus, and H. G. Delauze

Initiated by Lavoisier in 1789 (1), the history of hydrogen as breathable gas in a mixture was demonstrated by Zetterström a century and a half later when, in 1943-1944, the Swedish naval officer dove on hydrox to sea depths of 40, 70, 110, and 160 msw without any discomfort (2, 3). Systematic research into the biological effects of hydrogen began in the United States and France toward the end of the 1960s and continued at COMEX until 1970.

In the United States, Brauer exposed mice and squirrel monkeys to a trimix of $He-H_2-O_2$ in 1966-1968, for up to 24 h at pressures of 60 to 90 ATA (4-6). The saturation was perfectly well tolerated by the animals. Six volunteers breathed hydrox in a hyperbaric chamber at 60 m without the least difficulty (7). During the 1970s Edel (one of the initial volunteers) continued his research in Fife's laboratory, experimenting on dogs (a total of 1140 h of hydrox exposure at 305 msw), rats, and mice (5313 h), and on themselves in a chamber at 91 msw for 4 h 37 min in all. Fife's report (8), well substantiated by biochemical and histologic examinations on dogs, concluded that hydrox (97:3) was innocuous for animals at depths down to 300 msw and periods up to 48 h.

In France, animal experimentation was begun in 1969 on rabbits by Michaud et al. of GERS (French Navy) (9) and on *Papio papio* baboons at COMEX at depths of between 300 and 675 msw (HYDRA II) (10-12).

The Swedish scientists Örnhagen et el. (13) demonstrated in 1979 that hydrogen was not harmful to rabbits. Twelve animals were exposed several times for 24 to 48 h to 31 ATA of hydrox (P_{O_2} = 0.2 to 0.56 ATA), and all 12 survived normally without the slightest sign of acute or chronic intoxication.

The fact remains, however, that at the start of the 1980s, for what were chiefly technical reasons, experimental hydrox dives involving humans had not gone beyond Zetterström's 160 msw depth, so that an entire realm of human

physiology had yet to be explored by means of hydrogen-mix dives.

In 1983, after completing a final toxicologic investigation on 30 mice exposed to 61 ATA of hydrox for 40 h, which substantiated the harmlessness of this PH_2 (14), we decided to resume experimental human dives using hydrogen mixtures. This experimental series, HYDRA III, IV, and V, was carried out in the 2 yr from June 1983 to June 1985.

HYDRA III: SEA DIVES (15)

From June 27 to July 4, 1983, 16 COMEX divers in 8 pairs breathed hydrox in a wet bell for 5 min at 70-73 msw. The descent and ascent were carried out with the divers breathing heliox 80:20. None of them found that hydrox tasted different from heliox!

On July 1, 1983, Delauze and Bargiarelli followed the same procedure but to 91 msw, each breathing hydrox for 5 min. The decompression, following a conservative profile with hyperoxic mixtures from 18 msw on, took 3.5 h. This first positive experience using hydrox in operating conditions encouraged us to undertake the next experiment, HYDRA IV.

HYDRA IV (16)

In this experiment, conducted in November 1983, 6 divers were saturated on heliox to 300 msw with hydrox exposures of from 1 to several h at depths between 120 and 300 msw. These tests confirmed the breathing comfort and attenuation of muscular fatigue afforded by hydrogen. They also made it possible to establish, for the first time on humans, an evaluation of its particular narcotic power. The following is a brief outline of the conclusions presented in the HYDRA IV report and the papers given at the Xth EUBS Congress in Marseilles in October 1984.

Hydrogen toxicity at partial pressures of 12 to 25 ATA for exposures of 1 to 6 h was not shown by biochemical blood and urine examinations. This constitutes an initial confirmation of the results of much more severe animal experiments. For exposures of several hours, at 15 ATA, at least, hydrogen seems to behave like an inert gas relative to cell structures, while at the same time affecting psychic functions, as does nitrogen at even lower pressures.

The conditions of this first physiologic attempt did not permit exhaustive respiratory function investigations, and while the divers were able to appreciate the breathing comfort they qualified as "extraordinary" on hydrox, as well as the lack of tiredness during exercise, results of the measurements taken in these difficult conditions of immersion are more difficult to interpret. Examination of the heart rates measured during exercise, however, tends to indicate a lower cost of the heart at exertion on a hydrogen mixture.

According to the work published by Lambertsen and Bornmann (17) and by D'Aoust (18), switching the divers from experimental hydrox to their storage gas heliox should have produced the inert gas phenomenon of isobaric

counterdiffusion, with formation of circulating or stationary bubbles. During HYDRA IV, Doppler detection echography confirmed the existence of this bubble-producing mechanism. Under the conditions of this experiment, the phenomenon had no pathologic consequences, but it will bear watching in subjects saturated at greater depths.

Certain aspects of hydrogen narcosis are different from those of air narcosis, at least in the initial phases. The difference in intensity of the phenomenon can be evaluated, however, the hallucinant power of hydrogen being about one one-fourth that of nitrogen. In any event the results of HYDRA IV (16) enabled us to maintain that its narcotic potency—controllable because it depends on the P_{H_2}—indicates the use of hydrogen mixes because: a) it depends on the ambient pressure, which has a counter effect on the narcotic action of the gas (or a "pressure reversal effect"); and b) the narcotic power itself has a counter effect on the high pressure nervous syndrome (HPNS), like nitrogen—which would be a definite advantage for dives beyond 300 msw.

In December 1983, the Swedish Navy performed a heliox saturation dive at 120 msw where the divers had 3 exposures of 1 h each in hydrox mixture. This experiment showed the occurrence of the first symptoms of narcosis at that depth (9).

THE OBJECTIVES OF HYDRA V (20)

The purposes of the next hydrox tests are obvious: First, on the technical level, to constitute a hyperbaric system that could be pressurized with hydrogen as well as helium and which comprised a new environmental control system (ECS) capable of controlling not only temperature and relative humidity at all times, but also the P_{O_2} by automatic O_2 injection in the hydrogen mixture. Second, on the physiologic lever, they confirm the anti-HPNS effect of hydrogen, which would increase the diver's work capability both in the dry and in the wet, under pressure conditions (46 ATA) following a 38-h compression, identical to those of the preceding dives, ENTEX 5, 8, and 9, on helium or nitrogen trimix.

Operation of HYDRA V was long enough to permit a number of physiologic investigations, of which the chief ones were:

- regular blood and urine analyses to detect any incipient toxicity, albeit unlikely, of hydrogen during prolonged exposure;
- regular EEG checks and tremor measurement;
- respiratory function investigation during rest and work periods, both in the dry and in the wet;
- medical surveillance and observation of behavior;
- study of isobaric counterdiffusion phenomenon when switching from hydrox to heliox, and consequently determining what is feasible or prohibited in transferring from one gas to another;

- decompression of the subjects on hydrogen mix up to 200 msw for which a new decompression profile was established without benefit of previous experience, since nobody had ever before decompressed from a 450-msw saturation on hydrox.

MATERIALS AND METHODS

Adaptation of Hyperbaric Facilities

The hyperbaric facilities of the COMEX Research Center, particularly the chambers, were not initially intended for hydrogen use. We thus had to adapt them to this new deep diving technique. Activity areas in the Research Center were also redefined.

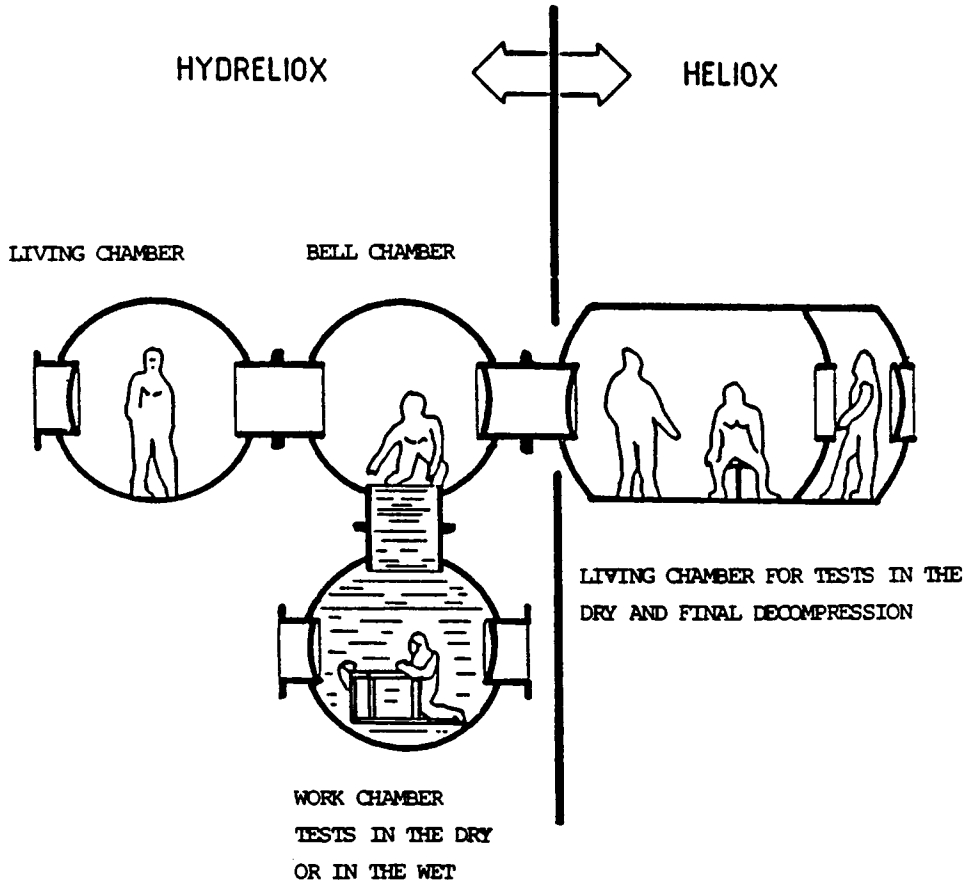

Fig. 1. General layout of saturation chambers.

Saturation Chambers

In accordance with HYDRA V experimental protocol we organized two separate and distinct zones (Fig. 1) a) for the unique use of hydrogenated trimixes (hydreliox) and b) for heliox, to be used both as a strategic retreat position in case of safety problems and for final decompression of the divers.

Hydreliox Zone

The EMS 600 modular saturation system was chosen for the hydrogen phase of the operation. This system is made up of three interconnected spheres which can be pressurized to different levels: sphere l: living chamber; sphere 2: sanitary chamber and bell simulation; and sphere 3: laboratory for tests in the dry or in the wet.

Internal lights were replaced by external ones fixed on the viewports and protected by thermal filters. All metal components were grounded. All O-rings on doors, viewports, locks, technical plugs, and manways were replaced by seals compatible with hydrogen use. Before the first team of divers was pressurized we made a rigorous test of system tightness at 46 ATA with hydreliox trimix. All other internal equipment was that normally used for physiologic experiments in heliox.

Heliox Zone

A 6-person cylindrical chamber with built-in lock was connected to sphere 2 of the EMS 600 to accommodate the divers after their stay in hydrogen. The transfer lock, which is normally used as a sanitary chamber, can be fitted with two bunks in the eventuality it should be necessary to pressurize a medical assistance team. The physiologic test equipment to carry out reference tests on heliox was identical to that used in the EMS 600.

The Divers

Six divers were selected for HYDRA V: 3 COMEX divers, 2 divers from the GISMER (Groupe d'Intervention Sous la Mer), French Navy, and 1 diver from the INPP (Institut National de Plongée Professionnelle). They were divided into 2 groups: team A: 3 divers, A_1 A_2 A_3; and team B: 3 divers, B_1 B_2 B_3.

METHODS AND PROCEDURES (Fig. 2)

Compression Techniques for Deep Dives Using $He-O_2$, and N_2-He-O_2, and H_2-He-O_2 Mixtures

After the human dives made in the CORAZ series (1975), rapid compression in 4 h to 300 msw with different percentages of nitrogen in heliox, CORAZ I: 9% N_2; CORAZ II, III: 4.5% N_2; and CORAZ IV: 0% N_2, we undertook a systematic study of the influence of nitrogen on the intensity of the HPNS in the monkey *Papio papio*. That was the CORASIN series, compression in 2 h to 600 msw. The results obtained have led us to develop a compression

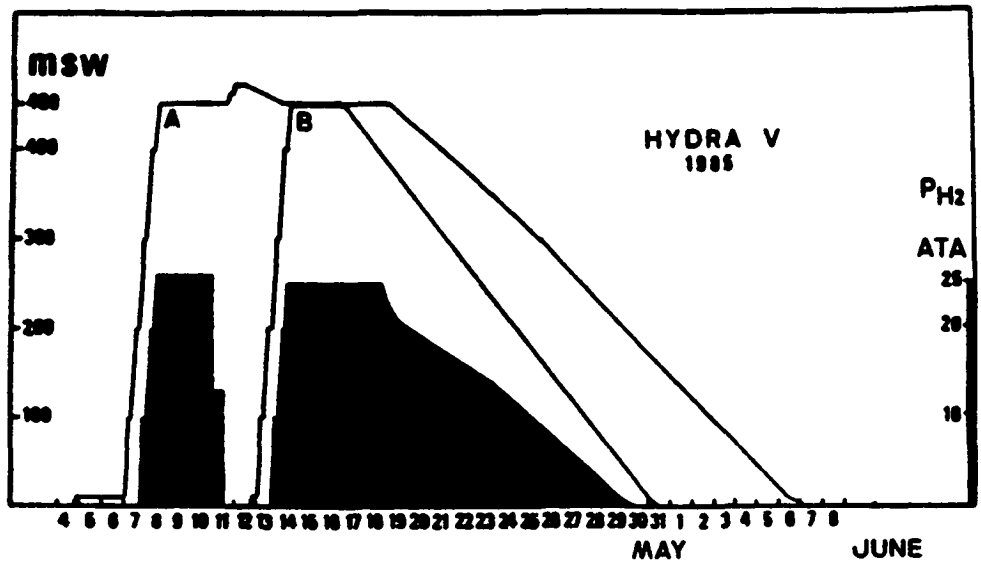

Fig. 2. General dive profile with hydrogen partial pressure in gas mixtures (PH_2). Two teams of 3 divers, A and B.

technique with the following characteristics: decreasing speed as depth increases; short duration, intermediate stages; and introduction of nitrogen before each stage, to obtain 8% at 600 msw. This method of compression made it possible to minimize the HPNS at 600 msw (CORASIN VIII) and to reach a 1000 msw depth (CORNELIUS I).

Extrapolation of this procedure to man, during two compression to 400 and 430 msw in JANUS IV, phase II and phase III, showed an increase in the modifications of the EEG during the first phase of compression (the most rapid), between 0 and 300 msw.

Reduction of speed at the beginning of compression in the experiments with the monkeys (CORNELIUS II and III) as far as 1100 m, using 5% N_2, minimized the clinical symptoms of HPNS and EEG modifications. A human dive to 450 msw was also carried out using this new procedure. Eight subjects were taken to 450 msw in 38 h (DRET 79/131) without clinical symptoms and without any important increase in theta EEG activity. The same compression procedures were used in ENTEX 5 and 8 (450 msw, 12 d) with 4.8% N_2 and in ENTEX 9 (450 and 610 msw) without adjunction of nitrogen in the heliox mixture.

ENTEX and HYDRA V Compression Procedures

0-10 msw: heliox = 0.4 ATA O_2
10-450 msw: 38 h
With stop every 100 msw, duration of each stop, 150 min
Decreasing speed with depth
0-1002 min/msw
100-200 2.5 min/msw
200-300 4 min/msw
300-400 5 min/msw
400-450 7 min/msw

Gas mixture in DRET 79/131, ENTEX 5, ENTEX 8
$He-N_2-O_2$/4.8% N_2 — Adjunction of 3.5 msw N_2 before each stop, 2.2 ATA of N_2 at 450 msw

in ENTEX 9 (between 10 and 450 msw)
$He-O_2$, without adjunction of N_2: P_{N_2} = 0.8 ATA; 1.7% N_2

in HYDRA V
Gas mixture: helium up to 200 msw, hydrogen between 200 and 450 msw,
H_2-He-O_2: 25 ATA H_2 = 54%
20.6 ATA He = 45%
0.4 ATA O_2 = 1
(0.05 ATA N_2 = 0.1%) elimination before compression from 0 to 10 msw

Compression duration:
He, 14 h (10-200 msw)
H_2, 26 h (200-450 msw)

Gas Switch from H_2-He-O_2 to He-O_2

After 4 d at bottom depth, the 3 divers of team A were transferred from a hydrox atmosphere—54% H_2 at 450 msw (25 ATA H_2) to a heliox atmosphere with an 8-h intermediate hydrox mixture stop—30% H_2 at 450 msw (14 ATA H_2).

Although it was progressive, the gas switch was too rapid and the divers on heliox were recompressed to 470 m with an increase of the P_{O_2}, from 0.4 to 0.6 ATA. After a slow decompression to 450 msw, they stayed at bottom depth for additional tests designed for comparison between heliox and hydrox.

Subsequently, the dive plan of team B was modified and converted into a complete hydrox dive procedure that will certainly be adopted for future operations: compression with hydrogen when deeper than 200 msw, stay under hydrox atmosphere at bottom, and decompression to 200 msw with H_2 progressive elimination.

Bottom Time Durations

	Team A (3 divers)	Team B (3 divers)
H_2-He-O_2 at 450 msw	72 h (3 d)	104 h (4 d 8 h)
He-O_2 at 450 msw	128 h (5 d 8 h)	0
Total	200 h (8 d 8 h)	104 h (4 d 8 h)

HYDRA V Decompressions

Team A: Three divers were decompressed on heliox from 450 msw to the surface in 14 d 10 h.

Decompression rate
- 450-15 msw = 45 min/m
- 15-0 msw = 60 min/m

P_{O_2}
- 450-350 msw = 0.6 ATA
- 350-120 msw = 0.5 ATA
- 120-15 msw = 0.6 ATA
- 15-0 msw = 24%

At the end of decompression, at 1 m from surface, 2 divers had slight pain in the knees. Team A was recompressed to 4 msw, on air with pure O_2 inhalations, and then decompressed to the surface (D.R.:90 min/msw).

Team B: Decompression was conducted with progressive elimination of hydrogen from 450 to 200 msw. From there to surface, the ascent was carried out in a heliox atmosphere. This saturation decompression was the first one ever performed with hydrogen and was closely monitored, using ultrasonic bubble detector technique.

Decompression duration: 19 d 10 h.
- on hydrox: 11 d 17 h (450-157 msw)
- on heliox: 7 d 17 h (157- 0 msw)

Decompression rate
- 450-350 msw = 70 min/msw
- 350-300 msw = 65 min/msw
- 300-250 msw = 60 min/msw
- 250-15 msw = 55 min/msw
- 15-0 msw = 120 min/msw

P_{O_2} { 450-100 msw = 0.5 ATA
100-15 msw = 0.6 ATA
15-0 msw = 24%

Team B arrived at surface on Friday, 7 June at 10:00 a.m., without problem. Total time exposure on hydrox: 17 d 3 h.

Investigations

- Clinical observations: COMEX-GISMER CEPISMER
  - Tremor quantified, EEG quantified, Sleep EEG } GIS/CNRS
  - Psychometric performance } OCTARES, Swedish Navy
  - Psychology } COMEX, CERB
  - Ventilatory investigations in dry conditions, at rest and during exercise on bicycle ergometer } CERB
  - Ventilatory investigations in wet conditions, at rest and during exercises on cyclorower ergometer } GIS, COMEX, CERB
  - Cardiac adaptation to effort: dry and wet conditions } NMRI (U.S. Navy)
- Bubble detection: CERTSM
- Scintigraphy: lungs, skeleton, heart: CERB
- Biology of blood and urine: CERB
- Ophthalmology: C.O.M.
- New regeneration systems with permanent reoxygenation of ambient gas to compensate diver's normal O_2 consumption } COMEX

Selection of the Divers

The medical examinations for the selection of the divers, made in 9 professional divers, included:

- clinical examination
- scintigraphy (bones, heart, and lungs)
- tests of cardiac and ventilatory adaptation to effort (with respiratory resistance)
- ophthalmologic tests.

Training

- psychometric tests
- technical diving training.

RESULTS AND DISCUSSION

Technological Imperatives

The preliminary study of the flammability of ternary hydrogenated mixtures, the rigorous control of hydrogen used as a breathing gas, and the adapting of hyperbaric equipment to hydrox constituted the most important elements in the preparation of HYDRA V. In accordance with the requirements set down, the procedures for the use of saturation diving equipment were defined so as to preclude the presence of any flammable mixture at any time and any place in the hyperbaric complex and its peripheral equipment. Due to the equally high level in the competence of the surface personnel and the quality of the installation, the 18 d "on hydrogen" passed without mishaps.

Study of Behavior and Medical Supervision

The harmlessness of hydrogen as a breathing gas during long exposure was confirmed. Our rough calculation of the P_{H_2} level tolerable at 450 msw was found to be valid and although the "hydrogen effect" was perceptible, especially in team B, we are inclined to believe that a lower P_{H_2} (23 ATA instead of 25) would have further normalized the neuropsychic state of the 6 men, *none of whom sustained any HPNS* disorders while breathing the ternary mixtures with 54% hydrogen.

We were expecting a resurgence of HPNS on heliox but not so pronounced, because we had taken the precaution of putting the 3 divers on an intermediate mixture of 30% H_2 for 8 h. With the switch to pure heliox at 46 ATA, on the basis of our experience with HYDRA IV, we were prepared for an effect of isobaric counterdiffusion, but we underestimated it, if only because of its variability from 1 individual to another and the precautions we had taken of an 8-h transit on 30% H_2.

The formation of circulating, generalized subcutaneous bubbles (Doppler: 2^+) in diver A2, although quite quickly curbed by recompression of 20 msw, constitutes one experimental result that should be taken into account for the future of industrial diving with hydrogenated mixtures. It is important to ascertain whether switching breathing mixtures (chambers on heliox, diving bell, and divers on hydrox, for instance) should be avoided in underwater work.

As for team A, the 3 team B divers would have been glad to remain on hydrox, they felt so good at 450 msw, "like heliox at 200 or 250 msw ...."

Neurophysiology and Psychology

Exposure to hydrox mixture of 54% H_2 did not serve to prevent electroencephalographic modifications, noted regularly at 450 msw both on heliox and nitrogen trimix, and more particularly in the 4 divers who had had experience at this depth. Neither did hydrox prevent disturbance of sleep patterns, but how sure can we be that a certain amount of dyssomnia was not due to the constrained and cramped environment and thermal discomfort? On the other

hand, on hydrox the absence of tremor was evidence of the disappearance (or masking) of HPNS, of which it is the primary and most constant manifestation. Tremor reappeared in the divers of team A when they went back to pure heliox.

Performance of the psychometric tests was erratic, certainly influenced, particularly in team B, by a slight degree of narcosis. Narcosis was evaluated by 3 cognitive tests which show a slight deterioration in performance, with broad individual variations and a temporary learning block during the period following arrival on the bottom. Nevertheless, the results of tests used previously in ENTEX 5, 8, and 9 for the same compression profile show a definite advantage of ternary hydrox over nitrogen trimix. Finally, the Visual Choice Reaction Time with 4 choices did not undergo any significant variation during the period on hydrox, which seems to confirm clinical absence of HPNS. As for the cognitive tests, aside from a feeling of tiredness the divers perceived on arrival at the bottom, these reveal (despite the variability of individual reactions) the state of stress caused by deep diving, whatever the nature of the breathing mixture. To which we should add that this stress, psychic rather than somatic, is not the most pronounced form of stress encountered in occupational medicine.

Respiratory and Cardiovascular Physiology

Three of the 6 subjects were thoroughly examined in the dry, the effects of hydrogen seem to be due more to its neurotropic effect than to its density. The hyposensitivity of the nervous system on hydrox seems to attenuate the impression of strenuous effort and to increase tolerance to a slight hypercapnia. If this should be confirmed, it would be advisable to lower the P_{H_2} to avoid overestimating performance possibilities.

In the wet, on the other hand, although comparative respiratory measurements were not possible, higher motivation among the 3 team-B divers for exercises in immersion, in ergonomic conditions better suited to their capacities. Hence, a satisfactory level of effort obtained on the cyclorower for periods of 25 to 31 min, with respiratory comfort and a ventilation of 30 liter·min^{-1} on hydrogen mix. The respiratory equivalent stayed relatively high, without CO_2 retention, for moderately energetic activity approximating that of working conditions.

From a cardiovascular point of view the experimental conditions did not permit precise quantification of the difference between the exercises carried out on the 2 mixtures.

For team A, the results on hydrox at 450 msw were closer to reference values that those on heliox, without, however, being statistically significant. All members of team B, fully equipped, were able to carry out in immersion what was asked of them, and in no case did the graphs show cardiac arrhythmia; however, it is true that we did not test at the divers' full effort capacity.

Decompressions

After their stay on heliox, team A decompressed with the same mixture at variable Po_2 and at constant speed up to 15 msw, following a well-established procedure.

The first venous bubbles were detected very early, at 425 msw for diver A2 (the one who had had trouble with the switch from hydrox to heliox at the bottom) but also for diver A3 (the one who had had the least trouble with this switch). None of this decompression was a surprise to us, not even the slight bends felt by A1 and A3 at 1.3 msw from surface. These little terminal "hitches" are not excluded in heliox decompressions, not even ours, which have proven to be perfectly well tolerated in numerous experimental saturations carried out by COMEX and GISMER.

Decompression of team B was carried out on a hydrogen mix with decreasing proportions of hydrogen up to 200 msw. Slow dehydrogenation was expected to prevent the problems of counterdiffusion encountered by team A (at 46 ATA). The fall in pressure, obtained by gradual elimination of hydrogen, started at 0.86 msw/h, under Doppler control. Observing an absence of bubbles at 350 msw we increased the rate of ascent to 1 and then to 1.09 msw/h with a Po_2 of 0.6 then 0.5 ATA. Not until 280 msw did a low bubble count appear in 2 of the 3 divers.

One must not forget that this was a "première" and that we accounted for only two elements to establish our decompression profile:

- the solubility of hydrogen in fat (2.5 times that of helium)
- the decompression of shallow bounce dives carried out by Edel (7) and Fife (8), decompression which turned out to be very tricky and responsible for osteoarticular accidents.

Hence our wariness. But in fact this decompression, probably too slow at the beginning, was conclusive, and in the future, desaturation by gradual elimination of hydrogen from the breathing mixture up to 200 m, without sudden change to heliox, will not need to be any slower than with pure heliox or nitrogen trimix.

Evolution of Biological Parameters

It is noteworthy and reassuring that after a critical situation resulting from the switch $H_2 \rightarrow$He, experienced by team A, and after final decompression of team B on hydrox up to 200 msw, the variations in the number of blood leukocytes and platelets did not suggest bubble pathology. This tends to confirm that in this respect hydrox is at least as well tolerated as heliox, and better than air. Variations in blood and urine chemical elements, always reversible, do not show any serious metabolic disturbances.

Only temporary hypophyseal thyroid anomalies, as well as an increase in serum ferritin, could be linked to a hepatic disturbance is described by Doran et al. (21) in divers having undergone experimental saturation at great depths on heliox, such as the 540 and 660 msw AMTE dives. Molecular hydrogen does not seem to induce any specific biological disorders nor any irreversible

cytotoxicity.

Pulmonary and Scintiscanner Examination

Comparative study of the 6 divers before and after the HYDRA V dive permitted us to conclude that, as far as the lungs were concerned, these long saturations with hydrogen and helium were remarkably well tolerated by both teams, even if a xenon 133 scan did reveal a decrease in perfusion at the apex.

Bone scanning permitted us to discover 1 case of tibial hyperfixation (diver B3), and for the 5 others a diffuse and symmetrical increase in isotope fixation. Here again experience shows us that such scintigrams, the cause of which has yet to be demonstrated (decompression, or biomechanical effects due to the constraint of confinement) (6), are so commonplace and so unstable that any notion of a pathologic process can be ruled out.

CONCLUSIONS

Introduction of hydrogen into breathing mixtures at high pressure gave positive results with HYDRA IV, to a depth of 300 msw, but in the interest of safety, the hydrox volume was restricted to 0.8 m^3 in a dome under which divers could stay for periods of not more than a few hours. HYDRA IV left a number of important questions, however, which we hoped to solve during HYDRA V:

1) Is it possible to adapt a conventional hyperbaric unit to the use of large volumes of hydrogen? Definitely; HYDRA V has proved that heavy saturation diving equipment, originally designed for helium, could be adapted to hydrogen and still satisfy safety requirements. Only some of the peripheral subunits need modification and these can be achieved without prohibitive expense.

2) Would exposure for long periods to hydrogenated mixtures, until a state of saturation is reached, be well tolerated by the human organism? Blood and urine analysis and medical examination before and after the dive are reassuring. Partial hydrogen pressures of about 30 ATA can be reached (and even exceeded) without danger of intoxication.

3) Does there exist an equilibrium between the surrounding pressure and hydrogen's narcotic effect that would allow us to maintain at 450 msw or more a suitable proportion of this gas in the mixture? That is, a percentage high enough to neutralize HPNS and to assure a gain in pulmonary ventilation. This balance does exist and HYDRA V has allowed us to define it.

4) Is it possible, taking certain precautions, to switch a diver saturated on more than 50% hydrogen at 450 msw to pure heliox? The answer, no, is an invaluable warning: The phenomenon of isobaric supersaturation and the rebound of HPNS prohibit diving with the two mixtures (living chamber on heliox and bell on hydrox), a complicated procedure in any case and one whose economic advantages are not certain.

5) Therefore: Can we propose an all-hydrox environment including decompression? On this last point hydrogen has not substantiated its poor reputation. On the contrary, progressive elimination of this gas to a content of less than 4% at 200 msw permits a desaturation rate similar to that of heliox.

Finally, it is always man himself who is the best judge. We reiterate a comment made by one of the most experienced divers, used to deep diving: "On hydrox at 450 msw I had the same impression of well-being and ease as on heliox at 200 msw." In short, everything confirms the fact that hydrogen permits us to gain an extra 250 msw.

HYDRA's technological success and the mass of physiologic data gathered open the way to a preindustrialization of diving with hydrogenated mixtures at the operating depths of tomorrow.

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AN ANALYSIS OF 14 SUCCESSFUL TRIMIX 5 DEEP SATURATION DIVES BETWEEN 150 AND 600 METERS

P. B. Bennett, H. G. Schafstall, W. Schnegelsberg, J. Holthaus, and R. D. Vann

Although Hannes Keller made a brief open-sea dive to 300 m in the early 1960s, it is only 17 yr ago, in 1969, that a full-scale simulated saturation dive to 300 m was made collaboratively by the U.S. Navy and the F.G. Hall Laboratory at Duke University (1). Since then, deep diving research has continued at many international centers to investigate the problems and control of the high pressure neurologic syndrome (HPNS) to permit successful ultra-deep diving (2) and to solve the difficulties of safe decompression from great depths (3). In 1981 this research culminated at Duke during the 4 deep *Atlantis* research dives (4), in a record simulated research dive by 3 men at the F.G. Hall Laboratory to 686 m (2250 ft) (5).

These dives were designed to compare the effectiveness in controlling the many signs and symptoms of HPNS such as tremors, nausea, dizziness, vomiting, increased EEG theta (6-8 Hz) activity, and performance decrements to 650 and 686 m by variation of the rate of compression or addition of 5 or 10% nitrogen to the helium-oxygen normally used in deep diving. An intensive series of pulmonary, psychometric, hematologic, and other tests were made (4, 5). In addition, much knowledge was gained on decompression from such great depths utilizing various continuous rates of decompression dependent on the oxygen partial pressure and the depth.

The least signs and symptoms of HPNS and decrement in performance from a cognitive and psychomotor view, as shown in Figs. 1 and 2, respectively, were obtained by use of trimix 5, i.e., 5% nitrogen in heliox. This was combined with a slow, exponential rate of compression and the regular use of stages at which several hours were spent without compression to permit adaptation to the pressure (4, 5).

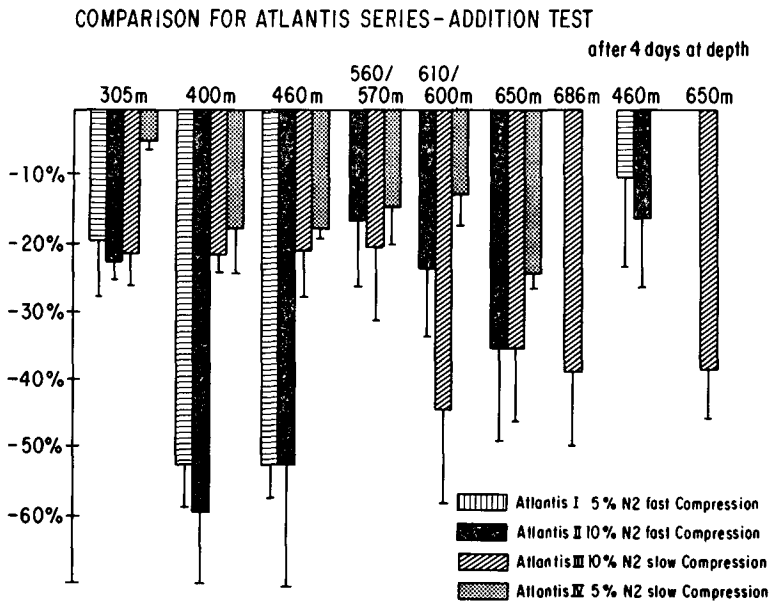

Fig. 1. Comparison of the mean percentage decrement of 3 divers for each of the dives Atlantis I, II, III, and IV at the arithmetic test involving memory and cognition. The larger decrements at 400 and 600 m for I and II due to fast compression is clear, as is the increasing decrement for dives greater than 500 m for dives II and III but the considerable benefit in Atlantis IV of 5% nitrogen and slow compression (4).

The GKSS Research Center, Geesthacht GmbH, near Hamburg, conducts preindustrial research and development on behalf of the Federal Republic of Germany in the fields of environmental protection, material technology, and underwater technology. Underwater research and development objectives are aimed at finding technical solutions for the safe and efficient performance of underwater work, including installation, maintenance, and repair. They include necessary diving techniques, underwater testing, safety of underwater work, and training of underwater specialists.

Early efforts in this regard started over 12 yr ago with the operation of the open sea habitat *Helgoland* and associated diving equipment. Between 1980 and 1983 a very large ocean simulator system was installed by Draeger, Lubeck. Designed by GKSS in cooperation with industry and universities, the GKSS Underwater Simulator Plant (GUSI) is one of the largest and most sophisticated systems in the world today. Manned diving tests are possible to a simulated salt water depth of 600 m, and unmanned tests down to 2200 m. The largest pressure chamber is 3.5 m i.d. and 12.7 m long. It can be filled with salt water and is connected by a vertical chamber to two living chambers. In addition, the temperature can be varied from 0 to 32°C (Fig. 3). Details of its main design data are given in Table 1.

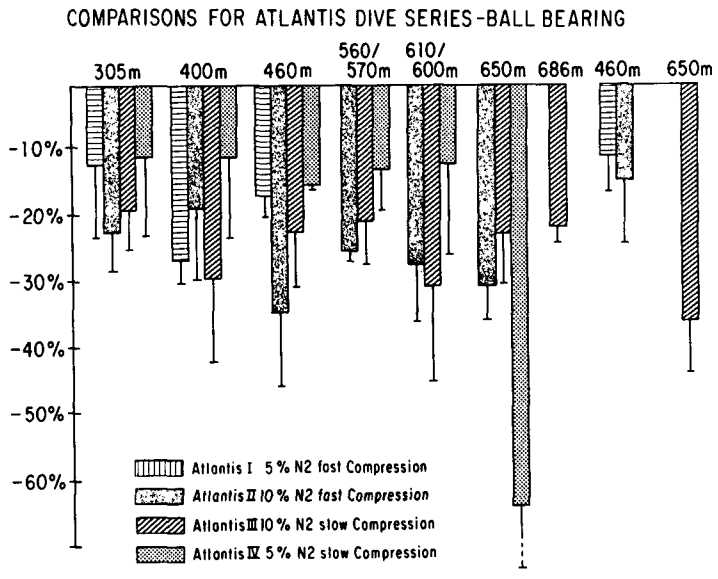

Fig. 2. Comparison of the mean percentage decrement of 3 divers for each of the dives Atlantis I, II, III, and IV at the ball bearing test of fine motor dexterity. Except for 650 m, the lowest nitrogen (5%) and slow rate of compression of Atlantis IV indicate the least psychomotor decrement.

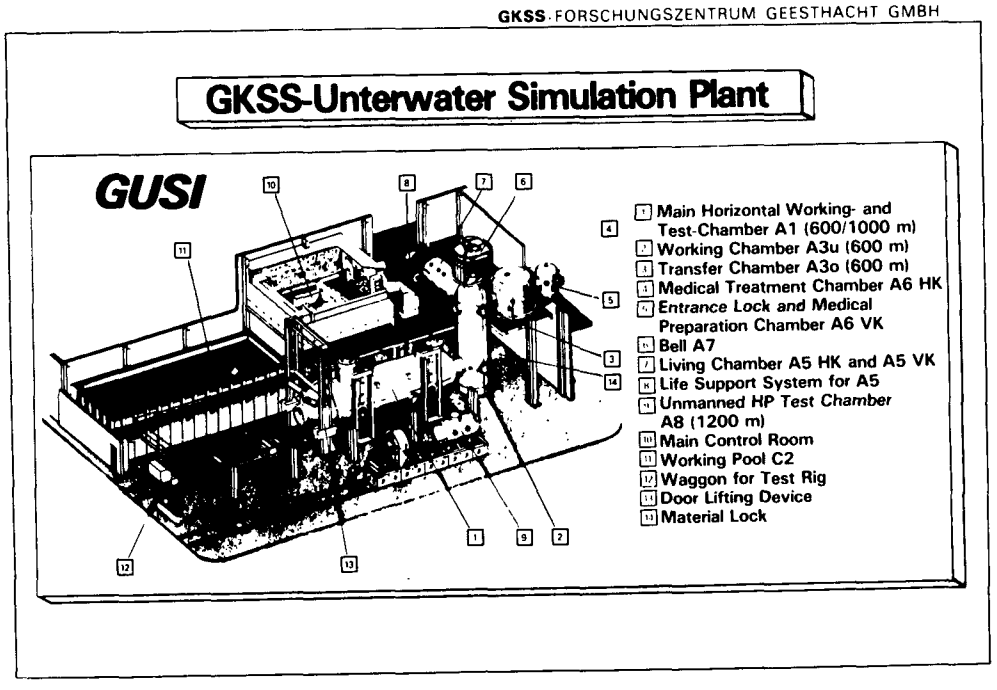

Fig. 3. The GKSS Underwater Simulator Plant at Geesthacht near Hamburg.

Table 1
GUSI: Main Design Data (See Fig. 3)

Ground floor	
Working and test chambers:	manned up to 600 m unmanned up to 1000-2200 m
A1:	100 m^3; 3.5 m diameter, 12.7 m length
A3u:	22 m^3; 2.3 m diameter, 4.7-7.4 m height
Working pool C2:	14 m × 5 m × 6 m
Upper floor	
Living chambers to 600 m depth:	
A30:	11 m^3, 2.3 m diameter, 2.7 m height
A5:	15 m^3, 2.1 m diameter, 4.3 m length
A6:	26 m^3, 3.2 m diameter, 2.7 m height
Operational conditions	
Medium:	seawater, freshwater, dry gas
Temperature:	0-32 ° C controllable
Diver capacity	
Max 10-12	
Special equipment	
Welding gas absorber	
Computerized chamber monitoring and gas analysis	
Data acquisition system	

As a consequence of the extensive experience of the Duke F.G. Hall Laboratory staff in deep saturation diving, they were contacted at an early stage in the operation of the German facilities and in training GUSI staff in saturation diving techniques to produce safe operational procedures to 600 m.

GUSI is designed for technical work at pressure and not primarily for medical research, and therefore needed conservative compression and decompression schedules to permit the divers to reach maximum depth and be able to start welding or other technical research work as soon as possible and to return safely to the surface. It was important also that there be no long-lasting deleterious effects from such exposures.

METHODS

It was decided that for optimal relief from the signs and symptoms of HPNS, and without the problems of nitrogen narcosis, compression would be with trimix 5 (i.e., nitrogen, 0.5 bar oxygen, and remainder helium) throughout. Indeed the same gas mixture was chosen for all GUSI dives, including decompression.

Evolving from the Duke *Atlantis* experience above, a compression profile was selected to 600 m (Table 2). A series of dives was then planned, using this profile, from 1983 to 1986 which would gradually work down to the maximum operating depth of 600 m, contingent upon there being no deleterious HPNS effects in the divers (Fig. 4).

Table 2
Duke-GUSI Compression Profile to 600 m with Trimix 5
(N_2 5% - 0.5 bar O_2 - He rest)

Travel 0-180 m	= 5 m/min (36 min)
Stop at 180 m	= 2 h
Travel 180-240 m	= 3 m/min (20 min)
Stop at 240 m	= 6 h
Travel 240-300 m	= 1.5 m/min (40 min)
Stop at 300 m	= 2 h
Travel 300-350 m	= 0.5 m/min (1 h 40 min)
Stop at 350 m	= 9 h
Travel 350-400 m	= 0.25 m/min (3 h 20 min)
Stop at 400 m	= 2 h
Travel 400-430 m	= 0.125 m/min (4 h)
Stop at 430 m	= 2 h
Travel 430-460 m	= 0.125 m/min (4 h)
Stop at 460 m	= 12 h
Travel 460-490 m	= 0.100 m/min (5 h)
Stop at 490 m	= 2 h
Travel 490-520 m	= 0.100 m/min (6 h 40 min)
Stop at 520 m	= 13 h
Travel 520-550 m	= 0.075 m/min (6 h 40 min)
Stop at 550 m	= 13 h
Travel 550-575 m	= 0.05 m/min (8 h 20 min)
Stop at 575 m	= 16 h
Travel 575-600 m	= 0.05 m/min (8 h 20 min)

The decompression procedures are shown in Table 3 and are based on evaluation of 1055 helium-oxygen man-decompressions and 189 nitrogen-oxygen man-decompressions (3) and equation

$$R = K^{*}P_{IO_2} \qquad (1)$$

where R is measured in m/h or fsw/h, P_{IO_2} is in atm (or bars), and the proportionality constant is in m/atm or fph/atm. The data suggested that it is necessary to reduce the value of K as the depth of dive increases. The schedules generated from the estimated K values are with 0.5 bar oxygen to a depth of 14 m, after which the oxygen is held at 0.21 bar until surfacing.

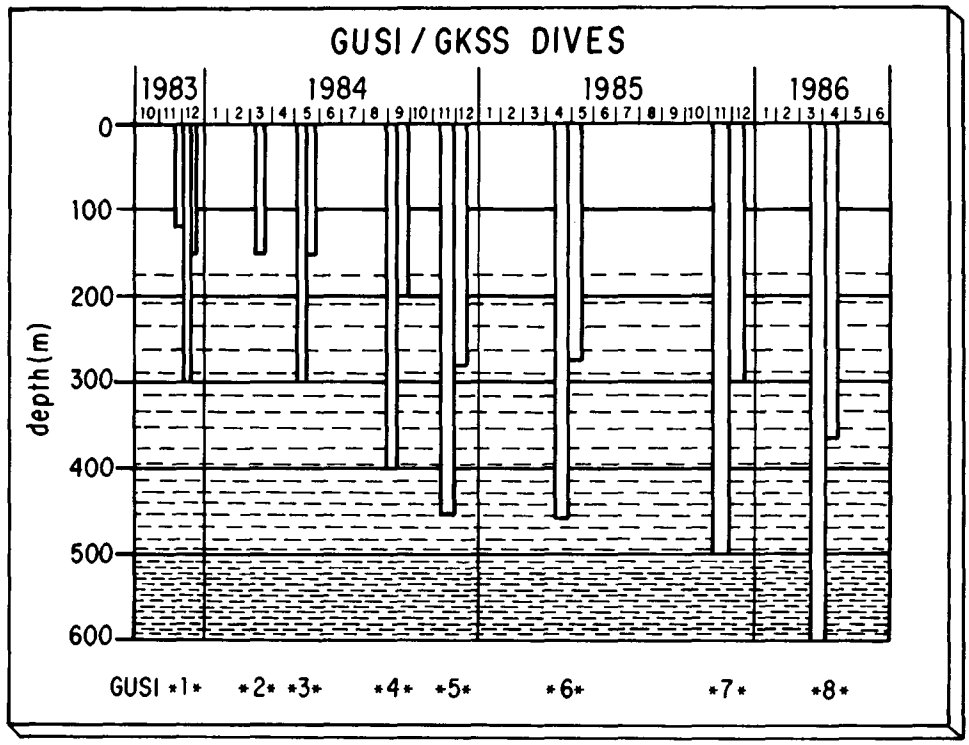

Fig. 4. Plan of the 14 deep dives at GKSS/GUSI from 1983 at completion of construction to 1986 at 600 m, the working maximum depth for divers.

From 1983 to September 1986 an extensive series of 8 major studies with 14 deep dives between 300 and 600 m were made using 13 German, English, French, and American divers, and involving 1341 man-d of saturation in 50 man-dives (Table 4). These divers were not specially selected and the majority had no experience of such deep saturation diving.

In most cases, while the 1st group of 4 divers were decompressing, a 2nd group were compressed to meet them for saturation at a shallower depth and then all divers decompressed together (Table 4).

Extensive clinical and psychologic testing of the divers was made annually leading to an offshore diving medical fitness certificate according to the UK-Norway Offshore Diving Standards. This included initial clinical psychologic

Table 3
Provisional Duke Saturation Decompression Schedules, msw

Saturation Depth, msw	KE, FPH/ atm	Rate of Ascent, min/0. 5 msw					Decompression Time	
		Until 14 msw	14-9 msw	9-6 msw	6-3 msw	3-0 msw	Day	Hour
0 - 30	12.0	17	21	25	30	40	0	22
30 - 60	11.0	18	23	27	33	43	1	17
60 - 90	10.0	20	25	30	36	47	2	18
90 - 120	9.5	21	26	31	38	50	3	18
120 - 150	9.0	22	28	33	40	53	4	21
150 - 180	8.5	24	29	35	43	56	6	7
180 - 210	8.5	24	29	35	43	56	7	7
210 - 240	8.0	25	31	37	45	59	8	15
240 - 270	8.0	25	31	37	45	59	9	16
270 - 300	7.5	27	33	39	48	63	11	13
300 - 330	7.5	27	33	39	48	63	12	16
330 - 360	7.0	29	36	42	52	67	14	20
360 - 390	7.0	29	36	42	52	67	16	1
390 - 410	6.5	31	38	45	56	73	18	0
410 - 450	6.5	31	38	45	56	73	19	18
450 - 480	6.0	33	41	49	60	79	22	10
480 - 510	6.0	33	41	49	60	79	23	19
510 - 540	5.5	36	45	54	66	86	27	11
540 - 570	5.5	36	45	54	66	86	28	23
570 - 600	5.0	40	50	59	72	94	33	20
600 - 630	4.5	44	55	65	80	105	39	1
630 - 686	4.0	50	62	73	90	118	48	6

Oxygen - 0.5 atm partial pressure deeper than 14 msw
- 21% from 14 msw to surface
Nitrogen - Not more than 5% deeper than 15 msw
- Not more than 0.79 atm partial pressure at less than 150 msw
Helium - Balance

evaluations by the experienced DFVLR aerospace group at Hamburg, chest and long bone x-rays, and a T_c 99 m technetate scan every 2 yr plus full-head EEG and neurologic evaluation (6). Immediately before and after every dive a further physical evaluation was made, usually by 2 physicians.

During the dive, monopolar EEG was recorded from the occipital region with eyes open and shut, and fast Fourier frequency analysis was provided.

Table 4
GUSI Duke Dives 1983-1986

Dives	Date	Dive Days	No. of Divers	Man Dives
GUSI 1 125, 300, 150 m	23 Nov 1983 to 16 Dec 1983	24	4	96
GUSI 2 150 m	12 March 1984 to 21 March 1984	10	3	29
GUSI 3 300, 150 m	7 May 1984 to 27 May 1984	21	3 (300 m) 3 (150 m)	86
GUSI 4 400, 200 m	2 Sept 1984 to 29 Sept 1984	28	3 (300 m) 3 (200 m)	135
GUSI 5 450, 265 m	14 Nov 1984 to 14 Dec 1984	31	4 (450 m) 4 (265 m)	173
GUSI 6 450, 265 m	23 April 1985 24 May 1985	32	4 (450 m) 4 (265 m)	198
GUSI 7 500, 450, 300 m	6 Nov 1985 to 10 Dec 1985	35	4 (500 m) - (450 m) 4 (300 m)	280
GUSI 8 600, 360 m	20 March 1986 1 May 1986	43	4 (600 m) 4 (360 m)	344
Totals		224	50	1341

Similarly, a neurologic examination was made on arrival at maximum depth and on arrival at the second work depth during decompression.

Daily check-off sheets were provided to identify any HPNS, nitrogen narcosis, or other adverse signs or symptoms, and included answers for presence of dreams, degree of sleep, and location of aches or pains on a manikin diagram. This was accompanied by regular verbal questioning of the divers.

Muscle strength was tested by a hand-held dynamometer which recorded peak grip and strength over a fixed time of 1 min. A 2-h clinical psychologic test package, of vigilance and memory especially, was made by Dr. Goeters of DFVLR before the dives to 500 and 600 m, at depth, and on return to the surface. These consisted of four tests. Test KBT (author Kirsch) required

steady and coordinated use of perception speed, memory capability, and calculating ability. Test MEK (author Kirsch) measured the memory capacity for visual information. Test UZA (author Winke) measured the memory capacity for auditory perception, and the CLE test (author Witt) measured the memory capacity for acoustically transmitted information.

In addition, especially in the 600-m dive, Dr. Richard Moon collected venous blood to study platelet and other changes, and urine volume and electrolytes were recorded. Body weight was recorded throughout the dives.

A practical test of the efficiency of the procedures was the ability of the divers to go to work as required after arrival at depth and carry out necessary chamber function and breathing apparatus tests as well as various forms of welding while wearing breathing apparatus.

RESULTS

The results indicated little or no HPNS during compressions and hardly any compression arthralgia. The divers were fit on arrival and functionally normal with no indications of nausea, visible tremors, or undue fatigue. At 500 m in GUSI 7, 1 diver did vomit immediately after breakfast a day after compression, but he then developed a viral infection with increased temperature which resulted in him being taken off work. At 450 m in GUSI 5, 2 French divers made a certification weld of a 36-in. steel pipe and finished ahead of schedule, affirming that the depth felt less than 100 m.

The performance tests showed little change to 500 m (Tables 5-8) at which the KBT test of perception, speed, memory, and calculating capability showed significant decrements at the 500 m and even at the 278-m depths, although the decrement was less at 278 m (Table 5).

Table 5
GKSS - DFVLR Test KBT (Goeters-Kirsch)
Perception, Speed, Memory, Calculation

	Mean	SEM	*t* Test
Predive	79.25	± 26.17	—
500 m (1985)	47.00	± 23.19	1%
278 m	70.50	± 27.04	1%
Postdive	97.00	± 28.52	—

At 600 m the performance decrement was more general, especially in the tasks requiring memory. However, this was not noticed in the ability of the divers to function at their welding and chamber function tests, although they remarked that extra concentration was required. They appeared normal with no adverse remarks on their check lists except for obligate mouth breathing being required at depths in excess of 450 m.

The EEG activity was remarkably free of HPNS-related theta activity (6-8 Hz) in all divers, even during the 600-m dive (Fig. 5). The particular subject shown in Fig. 5 was also a subject in the *Atlantis* dives, which unlike this dive did produce increased theta activity and HPNS with the faster compressions. Of special interest in the EEG was the abolition or reduction of the alpha activity (Fig. 6). Again in this diver no theta activity is seen but the large normal alpha activity (8-13 Hz) initiated with eyes closed, which usually is markedly reduced or blocked by opening the eyes, was eliminated by the pressure itself from 360 to 600 m.

Table 6
GKSS - DFVLR Test MEK (Goeters-Kirsch)
Memory Capacity for Visual Information

	Mean	SEM	*t* Test
Predive	17.75	± 4.57	—
500 m (1985)	20.00	± 6.88	NS
278 m	19.00	± 2.94	NS
Postdive	22.25	± 3.10	—

Body weight declined, especially in the 600-m dive, with some subjects being more susceptible than others. This weight loss, which continued into decompression, was partially reestablished closer to the surface by encouraging the drinking of more fluids (Table 9) and was fully restored shortly after return to surface.

Table 7
GKSS-DFVLR Test UZA (Goeters-Winke)
Auditory Vigilance and Perception

	Mean	SEM	*t* Test
Predive	41.75	± 3.09	—
500 m (1985)	38.00	± 5.16	NS
278 m	44.00	± 4.54	NS
Postdive	43.25	± 2.06	—

Dynamometer tests at 500-600 m showed an average reduction of between -3 and -13% in peak strength with little change in the duration strength test.

During the 50-man dives, three decompression incidents are of note. During GUSI 7, 1 of the divers at 7.8 m complained of difficulty in sleeping. He was a diver from the shallower, 300-m saturation, and some 20 min after

announcing this problem he reported left knee pain. He was given three 20-min periods of pure oxygen to breathe, and the pain resolved without recompression. After 6 h the decompression was continued on the original schedule.

Similarly, in GUSI 8, 1 of the 600-m divers awoke at 40 m complaining of constant left knee pain for 20 min. When it reappeared some hours later for the same time, the physician ordered three 20-min oxygen breathing cycles. Two days later recurring "niggles" of the right knee also resulted in a further three cycles of breathing treatment gas.

Table 8
GKSS-DFVLR Test CLE (Goeters-Witt)
Memory Capacity for Auditory Information

	Mean	SEM	*t* Test
Predive	181.75	± 23.41	—
500 m	167.25	± 20.88	NS
278 m	193.25	± 14.54	NS
Postdive	203.00	± 13.52	—

Finally, another of the 600-m divers presented a neurologic deficit in his right foot during the postdive examination on surfacing with "foot drop," muscular weakness, and sensory deficit on both sides of the foot. He was treated with recompression and oxygen, saturated at 18 m for 24 h, and recovered. It is pertinent that this diver had some evidence of foot drop in his predive physical and so it would seem the decompression from 360 m singled out this particular site of earlier injury.

DISCUSSION AND CONCLUSIONS

This is a unique number of 14 very deep dives made in a relatively short time of 2 to 3 yr. Trimix 5 (nitrogen 5%-O_2 0.5 bar-helium remainder), when combined with the Duke slow exponential compression profile with long stages, seems to permit divers to reach 600 m or shallower depths with remarkably little or no signs and symptoms of HPNS and to work effectively at welding or other tasks. Importantly, the presence of nitrogen in the breathing gas also does not appear to affect the integrity of the weld.

The divers showed no postdiving effects other than the usual debilitation for a week or so as a result of the confinement and lack of exercise and sunshine.

The performance tests indicated decrements at only the 500- and 600-m depths, and yet the divers' activity at welding and other tasks did not appear to be impaired. However, the results do indicate that performance efficiency can be expected to start to fall off at depths much in excess of 600 m. A part of this could well be due to the 5% nitrogen, but it is felt that this is the

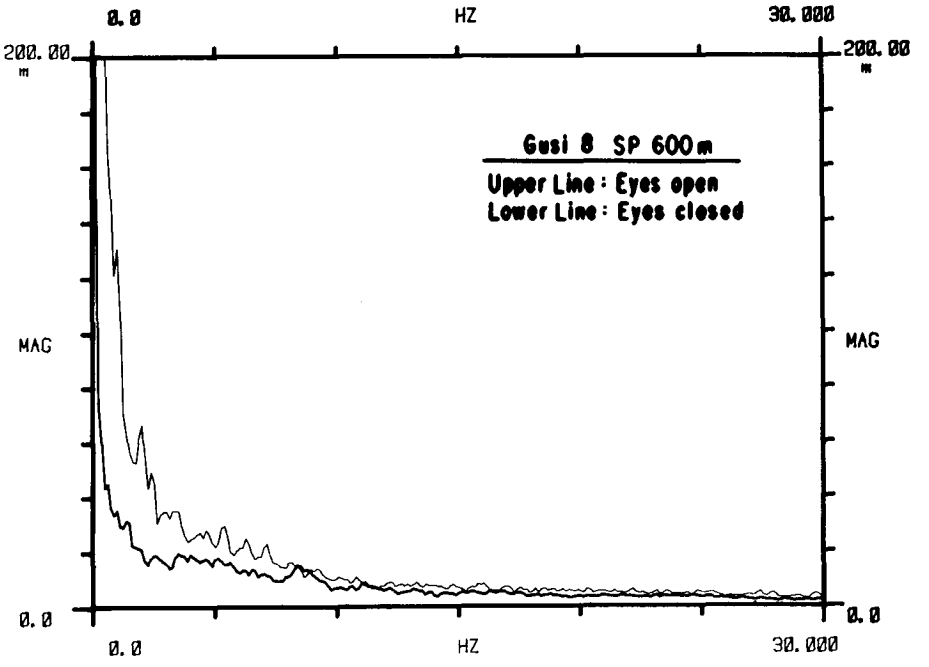

Fig. 5. Fast Fourier frequency analysis of the occipital EEG of a diver at 600 m. Upper line eyes open, bottom, eyes closed. No increase in slow wave activity seen. The other 2 subjects were the same.

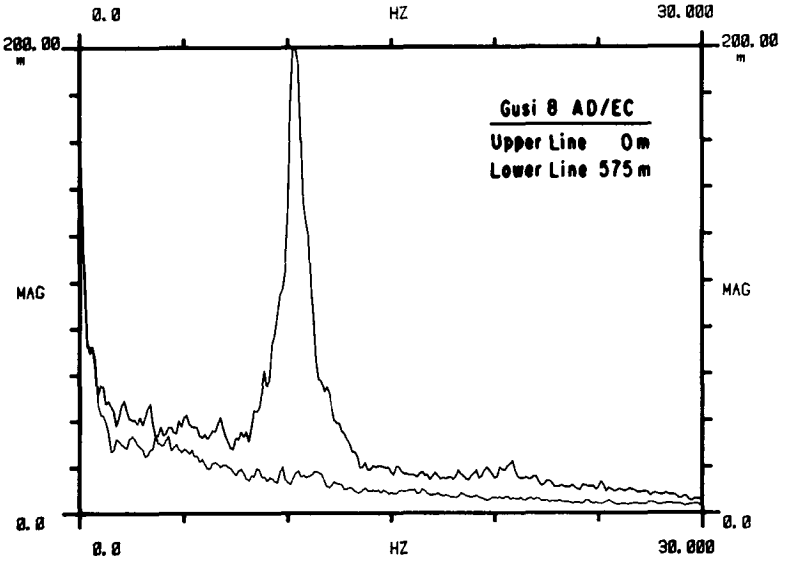

Fig. 6. Fast Fourier frequency analysis of occipital EEG of a diver with eyes closed at surface predive (upper line). It may be seen that the alpha frequency at 8-13 Hz has disappeared at 575 m.

Table 9
Body Weights, kg

	S1	S2	S3	S4	Mean
Control	95	81	76.5	93	86.4
599 m	92.3	79.1	74	88	83.4
474 m	91	76.8	74.3	86	82.0
441 m	91	76.8	72.8	85.9	81.6
360 m	89.8	75.7	72.1	84.7	80.4
259 m	89.1	76.7	73.0	84.5	80.8
134 m	88.8	75.3	73.3	84.3	80.4
Surface	90.0	77.5	73.8	84.0	81.3

price to be paid for control of the unpleasant signs and symptoms of HPNS which is the alternative and would be likely to be more incapacitating in some cases. Similar decrements were reported in *Atlantis* III (7) in 3 divers at 686 m breathing 10% nitrogen in heliox. This data also showed that divers can continue to function at such extreme depths, but changes became apparent in tasks requiring rapid problem-solving abilities or extended long-term memory. Extensive training could help to reduce these factors and may be important to the divers ability to react correctly in a sudden emergency.

The reduction or abolition of the alpha activity in the EEG at the greater depths is also an indication that physiologic changes are occurring, although there is no physical sign at this depth. The lack of HPNS-induced theta activity is most encouraging. On the other hand the loss of alpha activity may be due to a generalized reduction in electrical activity of the brain, which has been reported in previous deep dives, or an electrical alerting of the brain.

The linear decompression profiles were of necessity very long. Indeed, it seems that the deeper the dive the slower must be the decompression. Yet even with rates as slow an 0.75 m/h (2.5 ft/h) some signs and symptoms of decompression sickness were seen close to or at the surface, and this in the 2, 600-m divers even though they had spent over 2 d saturated during the decompression at 360 m. It might therefore be assumed that some gas from the 600 to 360 m part of the dive still remained in the divers, because the normal rate for a decompression from 360 m alone would be much faster at 3.3 ft/h, which was done by many of the divers without any adverse effect (Table 3).

Thus the diver in GUSI 7 who complained of decompression sickness had made 3 previous decompressions from the 300-m depth, to which the diver had been exposed, at decompression rates much faster and with no effect.

The decompressions are singularly difficult due to their length, yet they still cause decompression sickness. The length is a major problem because it involves many weeks for the diver living in a small space like an astronaut, with limited exercise and social interaction, and in an environment of boredom

to which too little attention has been paid in the past.

Nevertheless, these dives are a prominent milestone in the attempts to permit divers to live and work at greater depths, and indicate that 16 yr after the first dive to 300 m, man can work effectively, using the techniques described, at 600 m.

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TOPOGRAPHIC CHARACTERISTICS OF EEG DURING A SATURATION DIVE TO 31 ATA HELIUM-OXYGEN ENVIRONMENT

S. Matsuoka, S. Okuda, T. Ishikawa, S. Yamamoto, and M. Mori

Many electrophysiologic studies concerning saturation excursion dives in hyperbaric environments reveal an increase in the theta wave (1-5). However, in traditional EEG, no detailed analyses have been done to clarify the nature of the theta wave because of the restrictions imposed by the environment and the limitation associated with the induction electrode. Therefore, it is necessary to adopt a multielectrode induction recording system. With the 16-channel recording system we developed in 1972 (6), a topographic display of the EEG has been used to conduct underwater experiments in an attempt to establish the changes occurring in the EEG (in all frequency bands) at different sea depths (various atmospheric conditions) and to establish a correlation between the regions in which these changes occur.

In addition, the correlation studies between the topographic EEG and the compression rate in a helium-oxygen environment were done, at 80, 130, and 180 m, to compare them with studies done at 300 m.

MATERIALS AND METHODS

Six divers carried out a saturation dive to 300 m, 4 divers to 180 m, 6 divers to 130 m, and 4 divers to 60 m. The hyperbaric environment experiment was performed as a 31 ATA simulated human experiment for 1 mo. in the human diving simulator at Japan Marine Science and Technology Center (JAMSTEC), Yokosuka, from 1982 to 1985. Hyperbaric heliox dives to 31 ATA took place according to the schedule shown in Fig. 1.

The computer display system of the EEG consists of two parts (Fig. 2) (6, 7). One derives the equivalent potential for extraction of specific EEG activities superimposed on the background activities. The equivalent potential

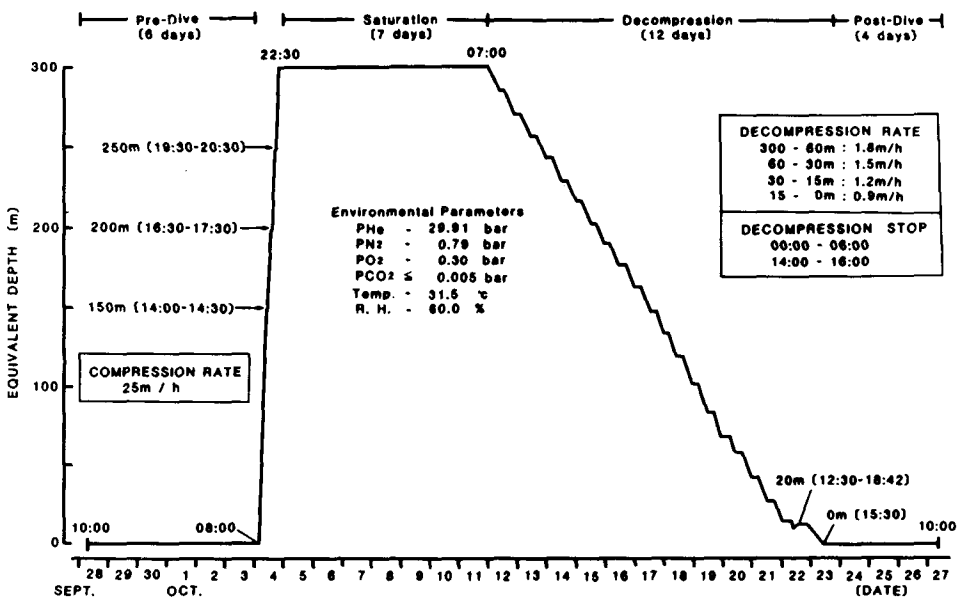

Fig. 1. Dive profile at *Seadragon* VI.

of a specific EEG activity is defined by the square root of average power over the desirable frequency band in the power spectrum of a certain time duration of EEG data. A 20-s EEG data period is a reliable length of time for estimation of the slow waves. Square roots of these normalized average powers are calibrated for each channel, and from this the equivalent potentials are derived.

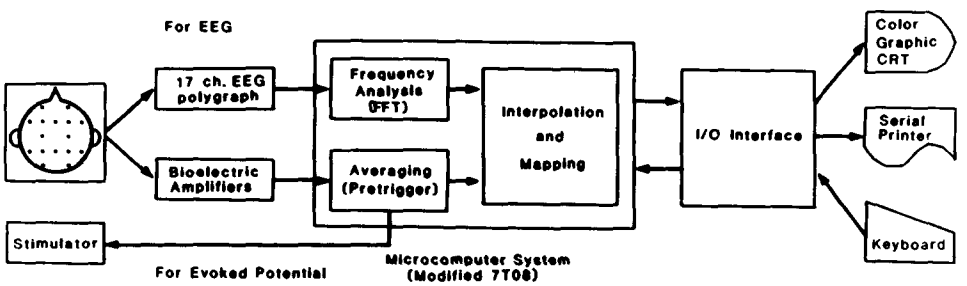

Fig. 2. System block diagram of topographic EEG and evoked potential.

The other part of the computer display system involves computation for mapping. Potential fields of EEG frequency bands are printed out on a line printer as shown in the topographic maps (Fig. 2). The electrodes of the 10-20 system are rearranged on a two-dimensional plane. The 16 channels of the EEG are recorded by a monopolar montage with a common reference electrode on the ear. Unmeasured potentials are estimated from neighboring potentials. The

potentials between grid points are approximated by a two-variable sampling function. The interpolated values are quantified to 11 levels and, finally, potential fields are mapped on color cathode ray tubes (CRT) (Fig. 3), or in a bar graph (Fig. 4).

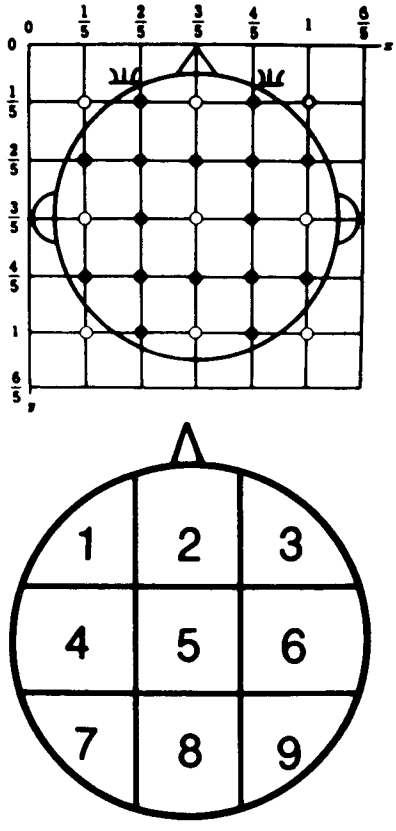

Fig. 3. Rearrangement of electrodes of the 10-20 system on a dimensional plane for mapping: black points show measured electrodes, and unmeasured ones at 9 grid points, marked with a white point, are assumed before hand to utilize the interpolation function. Lower: edited location of EEG.

On the other hand, on the basis of the potentials in 25 locations, two-dimensionally displayed with a computerized topographic EEG scanner, a division is made into nine blocks to determine the average value for each block, and we have analyzed the data using a distribution analysis technique (Fig. 3, *bottom*) (8).

RESULTS

All subjects participating in the saturation excursion dives were healthy, experienced divers, highly motivated and very cooperative.

The assumption is made that the EEG potential is determined by the depth factor on the one hand and the location factor on the other. In the

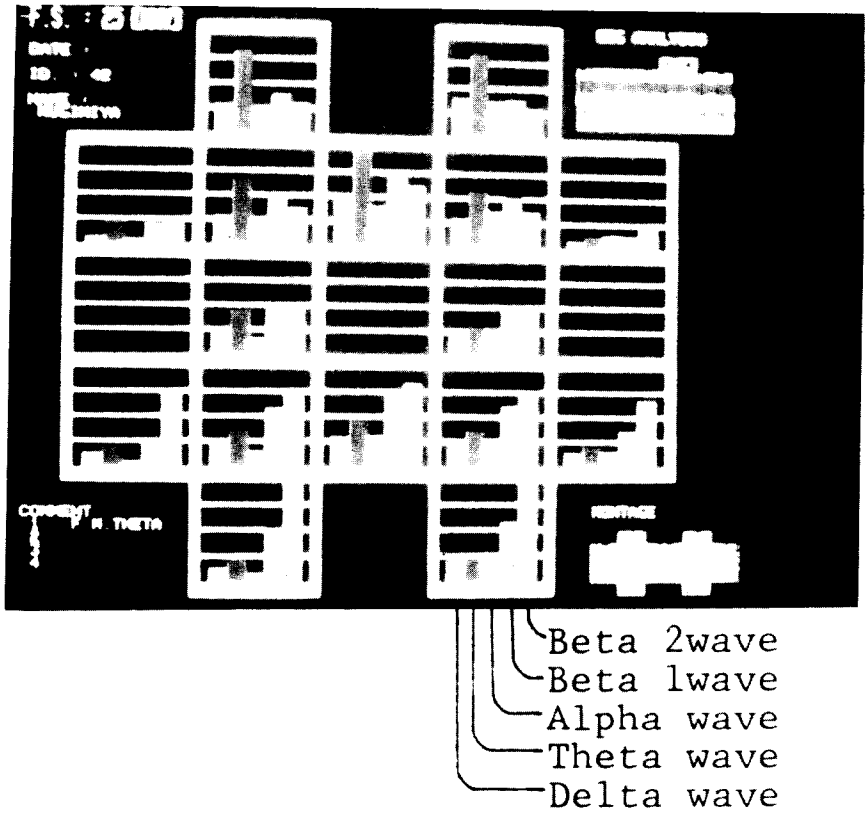

Fig. 4. The distribution of the equivalent amplitude of each frequency band at 10-s epoch of 16 electrodes shown in the bar graph. This characteristic histogram reveals a maximum amplitude of the theta band at frontal midline area (Fz) and reveals an episode of laughter at 21 ATA.

model given, a two-way (two-dimensional) layout is used in which Xij = μ + α_i + β_j + eij, where Xij in the EEG data, i the depth, and j the surface location. α_i is the effect due to the depth, β_j is the effect due to the location, and eij is the error. On this basis, we analyzed the data using our distribution analysis technique (8) and obtained a distribution analysis table (Table 1). From these results it has been ascertained that a depth-related effect and a location-related effect do actually exist. The pattern of the EEG obtained displayed changes depending on the variation in depth as well as with changing location.

Here we have tried to establish how the EEG pattern for each location changes with changing depth. For example, regarding the theta wave, Table 2 presents linear equations applicable when the changes in the data for the different positions of the theta wave have been fitted into a straight line and the corresponding correlation coefficients. The sign preceding a (i.e.,

Table 1
Results of Analysis of Variance of EEG Data

	Factors	Sum Square	*df*	Mean Squares	F value	Test
Theta	Depth	128.1226	6	21.3538	34.2866	**
	Location of EEG	114.6523	8	14.3315	23.0114	**
	Error	29.8945	48	0.6228		
	Total	272.6694	62			
Alpha	Depth	131.0352	6	21.8392	8.6910	**
	Location of EEG	2112.7031	8	264.0879	105.0946	**
	Error	120.6172	48	2.5192		
	Total	2364.3555	62			

** $P < 0.01$.

Table 2
The Variation of EEG (Theta Wave) and the Location

	1	2	3	4	5	6	7	8	9
MV	13.1250	12.7813	11.1938	9.5875	11.2813	8.6813	11.7525	11.0688	9.1813
SD	2.0554	1.6489	1.4808	1.2909	2.0462	1.0245	2.0947	1.1487	1.2438
r	-0.5612*	-0.7303**	-0.6797**	-0.7183**	-0.7716**	-0.7677**	-0.5264*	-0.7170**	-0.2411
a	-0.0110	-0.0116	-0.0097	-0.0089	-0.0151	-0.0075	-0.0106	-0.0079	-0.0029
b	11.4559	11.0146	9.7172	8.2272	8.9650	7.5274	10.1448	9.8604	8.7413

MV = Mean value; a and b = regression coefficients; * $P < 0.01$; ** $P < 0.05$.

denoting the slope of the straight line) is minus in all cases (i.e., theta) (Table 2). This means that with increasing depth, the potential will increase.

Let us next take the correlation coefficient and try to establish whether this straight line is meaningful or not. The double asterisks signify a level of significance of 1% and the single asterisk means a level of 5% significance. Underneath each table, we indicated the location that was considered significant is the evaluation. From these observations it becomes clear that the alpha wave has shown significant variations to the frontal prominence, the beta wave, to the occipital region on both sides, and the theta wave to the frontal midline region. Figure 5 reveals the corresponding plots relating to the different locations of the brain by a microcomputer display. Figures 6 and 7 show the characteristic pattern of the theta wave (Fmθ) above 250 m below sea level, predominantly in the frontomidline, in the numerical printout, and in color. Figure 8 illustrates the correlation between EEG and pressure. On the

other hand, during decompression of the saturation dives of 130 and 180 m, characteristic topograms of EEG were revealed that were the same patterns as Fmθ at 300 m.

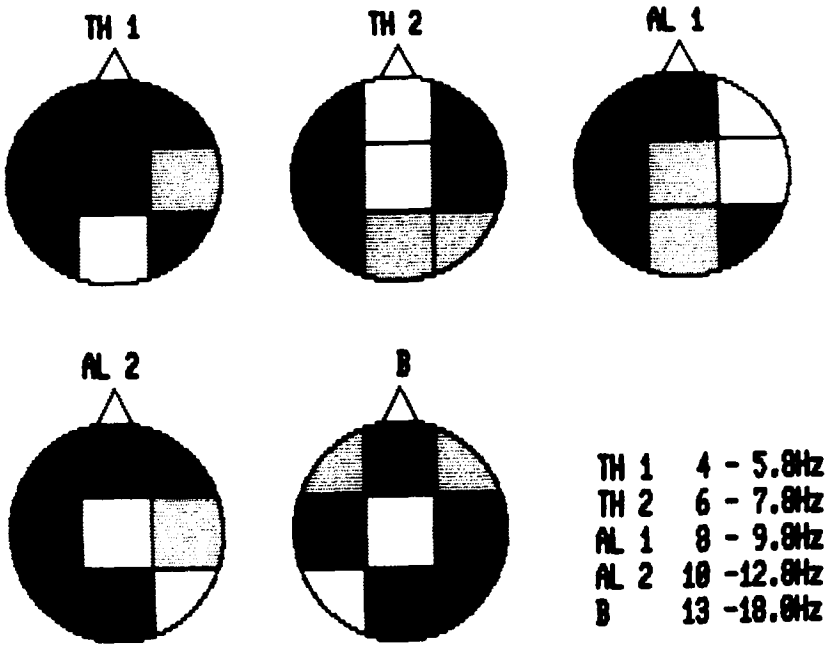

Fig. 5. The illustration of the corresponding plot, relating to the different locations of the brain (Th2); white = $\geq$ X + SD, dotted square = < X - SD.

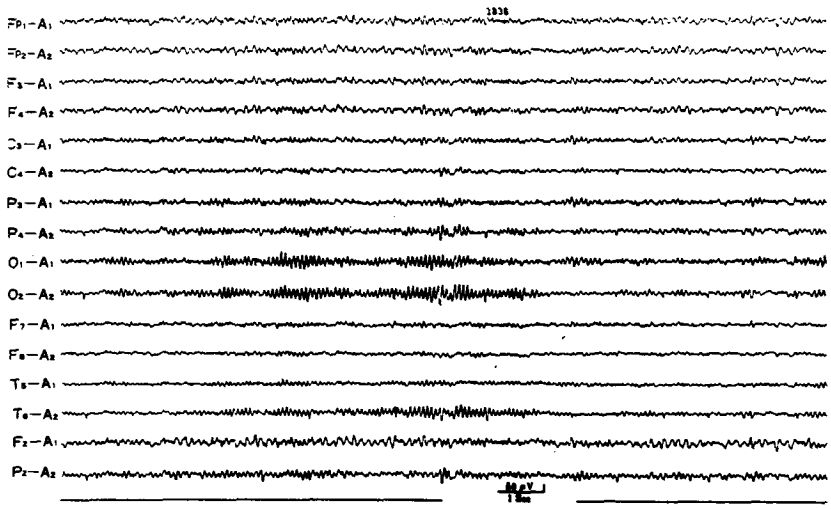

Fig. 6. EEG theta waves in the Fz, Fp_1, and Fp_2 at the time of laughter.

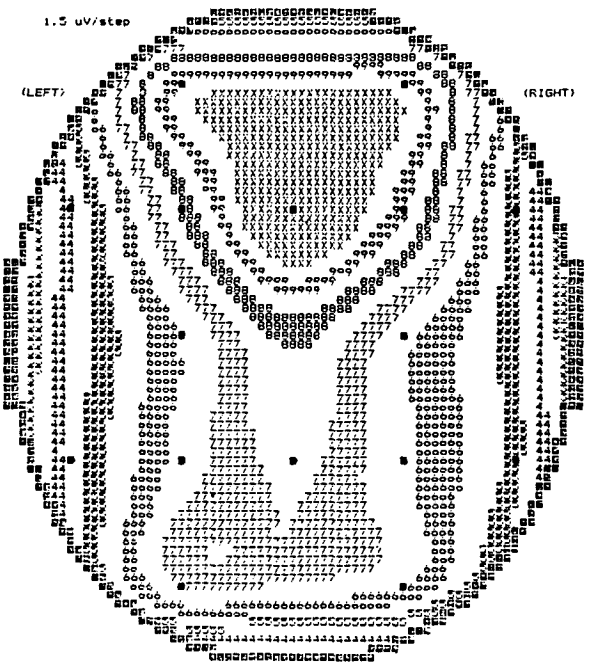

Fig. 7. The numerical printout of the topographic map of the theta wave in the Fig. 6 EEG.

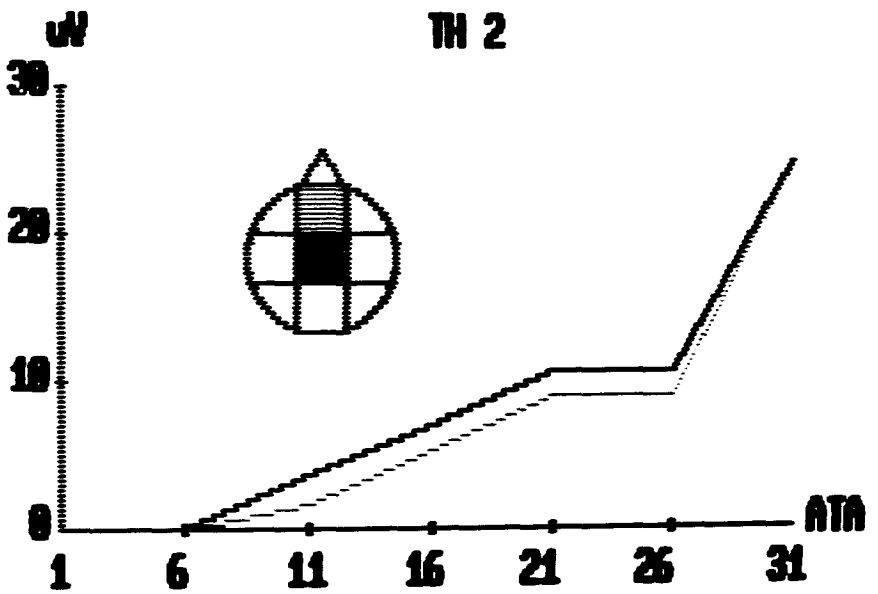

Fig. 8. Illustration of the correlation between EEG and pressure (ATA); *thick dark = high* potential of Th2; light dark = lower potential of Th2.

All the divers answered a questionnaire and kept a personal log of their signs and symptoms of HPNS, and characteristic features were noted in the emotional and mental status. For example, a transient episode of laughter or euphoric status at a depth higher than 21 ATA. At the same time, topographic EEG revealed a type of frontomidline theta wave that was associated with the diffuse alpha wave. Thus, an intimate correlation between frontomidline theta wave and laughter or euphoric state was also observed during compression and decompression processes in the saturation dive.

The divers became accustomed to this environment after 24, 32, and 80 h on the bottom. These results showed that there were significant individual variations in susceptibility to subjective signs and symptoms.

DISCUSSIONS AND CONCLUSION

We developed a two-dimensional display of the EEG and applied it to divers during the compression period of a 31 ATA saturation dive. Next, we applied a two-way layout of the analysis of variance, using all frequency bands to determine EEG characteristics at various dive depths and to establish a correlation between the region and EEG patterns. The distribution analysis technique revealed that the topographic pattern depended on the dive depth and indicated the most strikingly affected region during compression and decompression. Thus, significant correlations between the dive depth and EEG potential were observed at individual mapping locations, that is, the alpha wave showed a significant variation in type of diffuse alpha, and the theta waves for the frontomidline region which were developed paroxysmally in relatively brief bursts supplanting or intermixing in the normal background rhythms. If this is interpreted only in EEG terms, it becomes clear that this is part of the phenomenon of cerebral dysfunction. In addition, to elucidate the developmental mechanism of the theta wave, we compared the frontal intermittent rhythmic delta activity (FIRDA) (9) and the frontomidline theta wave (FMθ) (10, 11) observed in this experiment. Fmθ was mostly seen at Fz with the maximum amplitude. On the other hand, FIRDA was located more arteriorly than Fz. FIRDA abnormal rhythms result not from deranged function of cortical neurons but from deranged function of subcortical pacemakers driving cortical neurons. These activities show reactivity, being augmented by hyperventilation or after attenuation by "alerting" acts such as eye opening or mental activity. These marked reactivities are also affected by changing physiologic states (12). On the other hand, Westmoreland suggested regarding midline theta rhythm, that of the 36 patients, 28 had a seizure disorder, and the other 8 had various conditions unassociated with epilepsy. Although its mechanism of origin is uncertain, the midline theta rhythm seems to represent a nonspecific electroencephalographic pattern that can occur in a mixed group of patients with various diagnoses (12). Thus, this type of theta wave would seem to represent a nonspecific variant of theta activity that can occur in the normal EEG, or is related to certain mental activities (11), and in a

heterogeneous group of patients (12). In our cases, the Fmθ associated mostly with some characteristic features of HPNS, such as a transient episode of laughter or euphoric status at a higher depth than 21 ATA. An intimate correlation between Fmθ and laughter was also observed during compression and decompression processes of a saturation dive in a helium-oxygen environment. Therefore, Fmθ may be related to the same emotional activities caused by inert gas narcosis.

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INTERACTION OF INERT GAS NARCOSIS WITH THE COMPRESSION RATE EFFECT ON DEVELOPMENT OF HPNS SEIZURES

R. W. Brauer, J. Dutcher, and W. Hinson

THE COMPRESSION RATE EFFECT

Quantitative study of the effects of high pressures on the CNS depends in the last analysis on the use of appropriate and reproducible endpoints. One such is the onset of a specific type of seizure in a particular animal model: in our laboratories type I HPNS seizure in the CD-1 mouse has frequently served this function. Quite early it was shown that the pressure at which this event occurred (P_c) varied with the rate of compression P, approximately according to the equation

$$P_c(1) = P_c(2) + k \cdot \log (\dot{P}_1/\dot{P}_2). \qquad (1)$$

Here 1 and 2 refer to two different compression rates, $\dot{P}_1$ and $\dot{P}_2$. In the CD-1 mouse the effect of compression rate on convulsion threshold pressure P_c has a total scope of about 60 atm, i.e., convulsion threshold pressures with extremely slow compressions hardly exceed 120 ATA whereas with very rapid compressions the animals undergo HPNS seizures at about 60 ATA (1).

This effect is not dependent on the rate at which the seizure threshold is approached at the end of the compression, but rather on time actually spent at each pressure according to a set of differential equations developed by Brauer et al. (2), which adequately describe the effect on P_c of a wide variety of compression profiles. The model corresponding to this set of equations assumes that development of HPNS type I seizures in the CD-1 mouse is the result of a dual process: A direct effect of pressure causing or facilitating seizure development and reflecting intrinsic pressure susceptibility of the animals, and a secondary compensatory, approximately first-order reaction triggered by the

pressure and proceeding more rapidly at any given moment in direct proportion to the instantaneous pressure. The rate and magnitude of this compression are not the same for all of the different responses making up the complex HPNS, so that the clinical picture presented in animals during the course of compression can be expected to vary with changing compression profiles. An example of this is the fusion of the onset of type I and type II HPNS seizures in the mouse sat very slow compression rates, and their clear separation at faster rates (3).

Thus, we have HPNS seizures in the mouse developing in response to a dual process: A primary pressure-dependent induction of seizures, and a secondary, slower process which antagonizes this primary effect and postpones or prevents seizure onset. On this basis, seizure onset during very rapid compression should most closely reflect the intrinsic pressure tolerance of the test subject and almost be an uncomplicated measure thereof, whereas at slower compression rates a more complicated picture should emerge. This view is in accord with the available genetic data (4).

Pharmacologic data regarding the secondary compensatory process are available for the CD-1 mouse (5). The compression rate effect can be blocked completely by reserpine; this reserpine effect in turn is sharply potentiated by more specific antagonists against serotonine or norepinephrine, which are ineffective by themselves but become effective even in the absence of reserpine if used in pairs (6). All these reserpine or reserpinelike effects can be reversed by monoamine oxidase (MAO) inhibitors. Thus, the compression rate effect clearly involves a key step in which at least two of the three centrally acting monoamine neurotransmitters are involved, although it is not clear whether the rate-determining event is the release of the transmitters or some other slow event that results from the action of these neurotransmitters.

Compression rate dependence of HPNS seizure onset is not unique to the mouse but has been found in many (but not all) species of mammals (7). Furthermore, in the majority of species tested, reserpine reduces or abolishes the magnitude of these compression rate effects, making it safe to conclude that the concept of a dual mechanism, proved for the induction of HPNS seizures in the CD-1 mouse, will find parallels in many other mammalian species.

EFFECT OF INERT GAS NARCOSIS

The first demonstration of HPNS seizures showed that these develop at higher pressures when the compression atmosphere is H_2-O_2 than when it is He-O_2 (8). This ability to increase seizure onset pressures in mice and in squirrel monkeys is closely related to the anesthetic potency of the compression atmosphere, increasing in the order He:H_2:N_2:N_2O, and that in ternary gas mixtures (e.g., He-N_2-O_2 or He-H_2-O_2) the convulsion threshold pressure is roughly linearly related to the concentration of the narcotically effective component of such mixtures (9). Although at first these relations seemed to fit

the picture suggested by studies of the phenomenon of pressure reversal of anesthesia [i.e., of a direct antagonism at the molecular level between the action of inert gas narcotics and that of hydrostatic pressure on lipophilic tissue components, such as excitable membranes, cf. (10)], in the course of time more extensive and more quantitative data have cast some doubt on such direct application of molecular hypotheses to the description of the events in the intact animal. Two observations in particular are relevant in the present context: a) In several animal models the linear relation between hydrostatic pressure and partial pressure of the narcotically active atmosphere constituent predicted by the molecular hypotheses was not observed, cf. (11). b) The time course of injection of the narcotically active atmosphere component during a particular compression using a ternary gas mixture markedly affects the pressure at which HPNS convulsions (as well as other HPNS symptoms) would eventually make their appearance (12, 13).

Given the diversity of the components making up the HPNS, and that relative susceptibilities to pressure and to inert gas narcotic effects vary widely from one of these to the next [cf. (14)], it was tempting to consider whether the basis for such deviations from predictions deduced from the simple molecular model of HP-IG interaction might have to be sought in some form of interaction between different HPNS components. In that context, the compression rate effect is particularly attractive because it is known to represent an interaction effect involved in modifying HPNS seizure susceptibility.

INERT GAS NARCOSIS AND THE COMPRESSION RATE EFFECT

As shown above, the hypothesis of a dual mechanism behind development of HPNS type I seizures in the CD-1 mouse leads to the prediction that uncomplicated effects of inert gas narcosis on this phenomenon are most likely to be observed under conditions of very rapid compression. Conversely, if there are any effects of IG narcosis on the compression rate effect, these should become manifest when the compression rate is slowed progressively. Experience bears out both of these predictions: Convulsion threshold pressures during very rapid compression do increase linearly with the partial pressure of the narcotically active atmosphere component, Fig. 1, $^{1000}P_c$. Moreover, even when the compression rate is slowed greatly, provided the effect of inert gas narcosis on the compression rate is evaded as far as possible by injecting the narcotic only late during the course of the compression, convulsion threshold pressure again proves to be a linear function of the partial pressure of the narcotic, and the slope of the resulting dose-response line is parallel to that observed during very rapid compression experiments, Fig. 1, $^{60}P_c$(late).

On the other hand, if the narcotic in sufficient concentration is injected at the beginning of compression, the convulsion threshold pressures finally attained are substantially lower than when the same amounts of narcotic are introduced into the chambers only near the end of the compression procedure, Fig. 1, $^{60}P_c$(early). Indeed, when the compression rate is 60 ATA/h, convulsion

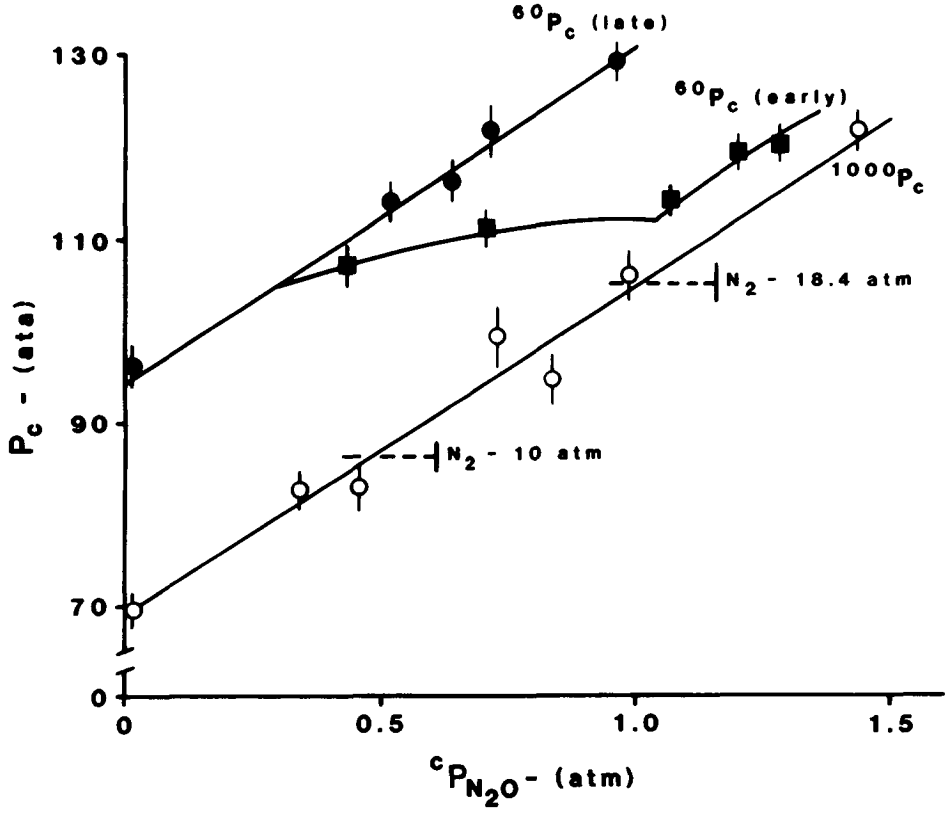

Fig. 1. Variation of the threshold pressure of CD-1 mice for HPNS type I seizures (P_c) as a function of the partial pressure of N_2O at the time of onset of seizures. Test conditions: $^{1000}P_c$-compression at 1000 ATA/h; $^{60}P_c$-compression at 60 ATA/h; "early" and "late" refer to the time of N_2O injection.

threshold pressures in the presence of 0.6 atm N_2O or more are only slightly greater than when the compression rate is 1000 ATA/h in the presence of the same amount of N_2O. By lengthening the compression time even more it can be shown that the scope of the effect of inert gas on the compression rate effect is limited, and that concentrations of narcotic beyond the level producing this maximal effect cannot eliminate a residual compression rate effect, which becomes increasingly important as compression time is lengthened (Fig. 2). When this narcosis-resistant component reaches its limiting magnitude, it accounts for about 50% of the total compression rate effect. Thus, the latter is seen to be compounded of two moieties; one subject to blockade by inert gas narcotics, and the other resistant to such agents. From the time relations

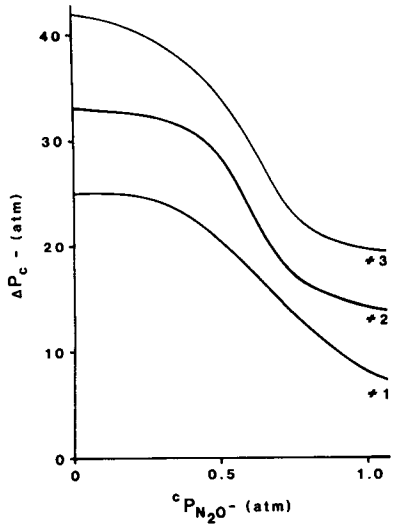

Fig. 2. Variation of compression rate effect on P_c, i.e., (P_c in presence of N_2O)-($^{1000}P_c$ in presence of same amounts of N_2O), with partial pressure of N_2O under different compression conditions: 1. = $^{60}P_c$ - as in Fig. 1; 2. = P_c (2.7) -interrupted compression - 2 h at 70 ATA; 3. = P_c (4.70) - interrupted compression but 4 h at 2.7 ATA. In all cases, N_2O was injected at the start of compression.

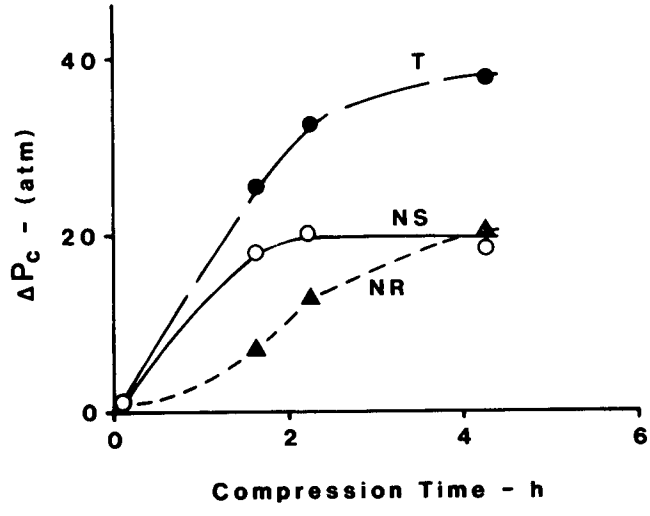

Fig. 3. Time course of development of total compression rate effect (T), and of its narcosis sensitive (NS) and narcosis insensitive (NR) components.

of Fig. 2, a diagram can be constructed representing the time course of development of these two moieties, and showing that during the early course of compression it is the narcosis-susceptible fraction that dominates the development of the compression rate effect (Fig. 3), whereas about 90 min further increase of the compression rate effect essentially reflects only growth of the narcosis-resistant component.

PRESSURE CONDITIONING AND THE EFFECT OF NARCOSIS

Multiday exposures to high pressure have long been suspected of inducing some remission of HPNS symptoms in man, reflecting a degree of acclimation or accommodation to high pressure. This effect has been explored recently using the seizure end point in the CD-1 mouse: Exposure to subconvulsant pressures for 1 d or more results in elevation of the seizure threshold as tested at moderate compression rate (i.e., $^{60}P_c$). The effect raises $^{60}P_c$ up to but hardly beyond the limiting value of P_c inferred from the earlier acute experiments (15) [cf. (2)] (Fig. 4). The same treatment increases the convulsion threshold observed in very rapid compression (i.e., $^{1000}P_c$). Indeed, this latter effect is so great that the difference between P_c at moderate and at rapid compression disappears, i.e., the compression rate effect vanishes (Fig. 4). These effects of prolonged pressure exposure, referred to as pressure conditioning, can, like the compression rate effect, be blocked by reserpine (15). The resemblances with respect to time course, maximum attainable value, and pharmacologic behavior suggest that the same response mechanism underlies both the compression rate effect and pressure conditioning. In the latter situation one must assume that prolonged pressure exposure results in maximum activation of the monoamine mechanism, so that during the course of the test compressions no further development of this effect can be expected; hence maximal values of P_c will be observed regardless of the compression rate used.

If compression rate effect and pressure conditioning are indeed two manifestations of the same underlying mechanism, then pressure conditioning should also be susceptible to the effect of inert gas narcotics. Experiment again bears out this expectation: In the presence of appropriate amounts of N_2 or N_2O the pressure conditioning effect on P_c, under moderate as well as very rapid compression, is blocked completely and the observed seizure threshold pressures are indistinguishable from those in the nontreated control mice (Fig. 5).

This result is more dramatic than could have been expected in view of the demonstration, in the preceding section, of a sizable fraction of the total compression rate effect that is resistant to the action of inert gas narcotics. To reconcile these two sets of results, we can hypothesize that such changes as would correspond to the IG-resistant component of the compression rate effect occurring during the early part of the pressure exposure might be reversible in the presence of inert gas narcotics during the latter part of conditioning exposures.

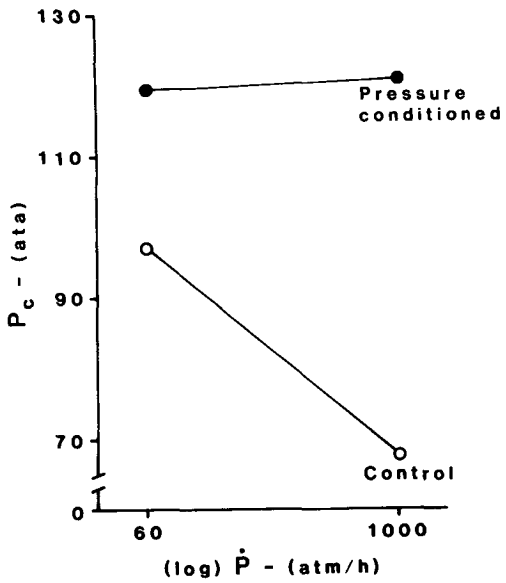

Fig. 4. Effect of pressure conditioning (2 d at 80 ATA in He-O^2 - (P_c) on dependence of P_c on compression rate (P). (Control was untreated mice compressed at the corresponding P.)

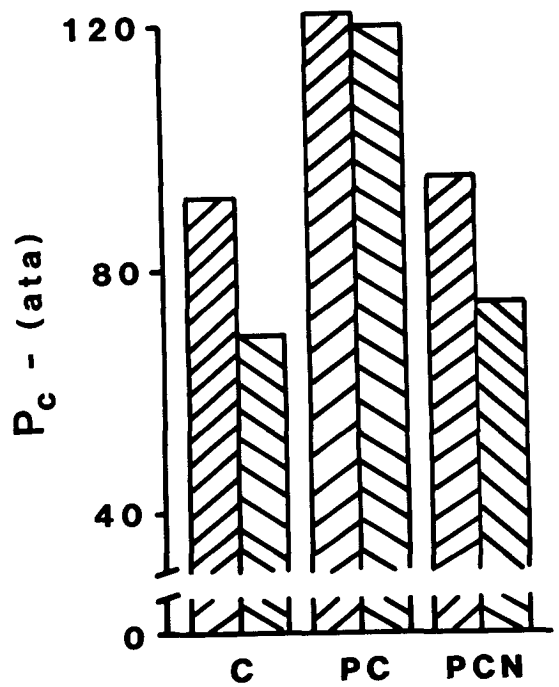

Fig. 5. Suppression of pressure conditioning effect on $^{60}P_c$ (□) and $^{1000}P_c$ (□) in the presence of N_2 in the conditioning atmosphere [18 ATA N_2, total pressure 80 ATA, conditioning time 2 d - (P_{cN})] compared to same parameters after conditioning in He-O_2 (P_c).

This hypothesis is attractive: Pressure conditioning has been shown to be reversible when pressure stress is removed, because in animals returned to 1 ATA after pressure conditioning there is rapid dissipation of the increased pressure tolerance. Under these conditions, the effect on P_c at 60 ATA/h disappears with a half time of about 7 h; the rate of dissipation of the increase of $^{1000}P_c$ is about 3 times as fast. If the concept of an antagonism between inert gas narcosis and high pressure effects could be extended to this situation, pressure conditioning should be reversible even at high pressure in the presence of adequate concentrations of narcotically active atmosphere constituents. This hypothesis is borne out by experiment: Mice that have been pressure conditioned by 2 d of exposure to heliox at 70 ATA lose their acquired increased pressure tolerances if, while they are still at 70 ATA, 1 atm of N_2O is injected into the chamber atmosphere and they continue to reside at pressure in presence of the narcotic for an additional period of time (Fig. 6) and cf. (16). The rates of dissipation of the increase in P_c due to pressure conditioning under these conditions are much slower than those observed at 1 ATA in air, but here too $^{60}P_c$ decreases far more slowly than $^{1000}P_c$, so that the compression rate effect temporarily increases by more than 50% over that in control animals. The slow rate of reversal observed under the conditions described implies that the partial pressure of narcotic utilized (1 atm N_2O) is substantially less than that required to fully neutralize the hydrostatic pressure to which the animals are exposed (70 ATA). This suggests that the relative "potency" of hydrostatic pressure in this situation is several times as high as that estimated in tests of pressure reversal of anesthesia where 1 atm N_2O is just about neutralized by 68 ATA, cf. (11).

Inasmuch as data of this kind are at hand for N_2 as well as for N_2O it becomes possible to compute relative potencies of hydrostatic pressure of these gases in relation to the production and reversal of pressure conditioning, and to compare the results to those for some other biological responses, cf. (11). Table 1 summarizes some of the relevant results and indicates that while the ratio of potencies of N_2 and N_2O is virtually the same for all the responses illustrated, the relative effect of hydrostatic pressure is much higher for HPNS type I seizure induction and for reversal of pressure conditioning than for blockade of pressure conditioning, for pressure reversal of anesthesia, or for suppression of voluntary running activity in mice.

SUMMARY AND CONCLUSIONS

The major results attained in this study and the conclusions they suggest are:

1. Narcotic gases can indeed block or greatly reduce development of the compression rate effect.

2. Compression rate effect in the CD-1 mouse involves two components of roughly equal importance; one susceptible to blockade by narcotic gases, the other resistant to them.

Table 1
Relative Potencies of N_2, N_2O, and Hydrostatic Pressure with Respect to Various Group A Responses

	Voluntary Running Activity*	Anesthetic Reversal*	Block P Condition†	$^{1000}P_c$†	Reversal of P Condition	HP Bradycardia**	Liposome Fluidity**
Pressure per se	-0.20	-0.20	- 0.22	- 0.59	< - 0.35	- 0.70	- 0.70
N_2	1.00	1.00	1.00	1.00	(1.00)	1.00	1.00
N_2O	1.94	20	22.5	22.5	22	22	26

* From (16); ** from (11); † present data.

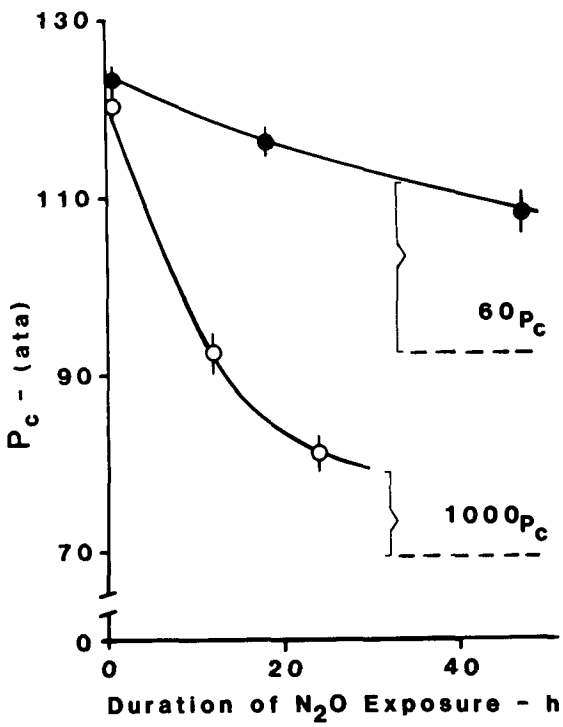

Fig. 6. Reversal of pressure conditioning (2 d, 80 ATA, He-O_2) by injection of 1 atm N_2O at the end of the 2nd d at pressure while holding total pressure constant to 80 ATA. Times shown represent duration of exposure to N_2O before decompression.

3. Like the compression rate effect, pressure conditioning (i.e., change in pressure tolerance as a result of multiday pressure exposure) can be blocked by including narcotically effective gases in the conditioning atmosphere. Unlike the compression rate effect, however, pressure conditioning can be *completely* blocked by such narcotic additions.

4. Pressure conditioning is rapidly reversible on return to 1 ATA, but can also be reversed at high pressure by the addition of a narcotic gas to the heliox atmosphere in which the animals had been pressure acclimated.

5. Under both sets of conditions, the increase in pressure tolerance to modest compression rates ($^{60}P_c$) is dissipated at one-third to one-quarter the rate for tolerance to rapid compression ($^{1000}P_c$).

Taken together, these data shed light on several issues:

- By confirming the substantial interaction effect in connection with the action of inert gas narcotics on HPNS seizures they provide at least a partial explanation for the observed departures from linearity of dose-response curves for this kind of inert gas effect in intact animals.
- By adding further evidence for the similarity of the action of pharmacologic agents on the compression rate effect and on pressure conditioning they strengthen the case for the hypothesis that both of these responses are based on a common mechanism.
- By demonstrating the much more rapid reversibility of the enhanced resistance of pressure conditioning to rapid vs. slow compression, they suggest a differentiation between pressure acclimation effects on available (monoamine) neurotransmitter stores near the synapse, and on transport or synthetic machinery for the same agents.
- The present data complete a matrix begun in an earlier publication (17), which now establishes conclusively the lack of symmetry between inert gas narcotic effects and the effects of high hydrostatic pressures on intact animals. This asymmetry becomes evident only when long-term exposure effects are taken into account. At the present stage, this would require substantial modification of the hypothesis that antagonistic effects of high pressure and of inert gas narcotics are exerted on common targets at the molecular level.

Finally, blocking pressure conditioning may provide an explanation for the exaggerated severity of HPNS symptoms observed when men were switched from a hydrogen-containing to a hydrogen-free atmosphere at a total pressure of 45 ATA after several days' sojourn in He-H_2-O_2 (18).

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DEVELOPMENT OF A NEW AMBIENT PRESSURE DIVING METHOD: EXPERIMENTS ON RHESUS MONKEYS UNDER THE HIGH GAS DENSITY HYPERBARIC ENVIRONMENT

K. Seki, H. Nakayama, M. Matsuda, and M. Hugon

In 1977, COMEX first demonstrated the possibility of open-sea diving up to a depth of 501 m (1). In 1981, Duke University carried out a simulation dive with trimix ($He-N_2-O_2$, N_2 = 4 to 10%) and succeeded, with the 3 divers returning to atmospheric conditions after the exposure at a depth of 686 m (2). Thus, saturation diving using a $He-O_2$ mixture has become a conventional diving method. In 1985, on the other hand, COMEX introduced a new diving method using a H_2-O_2 mixture (3) and showed the underwater-simulated diving at a depth of 450 m would be possible. Despite the evidence accumulated in these experiments, little work has been done to investigate effects of high density breathing gas on mammals.

Since 1977, we have conducted a series of experiments using cats (4-8) to test our hypothesis that physiologic adaptability enables mammals to be exposed to a high gas density hyperbaric environment (>24 h, ρ > 20 g/liter BTPS). The results seem to be a breakthrough in the research of diving technology, demonstrating that cats could survive in a compressed atmosphere of 67 g/liter BTPS (121 bars) without inducing significant signs of narcosis and high pressure neurologic syndrome (HPNS) (Fig. 1), if combinations of inert gases (He, N_2, and Ar) and ambient temperature are carefully controlled. The present study shows the effect of a high gas density hyperbaric environment on rhesus monkeys.

METHOD

The 4 male rhesus monkeys used in the experiments had been raised in the animal care room of the Japanese Marine Science and Technology Center

Fig. 1. Pupil reflex in response to light stimulation and normal breathing (8) was observed during the dive to 1200 m (CNRS-GIS and JAMSTEC 1983). The breathing gas was controlled as follows: ρ = 67 g/liter BTPS, PHe = 72.8 bar, PN_2 = 48 bar, and PO_2 = 0.2 bar. Unfortunately, this cat died of cold stress after 20 h 30 min exposure where ambient temperature was 33.4 ° C at 1200 m.

$$Cp \cdot \rho = 0.000021\, e^{0.45Ta} \quad (r^2 = 0.99)$$

Cp·ρ: heat capacity
Ta: ambient temperature

Based on the least square method to find the best fit curve, the heat capacity can be defined as an exponential function of ambient temperature and thus optimal ambient temperature for cats exposed to a 1200 m depth was estimated to be near 35.5 ° C

(JAMSTEC). They were housed in an individual cage set inside of the specific pathogen-free (SPF) barrier system and acclimated to the controlled environment where ambient temperature and relative humidity were maintained at 25 ± 0.5°C and 55 ± 5%, respectively. Their weight ranged between 5.6 and 8.6 kg and hence they were estimated to be 4 to 6 yr old. One month before each experiment, the selected monkeys were anesthetized with ketamine hydrochloride and electrodes for EEG, electrooculogram (EOG), electromyogram (EMG), and ECG and a sensor intracerebral temperature was implanted simultaneously.

Various physiologic variables were continuously recorded throughout the experiments (9). To avoid damage to the recording cables by the 2 monkeys, the electrode cable was cased in a metal pipe and slip ring (Airflyte Electronic Co.,) and protected with a wire net (Fig. 2). The rhesus monkeys were exposed to He-N_2-O_2 mixture at a depth of 51 bar (ρ = 27 g/liter BTPS). Ambient temperature was maintained at 34°C as shown in Table 1.

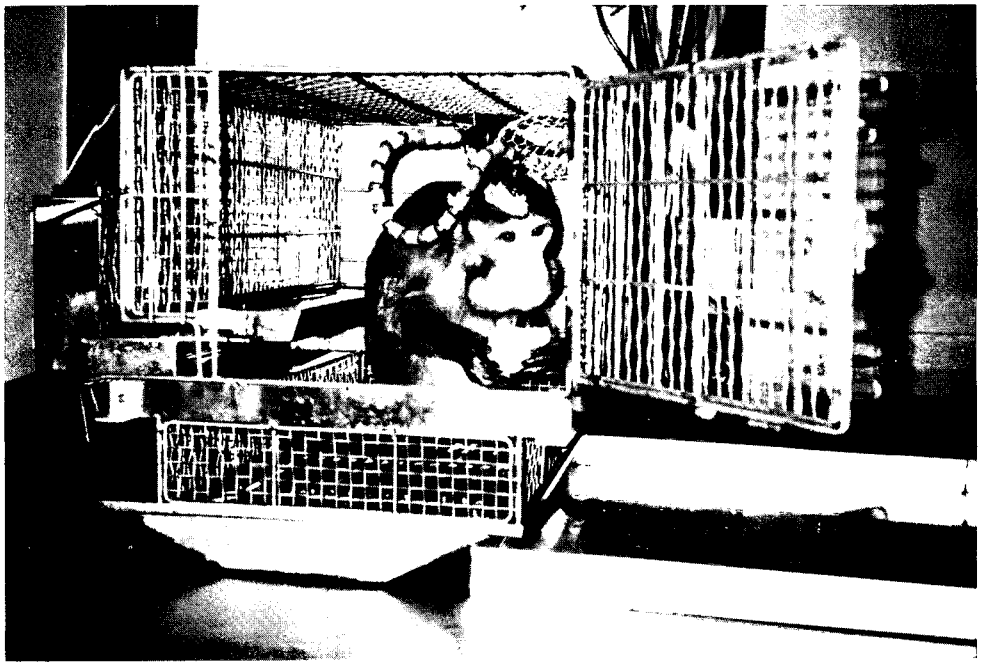

Fig. 2. A rhesus monkey inside the hyperbaric chamber cage, and a set-up of electrode cable and slip-ring, with protection. The protection system, which allowed the monkey to move freely (free-run system) was developed by Seki et al. (JAMSTEC).

After 25 to 48 h of observation at 1 bar air, compression at a rate of 12 bar/h was made successively with He introduction from 1 to 31 bar and with N_2 introduction from 31 to 51 bar. Upon compression, pressure was kept at 51 bar for 48 h (GENIUS II, 1984) (Fig. 3), 72 h (GENIUS III, 1984) (Fig. 4), and 96 h (GENIUS V, 1985) (Fig. 5). The controlled ambient gas consisted of 60% He and 40% N_2, and its O_2 partial pressure was kept at 0.21 bar.

Decompression was made for 66 h 45 min (GENIUS V), 69 h 15 min (GENIUS II), and 74 h 47 min (GENIUS III). During decompression, Po_2 value was kept at 0.8 bar, with the exception of GENIUS V. In that case, Po_2 was maintained at 0.21 bar during decompression from 51 to 31 bar, and again brought up to 0.8 bar after pressure dropped to 31 bar.

TABLE I PHYSICAL PARAMETERS IN HYPERBARIC ENVIRONMENT

JAMSTEC & CNRS-GIS 1981 - 1985

EXPERIMENTS	subjects	P (bar)	PO2 (bar)	PN2 (bar)	PHe (bar)	FO2	FN2	FHe	Ta (°C)	ρ	Cp	Cp.ρ
CONTROL (AIR)	cat & rhesus monky	1	0.21	0.79	0.00	0.21	0.79	0.000	25	1.1	1.01	1.11
ULYSSE-24&25 JAMSTEC	cat	51	0.21	20.0	30.79	0.004	0.392	0.604	34	27	3.51	94.77
GENIUS-II,III&V JAMSTEC	rhesus monkey	51	0.21	20.0	30.79	0.004	0.392	0.604	34	27	3.51	94.77
PACHA-31 CNRS-GIS	cat	101	0.21	40.0	60.79	0.002	0.396	0.602	(35) 33.4	55	3.52	193.6
PACHA-32 CNRS-GIS	cat	121	0.21	48.4	72.39	0.0017	0.40	0.5983	(35) 33.4	67	3.52	235.84
(1988)	cat	201	0.21	80.0	120.79	0.001	0.399	0.600	(37)	110	3.52	387.2
(1990)	cat	501	0.21	200	300.79	0.0004	0.399	0.6006	(37)	275	3.52	(H_2O: 4180) 968.00

( ) : project
P : ambient pressure
F : fractional concentration
ρ : density (g/l BTPS)
Cp : specific heat(J/g°C)
Cp.ρ: heat capacity (J/l°C)

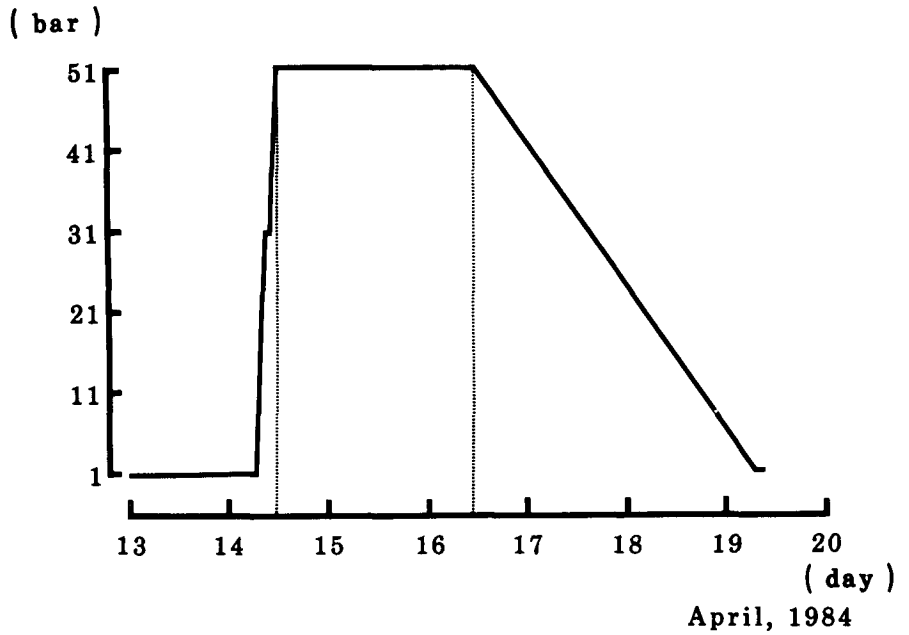

Fig. 3. Dive profile of GENIUS II (He-N_2-O_2, 51 bar, JAMSTEC, 1984). From 12 to 14 April: 43 h confinement under 1 bar air. Compression was made on 14 April:

1-31 bar, 120 m/h (with He)
hold in 31 bar for 30 min to change inert gas.
31-51 bar, 120 m/h (with N_2).
Stay at bottom for 48 h 20 min.
Decompression:
51.0-50.0 bar, 10.0 m/h
50.0-33.9 bar, 8.57 m/h
33.9-18.1 bar, 7.50 m/h
18.1- 2.0 bar, 6.67 m/h
2.0- 1.5 bar, 6.0 m/h
1.5- 1.0 bar, 5.46 m/h

RESULT

The 4 monkeys successfully survived the exposure to the high gas density hyperbaric environment. As visually observed, the behavior activity of all the monkeys seemed to decrease extremely on the 1st d at 51 bars, but on the 2nd d they returned to the normal state observed during predive. In spite of the changes in behavior, all the monkeys drank water, ate food, defecated, and urinated normally.

Heart frequency recorded by polysomnography during sleep and awake

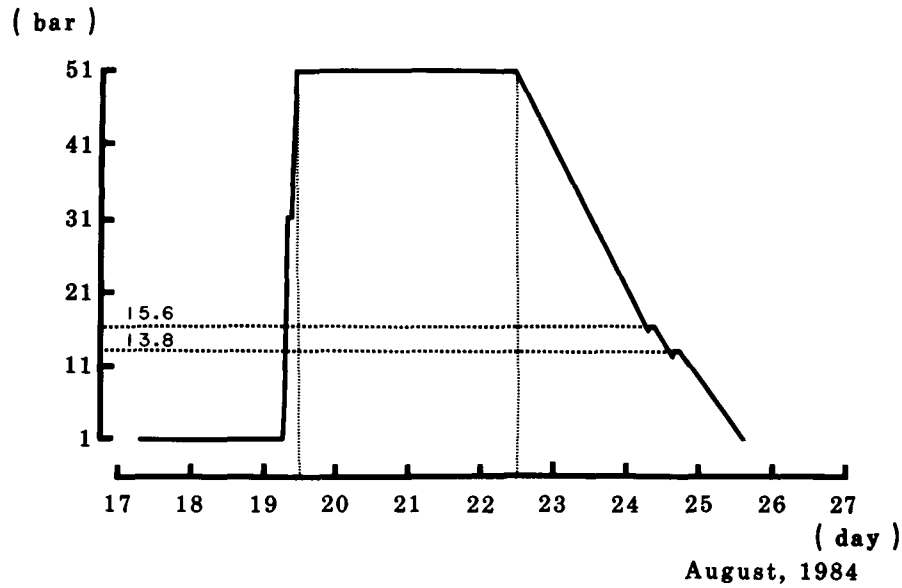

Fig. 4. Dive profile of GENIUS III (He-N_2-O_2, 51 bar, JAMSTEC, 1984). From 17 to 19 August: 48 h confinement under 1 bar air. Compression was made on 19 August:

1-31 bar, 120 m/h (with He)

hold at 31 bar for 30 min to change inert gas.

31-51 bar, 120 m/h (with N_2).

Stay at bottom for 72 h.

Decompression:

51.0-50.0 bar, 10.00 m/h

50.0-33.9 bar, 8.57 m/h

33.9-18.1 bar, 7.50 m/h

18.1- 2.0 bar, 6.67 m/h

2.0- 1.5 bar, 6.00 m/h

1.5- 1.0 bar, 5.46 m/h

Recompression:

14.7-15.6 bar at 08:21 on 24 August.

12.9-13.8 bar at 10:12 on 24 August.

stages showed that tachycardia occurred at 51 bars more frequently than at 1 bar (Fig. 6); respiratory frequency indicated the same tendency to increase significantly at 51 bars (Fig. 7). As to narcotic effects induced by the N_2 partial pressure of 20 bars, monkeys were affected less than cats. Because the recording electrode cable was damaged at times during each experiment, completely continuous observations on their sleep stages could not be obtained. However, according to the definition of sleep stages (9), all the monkeys registered slow wave sleep (SWS) and paradoxical sleep (PS) at 51 bars.

Body weight before and after each experiment showed a 4 to 11% decrease in 5 cases out of 6; 1 case (GENIUS V) resulted in an increase of 6%.

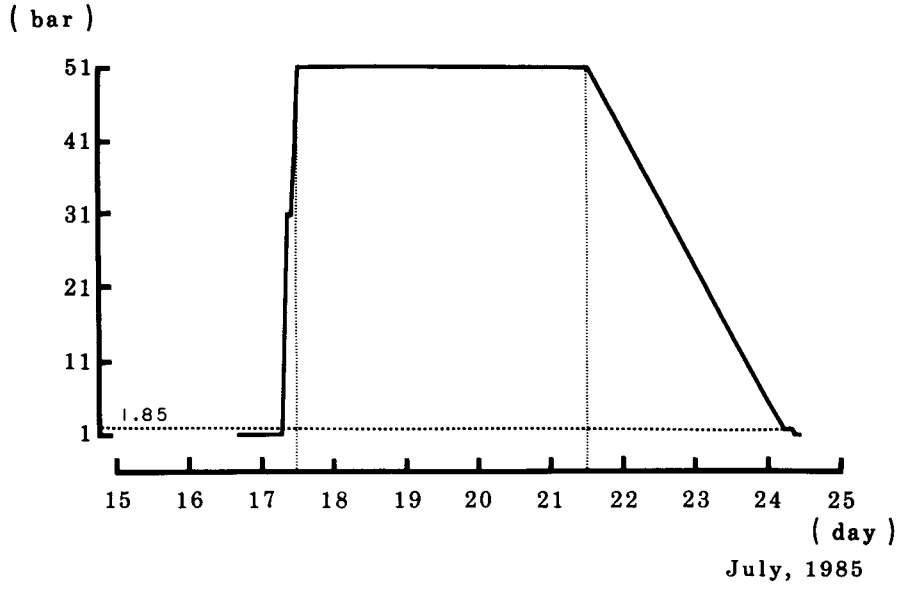

Fig. 5. Dive profile of GENIUS V (He-N_2-O_2, 51 bar, JAMSTEC, 1985). From 16 to 17 August: 25 h confinement under 1 bar air. The compression was made on 17 July:

1-31 bar, 120 m/h (with He)

hold at 31 bar for 30 min to change inert gas.

31-51 bar, 120 m/h (with N_2).

Stay at bottom for 96 h.

Decompression:

51.0-50.0 bar, 10.00 m/h

50.0-33.9 bar, 8.57 m/h

33.9-18.1 bar, 7.50 m/h

18.1- 2.0 bar, 6.67 m/h

2.0- 1.5 bar, 6.00 m/h

1.5- 1.0 bar, 5.46 m/h

Recompression:

1.33-1.85 bar at 06:25 on 24 July.

During decompression, 1 monkey showed signs of decompression sickness at 5 m, but after recompression treatment he was brought back to the surface safely. After their return to the 1 atmospheric pressure environment, no monkey showed any signs of decompression sickness.

DISCUSSION

Since HPNS was described in numerous studies (1, 2, 10), addition of N_2 has been suggested to prevent HPNS during deep dives with He-O_2 mixtures

because of the antagonism of narcotic agents and pressure. In addition, the complicated mechanism of HPNS and associated disorders promoted further studies to find the method of compression (1, 2, 10). Results from those studies made deep dives with trimix practical and safe. It was however believed that density was a limiting factor (11-13), and that no animals could tolerate

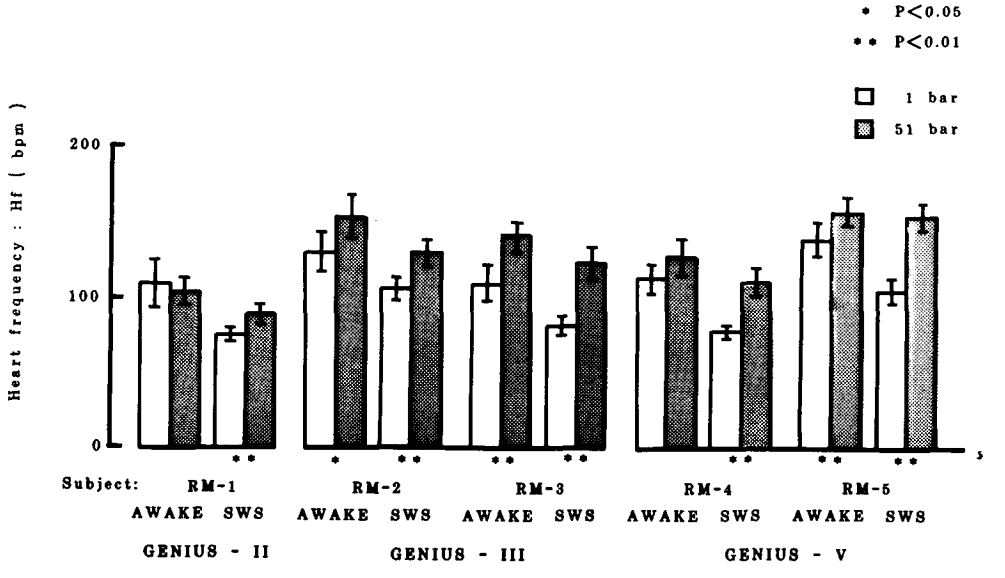

Fig. 6. Heart frequency during SWS and awake stages at 1 bar air and 51 bar He-N_2-O_2.

prolonged exposure (> 24 h) to hyperbaric high gas density mixtures in which density exceeded 20 g/liter BTPS.

In an attempt to develop the best possible procedure for high gas density diving, we have studied extensively effects of high density gases on cats (3-7). Considering all the physiologic and psychophysiologic factors involved in HPNS and N_2-narcosis and those outlined by Lambertsen (14) and Zhao et al. (15), this new method, called high gas density hyperbaric environment diving (HGDHED), has been proposed (8, 16, 17).

The present study demonstrates the effects of high density breathing gases on rhesus monkeys, and confirms that density is not a limiting factor because helium counteracts the narcosis. Notable adaptability to hyperbaric high density gases has been observed. The animals endured the decompression resulting from changes in pressure during decompression and returned to the surface safely. Thus, HGDHED may improve current deep diving technology.

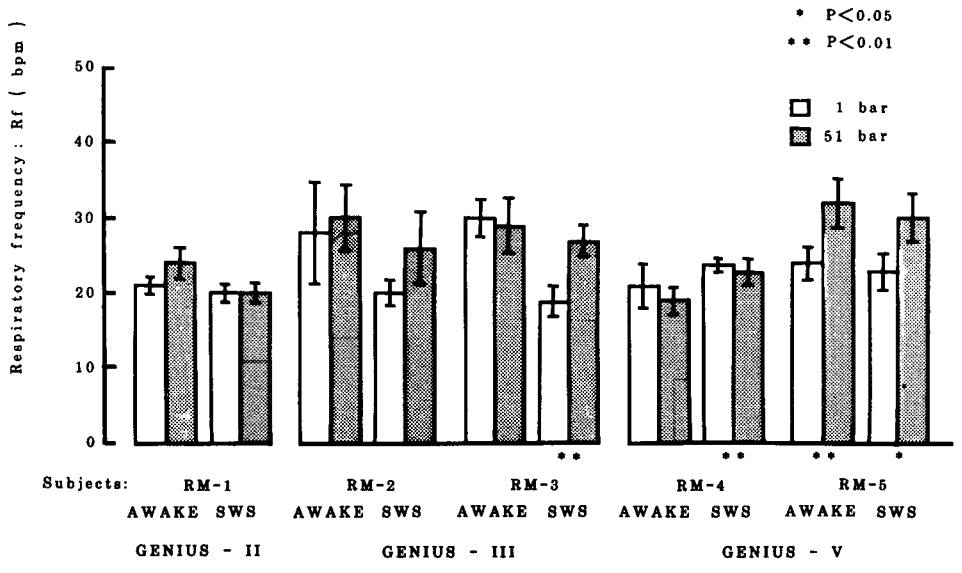

Fig. 7. Respiratory frequency during SWS and awake stages at 1 bar air and 51 bar He-N_2-O_2.

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GAS-PHASE DIFFUSION OF O_2 IN HELIUM AND NITROGEN UNDER PRESSURE

C. V. Paganelli

It is a well-known physical principle that increased ambient pressure reduces the rate of diffusion in the gas phase. Gas-phase diffusion plays a role in exchange of O_2 and CO_2 in the terminal portions of the alveoli of the lung. The phenomenon of stratified inhomogeneity has been attributed in part to possible gradients of O_2 and CO_2 partial pressures in the alveoli and has served to focus interest in recent years on modeling gas-phase diffusion processes in the alveoli. Gas mixing and diffusion in the lung have been reviewed extensively by Scheid and Piiper (1) and in several chapters of the monograph edited by Engel and Paiva (2). The influence of high ambient gas pressure on pulmonary diffusive gas mixing and gas exchange has been treated experimentally and theoretically in recent studies by Ohta and Kodaka (3), Van Liew et al. (4, 5), and Hlastala et al. (6). Such studies require knowledge of the pressure dependence of gas-phase diffusion coefficients for calculation and interpretation of measurements.

It is generally assumed that gas-phase diffusivity (D) varies inversely with absolute pressure (P) or gas density, at least at moderate pressures. Put another way, the product D•P should remain invariant as P changes, as predicted from kinetic theory and the Chapman-Enskog equation. However, as Reid et al. (7) point out, most diffusion experiments carried out at elevated pressures have been limited to measurement of self-diffusion coefficients, which tend to confirm the constancy of the D•P product. These authors further remark that few data are available for binary diffusion at pressure; some binary systems display a quite different dependence of diffusion coefficients on pressure. For example in methane-argon mixtures, D•P decreases substantially in the pressure range 1 to 100 ATA (100-10,000 kPa). Reid et al. (7) conclude that "It is not clear how one may best estimate the binary

diffusion coefficient at high pressures." In the absence of a universally reliable method of prediction it becomes necessary to test the inverse relation between D and P experimentally for the gas mixtures one wishes to use. The present studies were designed to investigate how pressure affects diffusivity of $He-O_2$ and N_2-O_2 in the range of 7 to 90 ATA (700-9000 kPa), a region in which human diving activities are carried out.

MATERIALS AND METHODS

A modification of the classical method of Loschmidt (8) described by Wilson (9) was used to measure diffusion coefficients at pressure in binary mixtures of $He-O_2$ and N_2-O_2. Worth et al. (10) used a similar technique to measure diffusivities at 1 atmosphere, and preliminary data for $He-O_2$ obtained from this method were recently reported (11). The apparatus is shown schematically in Fig. 1. Our Loschmidt tube was a long, straight cylinder made up of six stainless steel segments of equal length, jointed together by ball valves whose orifices are matched to the tube's inner diameter. The overall length of the tube including valves was 0.805 m; its inner diameter was 4.7 mm. The large ratio of length to diameter is intended to minimize effects of radial diffusion and convection. The tube was suspended vertically in a well-stirred, constant-temperature bath at 37°C and carefully shielded from vibration. To ensure that no significant temperature gradients were present in the water bath, temperatures at several points along the diffusion tube were measured by copper-constantin thermocouples calibrated against an NBS-certified Hg thermometer. The temperature did not vary more than 0.06°C over the entire length of the tube. A filling manifold with suitable pressure gauges and vacuum pump permitted filling and sampling of each of the six segments of the tube without contamination from residual gas from other segments or from the dead space in the filling manifold.

With the centrally placed valve closed, pure O_2 at the desired pressure was introduced into the lower half of the tube and either pure N_2 or He at the same pressure, into the upper half. The denser gas (O_2) was always placed in the lower half of the tube to prevent convective mixing. The two halves of the tube were connected through the manifold to a common remote point to ensure that pressure equilibration was achieved. To begin an experiment, the central valve was opened and the gases were allowed to mix by diffusion for a timed interval, usually of the order of several hours. Experiments at the highest pressures employed (ca. 90 ATA) required on the order of 7 to 8 h of diffusion time. At the end of the interval, all valves were closed, partitioning the tube into six segments of equal volume. A further time was allowed for each segment to approach equilibrium, and then each segment was sampled by permitting the pressurized contents to expand into a gas-tight syringe. Duplicate or triplicate samples were taken from each segment and analyzed either by mass spectrometry or gas chromatography. The average gas composition of each segment was considered to be that at the midpoint of the

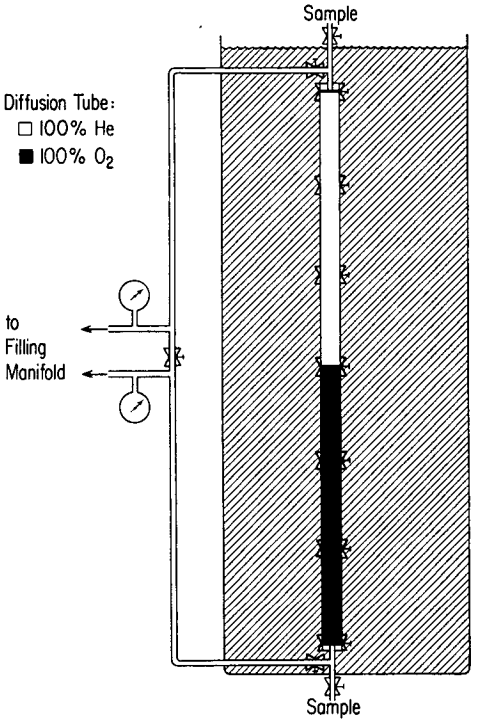

Fig. 1. Schematic drawing of diffusion tube in thermostatted water bath. Seven ball valves partition the tube into six segments. Gas samples are withdrawn sequentially from each segment at both top and bottom of tube. See text for details.

segment for purposes of plotting concentration profiles in the diffusion tube. Thus six points were obtained in the concentration profile for each diffusion experiment. A representative experiment showing diffusion of O_2 into He for 7 h at 90 ATA is shown in Fig. 2.

To analyze the results of diffusion experiments, two forms of Fick's second law were employed. The first was that for one-dimensional diffusion in a closed system for two gas columns of equal length, given by Jacobs (12):

$$u = \frac{u_0}{2} + \frac{2u_0}{\pi}\left(\cos\frac{\pi x}{H}\, e^{-\frac{\pi^2 D t}{H^2}} - \frac{1}{3}\cos\frac{3\pi x}{H}\, e^{-\frac{9\pi^2 D t}{H^2}} + \ldots\right)$$

where u= fractional concentration of O_2 at any point x in the diffusion tube;
u_o = initial fractional concentration of O_2 (= 1.00) in the lower half of the diffusion tube;
x = distance coordinate along with length of the diffusion tube, cm;
H= length of the diffusion tube, cm;
D= binary diffusion coefficient of the gas pair used in the experiment (either He-O_2 or N_2-O_2), $cm^2 \cdot s^{-1}$; and
t = time, s.

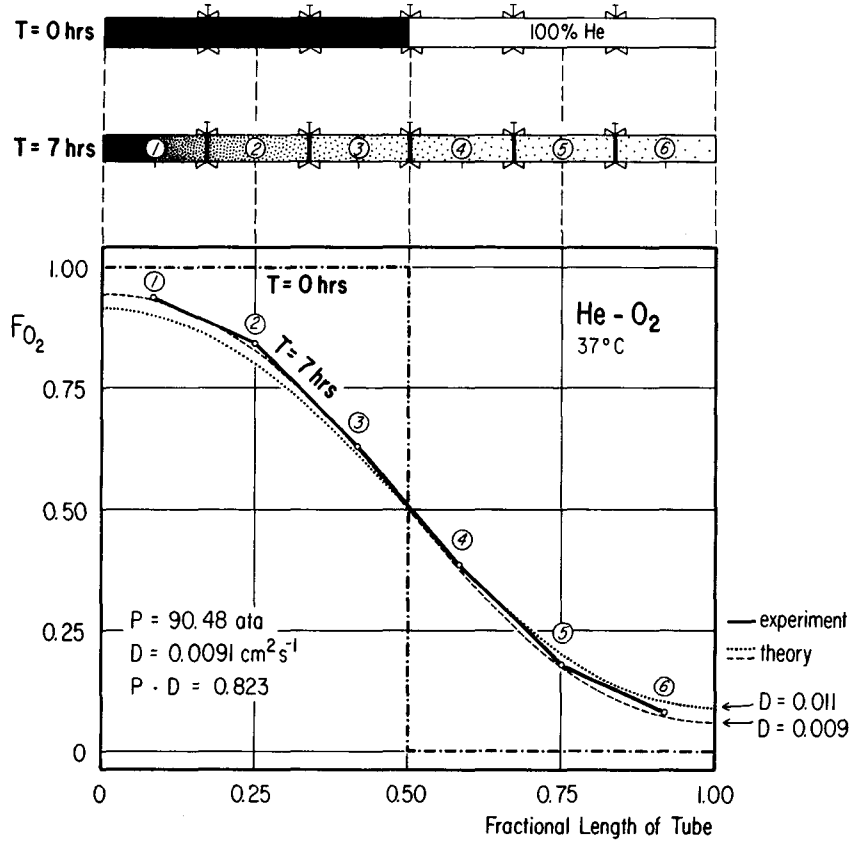

Fig. 2. Theoretical and experimental O_2 profiles in diffusion tube at 90 ATA after 7 h. Theoretical profiles were calculated from Eq. *1* with the assumed values of D shown. See text for details.

Equation *1* was used to calculate theoretical concentration profiles of O_2 along the length of the diffusion tube from assumed values of D for a particular binary gas mixture and pressure. The *dotted* and *dashed* lines in Fig. 2 show two such theoretical profiles for He-O_2 at 90 atm. In Fig. 2 the diffusion tube is shown schematically at the top of the figure at the beginning of the experiment (t = 0 h) and at the end (t = 7 h). The abscissa is given in units of x/H, or fractional length of the diffusion tube, and the ordinate, labeled Fo_2, is fractional O_2 concentration at any point (u/u_o in Eq. *1*). The concentration profile at t = 0 is the square wave shown by the *dotted-and-dashed* line. The *circles* 1 through 6 indicate experimentally determined values of Fo_2 in each of the six segments of the diffusion tube. The fit between experimental and theoretical concentration profiles was in most cases reason-

ably good and provided a qualitative check on our experimental procedures.

An integrated form of Eq. *1*, also given by Jacobs (12), was used to calculate values of D at pressure from the measured amount of O_2 present in the lower half of the diffusion tube and the amount transferred to the upper half by the end of the experimental time interval:

$$\frac{Q_1 - Q_2}{Q_1 + Q_2} = \frac{8}{\pi^2}\left(e^{-\frac{\pi^2 D t}{H^2}} + \frac{1}{9}e^{-\frac{9\pi^2 D t}{H^2}} + \ldots\right) \qquad (2)$$

where Q_1 = total quantity of O_2 in the bottom half of the diffusion tube at time t; and

Q_2 = total quantity of O_2 in the top half of the diffusion tube at time t.

The remaining terms in Eq. *2* are defined as for Eq. *1*. Thus $Q_1 + Q_2$ is the total quantity of O_2 present in the system at any time, and $Q_1 - Q_2$ is the amount transferred from one-half of the diffusion tube to the other during the diffusion process. Equation *2* was solved for D by iteration from measured values of Q_1, Q_2, H and t.

RESULTS AND DISCUSSION

Experimental results for the measured diffusivities of O_2 in He or N_2 at 37°C at pressures ranging from about 7 to 90 ATA are given in Table 1. The values of D in Table 1 at any given pressure are the means of between 3 and 11 determinations; most commonly, 4 to 5 measurements of D were made at a single pressure in both He-O_2 and N_2-O_2. The D•P product for both gas mixtures remains reasonably constant in the pressure range tested, a fact that is more easily seen in Figs. 3 and 4, in which D•P is plotted against P. Linear regression analysis of the data for both gas mixtures showed that the slope of such plots did not differ significantly from zero. (For He-O_2, $t = 1.22$, $P > 0.2$, $n = 63$; for N_2-O_2, $t = -0.05$, $P > 0.96$, $n = 40$). When the regression lines in Figs. 3 and 4 are extrapolated to 1 ATA, the values of D for He-O_2 and N_2-O_2 are 0.814 and 0.250 $cm^{-1} \cdot s^{-1}$, respectively, in reasonable agreement with those taken from the literature and shown in Table 1.

The results of our experiments confirm within experimental error the prediction of Chapman-Enskog theory that binary diffusivity in both He-O_2 and N_2-O_2 varies inversely with absolute pressure in the range of pressures tested, which corresponds to that encountered in human saturation diving.

Preliminary experiments in a ternary mixture containing O_2, N_2, and He show that the rate of O_2 diffusion is also reduced as pressure is increased, but elucidation of the quantitative relation between pressure and diffusion is this ternary system awaits further experimentation.

Table 1
The Effect of Pressure on Binary Diffusion Coefficients in $He-O_2$ and N_2-O_2 at 37 ° C*

	$He-O_2$ at 37 ° C			N_2-O_2 at 37 ° C	
P, ATA	D, cm^2/s	D•P	P, ATA	D, cm^2/s	D•P
1.00	(0.782)	(0.782)	1.00	(0.231)	(0.231)
6.80	0.120	0.817	10.24	0.0243	0.249
10.20	0.0801	0.817	13.62	0.0185	0.252
13.61	0.0596	0.811	20.42	0.0121	0.248
20.41	0.0397	0.810	27.21	0.00909	0.248
34.01	0.0241	0.819	34.01	0.00718	0.244
50.00	0.0163	0.817	40.82	0.00617	0.252
61.22	0.0135	0.827	47.61	0.00511	0.243
68.03	0.0124	0.841	50.00	0.00491	0.246
76.53	0.0107	0.821	61.22	0.00406	0.249
90.48	0.0091	0.820			

* Values of D at each pressure are the means of between 3 and 11 experiments. The values of D in parentheses at 1 ATA were taken from the literature for comparison. For $He-O_2$ see (13-16), and for N_2-O_2 (17-19). Literature values of D measured at temperatures other than 37 ° C were corrected to that temperature using the ratio of absolute temperatures raised to the 1.5 power.

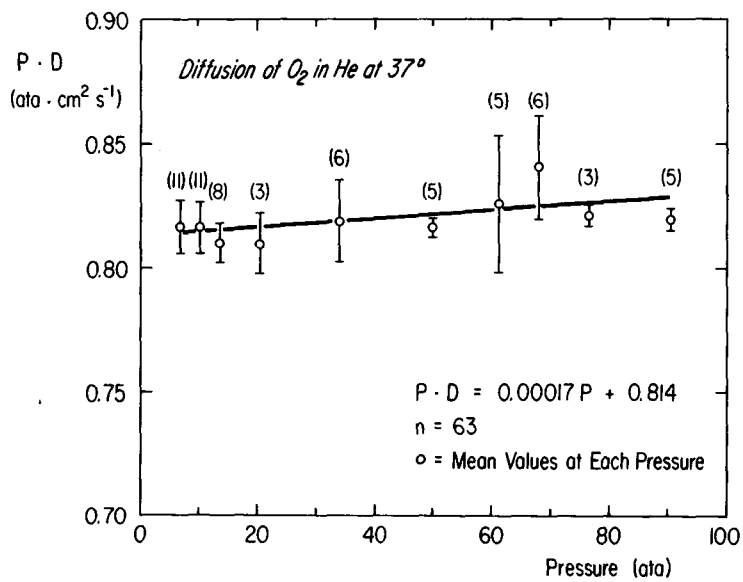

Fig. 3. D·P product for $He-O_2$ vs. P in ATA. n = Number of experiments at each pressure. Error bars represent ± 1 SD; least-squares line is drawn using all 63 points rather than mean values.

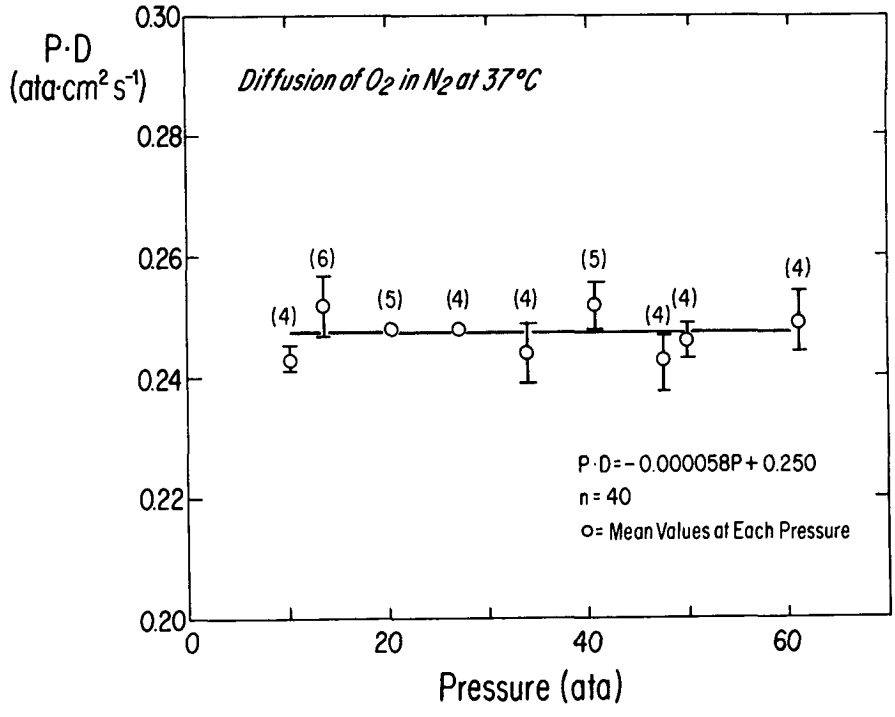

Fig. 4. D·P product for N_2-O_2 vs. P in ATA. n = Number of experiments at each pressure. Error bars represent ± 1 SD; least-squares line is drawn using all 40 points rather than mean values.

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SESSION 7: RESPIRATORY AND CARDIOVASULAR EFFECTS

9th International Symposium on Underwater and Hyperbaric Physiology
Undersea and Hyperbaric Medical Society, Bethesda, MD, 1987

COMPENSATIONS FOR FLOW LIMITATION WHILE BREATHING DENSE GAS

H. D. Van Liew

During very rapid exhalation, airways near the top of the tracheobronchial tree become narrowed at "choke points"; when this "dynamic airway compression" occurs, flow is limited—additional muscle force does not yield greater flow (1). It has been predicted on theoretical grounds that flow through a particular choked airway should be inversely proportional to the square root of gas density (1, 2), and measured maximal expiratory flows can be shown to conform to the prediction (3). Therefore, the flow limitation due to development of choked airways is a risk for persons who breathe dense gas, particularly during exercise (3).

CALCULATIONS

To study possible compensations for flow limitations, simulations were performed, using a microcomputer and a program in Microsoft BASIC, of the time course of respiratory variables, including lung volume, flow, and pressures for overcoming elastic recoil, for causing flow, and for acceleration of gas. A primary assumption was that volume was a sine wave function of time except when flow limitation was occurring. For each lung volume, magnitude of the limiting expiratory flow (F_{lim}) was calculated by assuming that it was a function of volume above residual volume (V), vital capacity (VC), and relative gas density (RGD, density in a particular environment as multiple of density of normal air):

$$F_{lim} = (12/RGD^{0.5})(V/VC). \quad (1)$$

If, in the process of calculating volume as a sine wave function of time,

the time rate of change of volume reached F_{lim}, then the breath's flow was set equal to F_{lim} and the ensuing volume pattern was calculated from the integral of flow.

During nonlimited flow, pressure for flow was calculated from the assumptions that airway conductance a) increases with lung volume according to data of Bouhuys and Jonson (4) and b) is inversely proportional to the square root of RGD (5). During flow limitation, it was assumed that people do not exert excessive pressure over that needed to cause flow through the choked airways and that this minimal pressure is twice the pressure for nonchoked airways.

RESULTS AND DISCUSSION

Figure 1 illustrates the reciprocal-square-root relationship between F_{lim} and gas density on a conventional plot of maximal expiratory flow vs. lung volume (the MEFV diagram) for three specific environments: the RGD = 1 curve is for a normal air environment, RGD = 6 simulates the air environment at 6 ATA used in the study of Hesser et al. (6), and RGD = 23.5 simulates the crude neon environment used in studies at 37 ATA by Lambertsen et al. (7). The *straight-line* parts were calculated from Eq. *1* and *left-hand* parts were drawn by hand based on data of Hesser et al. (6).

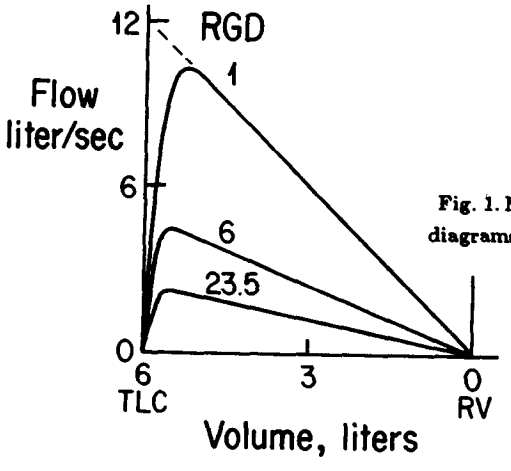

Fig. 1. Maximal expiratory flow vs. lung volume (MEFV diagrams) for three relative gas densities.

Breathing Normal Air

Figure 2 shows simulated variables for tidal volume of 3.1 liter and frequency of 50 breaths/min, the reported breathing pattern for men at RGD =

1 who were exercising at a rate that was 40% above their maximal oxygen consumption (6). The simulated flow is the *solid* trace in the *middle panel.* The *dotted* limiting-flow curve was calculated by Eq. *1*, as was the *straight portion* of the Fig. 1 curves; because conductance of choked airways increases with volume, as seen in Fig. 1, the *dotted* limiting-flow curve in Fig. 2 peaks when lung volume is greatest. The *dashed* trace at the right shows what flow would have been if flow limitation had not occurred; the simulated flow would have exceeded the limiting flow by the magnitude shown by *heavy shading.*

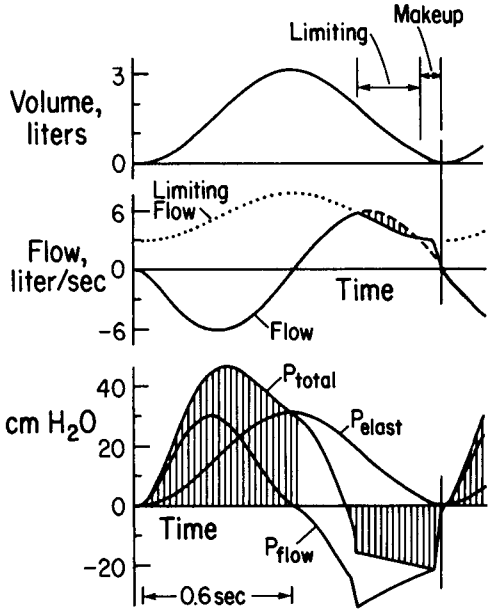

Fig. 2. Volume above resting volume (upper), flow (middle), and pressures (lower) for the tidal-volume/frequency pattern reported for exercising men in a normal environment. Verticals at the right show the beginning of the next breath.

The flow limitation makes the volume trace during expiration less curved than it would have been; it deviates slightly from the sinusoidal pattern that was assumed for nonflow-limited volume vs. time. Two time phases are of interest—the *arrow* labeled "limiting" shows where flow is less than it would have been if the sinusoidal pattern of volume vs. time could have been followed; later, the *arrow* labeled "makeup" shows where flow is greater than it would have been. The relatively high flow in the makeup phase allowed the volume to reach its preinspiratory value at the same time as it would if the flow had not been limited; the next breath begins at the same time as if flow had not been limited.

At any time, total pressure (*lower panel*, Fig. 2) is the sum of pressure for overcoming elastic recoil of the respiratory system, for causing air flow,

and for accelerating and decelerating gas. The latter is so small that it would appear as slight thickenings of the zero-pressure baseline in all the figures of this communication; it is considered negligible and is not shown. During flow limitation, flow pressure differs markedly from the approximately sinusoidal nature it had before. The total pressure trace is *shaded* where effort on the part of the subject is required—during all of inspiration and during the last part of expiration. Where the total pressure is not shaded, expiration could be due to elastic recoil.

Air at 6 ATA

Figure 3 shows pressures and the flow-volume diagram for the actual breathing pattern reported for men doing the same very heavy exercise as in Fig. 2, but breathing air at 6 ATA (6). Elastic pressure excursions were less than in Fig. 2 because tidal volume was less. Flow pressure on inspiration was about the same as in Fig. 2 because, although frequency was less, pressure to cause flow in the dense environment was greater. In addition to a markedly reduced total ventilation (77 instead of 149 liters/min), the lung volume before and after the breath was above the resting volume by 18% of the VC. Such an increase of lung volume, which is designated an "enlarged preinspiratory volume," may be a common response during exercise in dense-gas environments (3, 8). The enlarged preinspiratory volume makes it necessary for the person to exert a sizable pressure (10 cm H_2O) at the very beginning of inspiration to overcome elastic recoil. In the MEFV diagram in the *lower panel* it can be seen that if the lung volume had not increased, flow during the midpoint of expiration would have been about 50% lower than that shown.

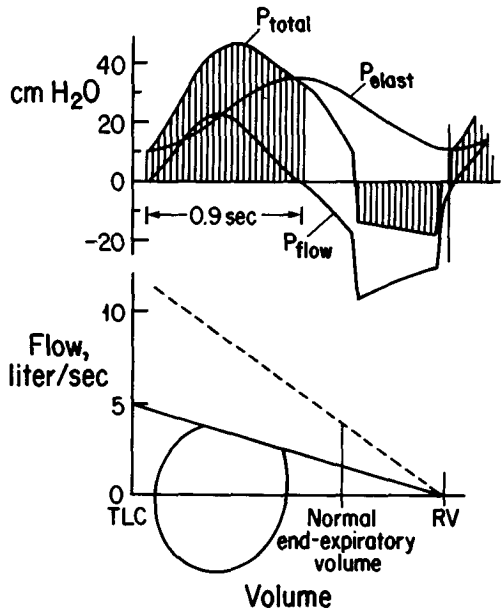

Fig. 3. Simulated results for the frequency/tidal volume pattern observed in the same men doing the same exercise as in Fig. 2 but at RGD = 6. Upper, pressures vs. time; lower, flow vs. volume.

Figure 4 shows simulations of the pressure patterns for two alternatives to the response that was depicted in Fig. 3. The *upper panel* illustrates an impossible case. If the men had not changed their lung volume, the flow limitation would have prolonged expiration. The breath would have continued beyond the *vertical line*, that shows when the breath must end to have the respiratory frequency that was reported.

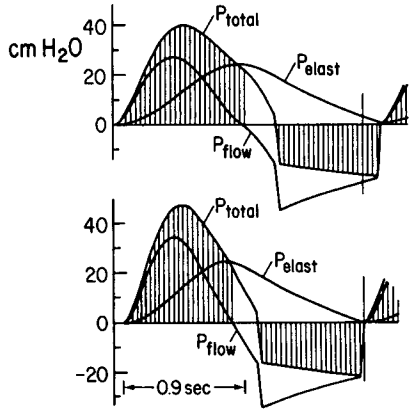

Fig. 4. Pressures vs. time for two alternatives. Upper, no response; lung volume is normal and inspiratory duration is the same as in the Fig. 3 breathing pattern. Lower, lung volume does not increase but inspiratory flow is greater than in the Fig. 3 pattern.

The *lower panel* of Fig. 4 shows the result of shortening the duration of inspiration to provide more time for the limited flow during expiration. A higher inspiratory flow is achieved at the cost of higher inspiratory pressure, and the duration of the breath is not prolonged.

Figure 5 shows another impossible case, a simulation of the result if the men at exercise at RGD = 6 had increased their preinspiratory volume more than shown in Fig. 3; flow limitation occurs only momentarily during expiration. Such a response would have increased the *shaded area* that indicates inspiratory effort even more than in Fig. 3, and caused expiratory effort to be very small. However, the end-inspiratory volume exceeds total lung capacity, as seen in the *lower panel*. Such a large preinspiratory volume would have been possible only if tidal volume had been smaller; if total ventilation was to be maintained, frequency would have to be greater. The higher frequency would have required higher flows, which would have made inspiratory effort greater and would have increased the duration of flow limitation during expiration.

Density 23.5 Times Normal

Figure 6 shows simulation of a tidal volume-frequency pattern that could have occurred in the experiments of Lambertsen et al. (7), in which men worked in an environment of neon and helium with adequate O_2 when RGD equaled 23.5. Total ventilation was reported to be 40 liters/min. Lung volume data were not available in the report; the same preinspiratory volume as in Fig. 4 was used arbitrarily. If lung volume had not been increased, the subjects could have attained the ventilation that was observed only by marked decrease

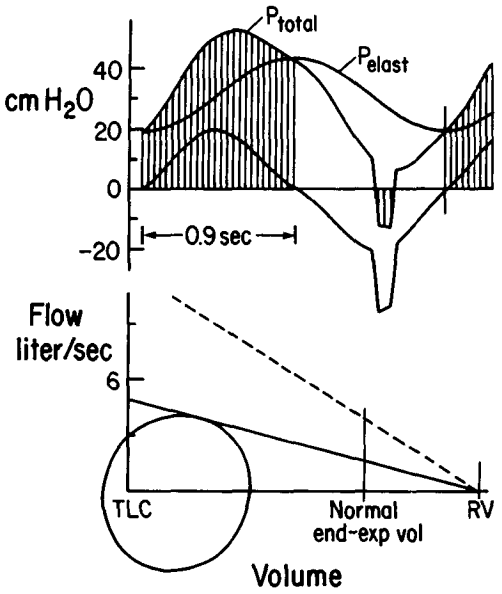

Fig. 5. Pressures vs. time and flow vs. volume for yet another alternative response. Increase of preinspiratory lung volume is almost twice that in Fig. 3.

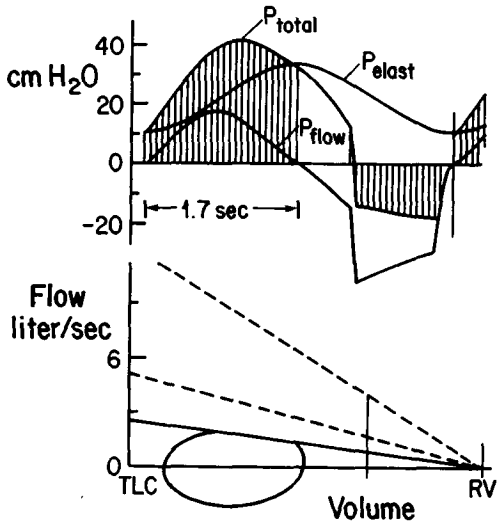

Fig. 6. Simulation of pressures vs. time and flow vs. volume of a possible pattern for the ventilation that was observed when men exercised in an environment in which RGD was 23.5 times normal.

of inspiratory duration to compensate for the time that the expiration would have been flow limited.

Effort

If it can be assumed that the metabolic cost of a muscular activity increases in proportion to the product of force generated and its duration, the *shaded areas* of the figures may serve as crude indicators of the effort

required for the breaths, although pressure is not strictly proportional to muscular force because of the length-tension characteristics of muscle. To compare the *shaded* pressure-time areas of the various figures, note that a given length on the time scale for Figs. 3-5 depicts the same time, but the same length on Fig. 2 depicts two-thirds as long a time and on Fig. 6 depicts almost twice as long a time.

Figure 2 has the smallest effort-related area for inspiration because of its shorter time. Inspiratory *shaded area* is larger in Fig. 3 than in the two patterns in Fig. 4; Fig. 5 has a larger inspiratory area than Fig. 3 and Fig. 6 has the largest of all because of the long duration. It may be that the subjects at RGD = 23.5 were limited by the inspiratory effort they could apply. Figure 5 has the smallest expiratory effort-related area because almost all of expiration is passive. Figure 3 expiratory effort is about half as large as those of Figs. 4 and 6 because the active part lasts only about half as long.

One could ask whether the observed pattern, that is depicted in Fig. 3, has an unequivocal advantage in terms of effort expenditure. The answer seems to be negative—what Fig. 3 pattern gains in low expiratory effort, compared to Fig. 4 patterns, it loses in high inspiratory effort.

CONCLUSION

To achieve the tidal volume and breath duration for an adequate respiratory ventilation, respiratory muscles exert forces in a certain time pattern. The respiratory flow limitation that occurs when breathing dense gas necessitates a change in the pattern of the inspiratory vs. expiratory muscles and of the timing. One of the responses to flow limitation that was simulated exceeded possible lung volumes and another put a high load on both inspiratory and expiratory musculatures. The response that has actually been observed is to increase the preinspiratory lung volume; this increases the inspiratory effort to overcome elastic recoil forces but allows for a relatively rapid expiration and moderate expiratory effort.

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ALVEOLAR GAS-PHASE DIFFUSION LIMITATION IN THE HYPERBARIC ENVIRONMENT

M. P. Hlastala, J. Ohlsson, and H. T. Robertson

In addition to the effects of increased work of breathing, pulmonary gas exchange in a hyperbaric environment may be handicapped by impaired diffusion related to the increased density of gas at pressure. At 1 ATA, studies of exchange of inert gases have indicated that gas-phase diffusion limitation may play only a minor role in gas exchange efficiency (1-3). However, it is likely that any diffusion limitation in the gas phase of the lung that does exist would be more important at high pressure when the increased density decreases the diffusion coefficient of any gas (4). Most of these previous studies have used methodologies that are limited to indicator gases delivered via the inspired air. Under such experimental conditions, many complicating factors conceal the importance of the diffusion limitation effect relative to overall gas exchange.

The present theoretical study was undertaken in an attempt to use recent data quantitating gas-phase diffusion conductance obtained using the elimination properties of gases of differing molecular weight but similar blood solubility. These data allow accurate assessment of the magnitude of gas-phase diffusion limitation, which is then used to estimate the importance of such an effect in the presence of increased gas density.

THEORY

We have developed a method to assess gas diffusion limitation by examining elimination of gases with different physical properties from the blood by the lung (1, 2). If an inert gas is dissolved in mixed venous blood, it will equilibrate between the alveolar gas and end-capillary blood according to the blood-gas partition coefficient (λ) and the ventilation-perfusion ratio

($\dot{V}_A/\dot{Q}$) of the alveolus. The relative retention (P_a/P_v) and excretion (P_E/P_V) of the gas have been described by Farhi (5):

$$P_v R = \frac{P_a}{} = \frac{\lambda}{\lambda + \dot{V}_A/\dot{Q}} \quad (1)$$

$$P_V E = \frac{P_E}{} = \frac{\lambda}{\lambda + \dot{V}_A/\dot{Q}} \quad (2)$$

However, these relationships hold only when there is diffusion equilibrium between the alveolar gas and the end-capillary blood. If there is a gas-phase diffusion limitation in the lung (a difference is partial pressure between bulk alveolar gas and the gas at the alveolar-capillary membrane), then a modified set of equations describes the retention and excretion by the lung (3):

$$R = \frac{F\lambda}{F\lambda + \dot{V}_A/\dot{Q}} \quad (3)$$

$$E = \frac{\lambda}{F\lambda + \dot{V}_A/\dot{Q}} \quad (4)$$

$$\text{where } F = 1 + \frac{\beta_g \dot{V}_A}{D^*} .$$

β_g is the capacitance coefficient of gas in gas (6), $\dot{V}_A$ is the alveolar ventilation, and D^* is the gas-phase diffusion conductance. D^* is inversely related to the square root of the molecular weight of the indicator gas.

According to Eqs *1* and *2*, gases with identical solubility but different molecular weights would be expected to have identical retention and excretion by the lung if there were no gas-phase diffusion limitation. On the other hand, according to Eqs *3* and *4*, the presence of a gas-phase diffusion limitation ($D < \infty$) would cause the higher molecular weight gas to have a greater retention and lower excretion than the lower molecular weight gas.

METHODS

To detect the presence of a gas-phase diffusion limitation, partial pressures of intravenously infused acetylene, Freon-22, and Forane (Isoflurane) [gases with similar solubility in blood but different molecular weight (MW), *see* Table 1] were compared in arterial and mixed venous blood and mixed expired gas in 13 anesthetized (pentobarbital) and ventilated (tidal volume of 15 ml/kg, Pa_{CO_2} of 33-38 mmHg) mongrel dogs (wt 20-27 kg) at 1 ATA (2).

The experiment was designed to isolate the MW-associated differences in inert gas exchange by the choice of gases with similar partition coefficients and different MW. If there is no diffusion limitation present, then the retentions and excretions of gases with different MW and identical solubility will be

Table 1
Gas Physical Properties

Inert Gas	Blood-Gas Partition Coefficient, λ	MW
Acetylene	0.91	26
Freon-22	0.84	86.5
Forane	1.36	184.5

identical. If there is a diffusion limitation, then the retentions of the higher MW gases will be higher and their excretions lower. The retentions and excretions of the gases were analyzed according to the following relationships, which are derived by Robertson et al. (2) from Eqs *3* and *4* for homogeneous lung:

$$\ln [(1-R)/R] = \beta_1 \ln\lambda + \beta_2 \ln(V_A/Q) + G \quad (5)$$

$$\ln [(1-E)/E] = \alpha_1 \ln\lambda + \alpha_2 \ln(V_A/Q) + H, \quad (6)$$

where $G = 0$, $H = 0$ for gas 1, $G = G_2$, $H = H_2$ for gas 2, and $G = G_3$ and $H = H_3$ for gas 3. Each gas with a different MW will have different values for G and H. The β_1 and α_1 allow correction for the relationship of retention and excretion with partition coefficient (λ), and β_2 and α_2 allow correction for the relationship of retention and excretion with V_A/Q. Analysis of covariance was used to identify small inert gas diffusion-related changes, given that the predominant influences on gas exchange are determined by the ventilation:perfusion ratio, partition coefficient, and experimental run.

RESULTS

The values for each parameter calculated from both retention and excretion data for acetylene and Forane are shown in Table 2.

A molecular weight-dependent difference was observed that made the retention (P_a/P_v) of Forane (MW 184.5) 12% higher than that of acetylene (MW 26) if the two gases had identical partition coefficients.

Transforming Eq *3* and *4* and comparing with Eq *5* and *6* provide the relationship between retention and excretion of the specific gas with MW:

$$G = K_1 - \ln (1 + K_2\sqrt{MW}) \quad (7)$$

$$H = K_3 + \ln (V_A/Q + \lambda K_4\sqrt{MW}) \quad (8)$$

Table 2
Regression Coefficients

	Retention		Excretion
β_1	−0.584	α_1	−0.891
β_2	0.878	α_2	0.689
G_2	−0.049	H_2	0.057
G_3	−0.239	H_3	0.156

ln[(1-R)/R] and ln[(1-E)/E] are linearly related directly to G and H, which are related to the logarithm of the square root of the MW of the gas. Using retention, excretion, G and H values determined for each gas (along with the known MW of the gas), two equations are known for both Eqs *7* and *8*. This allows calculation of K_1, K_2, K_3, and K_4. The expected retention and excretion for both a hypothetical zero MW gas and for oxygen (MW = 32) are then calculated assuming that both gases have a value equal to that for oxygen. Assuming inspired and mixed venous values of 150 and 40 mmHg, respectively, the MW of oxygen results in an alveolar P_{O_2} that is 1.20 mmHg higher and an arterial P_{O_2} that is 5.19 mmHg lower than it would be if there were no gas phase diffusion limitation. The result is an alveolar gas-phase diffusion limitation of 1.20 mmHg for oxygen.

At high pressure, the diffusion resistance of any gas in the gas phase of the lung can be estimated as increasing in proportion to the density of the ambient environment. It must be assumed that the diffusion (A-a)P_{O_2} calculated from these three-gas data is due solely to gas-phase diffusion limitation. The relationship between the diffusivity of O_2 in air and that in trimix (used at pressure) can be calculated from the Chapman-Enskog equation (7). The (A-a)P_{O_2} values at pressure can be calculated from Eqs *5*, *6*, *7*, and *8*. For 5% trimix at 47 ATA and 8% trimix at 88 ATA, the values are:

	Diffusion coefficient, cm^2/s	Alveolar ΔP_{O_2}, mmHg
Air — 1 ATA	0.219	1.2
5% Trimix — 47 ATA	0.0152	12.0
8% Trimix — 66 ATA	0.0102	15.7.

These P_{O_2} differences would represent the components due to diffusion limitation. Any additional $(A-a)P_{O_2}$ due to ventilation-perfusion heterogeneity would superimpose on the diffusion limitation value to result in the total $(A-a)P_{O_2}$.

DISCUSSION

Arterial P_{O_2} has been measured at extreme pressures with equivocal results (8-10). The $(A-a)P_{O_2}$ either increases, decreases, or remains unchanged when ambient pressure is increased. These data are sparse and variable. However, the predicted difference in alveolar P_{O_2} at high pressure is within the range of the various experimental measurements.

The accuracy of both the measured $(A-a)P_{O_2}$ values at pressure and the three gas data at 1 ATA are subject to error. A significant weakness of the extrapolated $(A-a)P_{O_2}$ values calculated in this paper is that the values at pressure are calculated by multiplication of the experimentally determined values of 1 ATA by a factor correcting for density. Thus, any error present will be increased substantially.

An important error is the indirect nature of the experimental determination of $(A-a)P_{O_2}$ in previous experiments. It is impossible to directly measure the mean alveolar P_{O_2} used for gas-exchange calculations. Thus, the standard approach is to assume that alveolar P_{CO_2} and arterial P_{CO_2} are identical. Alveolar P_{O_2} is then calculated using the standard alveolar gas equation.

$$P_AO_2 = P_IO_2 - \frac{P_aCO_2}{R}\left[F_IO_2 + \frac{(1 - F_IO_2)}{R}\right] \quad (9)$$

$$(A-a)P_{O_2} = P_AO_2 - P_aO_2. \quad (10)$$

The equation assumes that there is diffusional equilibrium within the lung and that normal $\dot{V}_A/\dot{Q}$ heterogeneity does not produce a significant $(A-a)P_{CO_2}$. If there is a diffusion limitation in the gas phase of the lung, then arterial and mean alveolar P_{CO_2} will not be in equilibrium. In addition, the approach neglects any $(A-a)P_{O_2}$ due to $\dot{V}_A/\dot{Q}$ heterogeneity, so the equation will not accurately reflect the true $(A-a)P_{O_2}$. In the absence of the true measure of alveolar P_{O_2}, it is impossible to make an accurate measurement of the effect of diffusion limitation on oxygen exchange using standard gas exchange techniques.

The question of diffusion limitation within the gas phase of the lung has been under debate for years. That there is some MW-dependent difference in gas exchange is apparent; however, the mechanism of such a limitation is not clear. There are three possible sites for this limitation: diffusion with the airways (altered anatomic dead space); convection-diffusion interaction within the airways; and gas-phase diffusion limitation within the acinus (alveolar gas). The findings of this study do not differentiate between these separate

mechanisms.

A partial explanation for the apparent gas-phase diffusion limitation may be the difference in anatomic dead space for the gases of different MW (11. 12). During inspiration, gas transport is determined both by convection and diffusion. Gas is carried into the lung by convection and a back diffusion occurs that counteracts the convective movement. At some point in the airways, the cross-sectional area increases sufficiently to reduce the convective flow to a point where it equals the reverse (mouthward) movement caused by diffusion. At this point, a front between the inspired gas and alveolar gas is established. For a review of the mechanisms associated with this front, see a recent review by Scheid and Piiper (13). If gases such as acetylene and Forane are contained in the alveolar air, then the positions of each diffusion front will be different because of their different diffusivities (14). The lower MW gas has a smaller effective anatomic dead space and thus a slightly greater alveolar ventilation. This means that each lung region will have a different effective ventilation for each gas. This would have the effect of causing a different retention curve for each gas resulting in a slight difference in retention.

Another possible site for the MW-dependent difference in elimination is within the smaller airways. Paiva and Engel (14) have shown that when the diffusion front is established at a bifurcation of two airways feeding regions of different ventilations, a sloped alveolar plateau is established on exhalation which appears as though there is a gas-phase diffusion limitation. If two different gases are inhaled, the different front positions for the two gases establish different slopes to the alveolar plateaus. Thus a MW-dependent elimination is established due to the convection-diffusion interaction mechanism within the smaller airways.

The final potential site for the gas-phase diffusion limitation is the alveolar region itself. Early calculations by Rauwerda (15) indicated that distances are very small in the acinar region. Diffusion equilibration takes place very rapidly, thus negating any possible diffusion limitation. Subsequent calculations by other investigators have substantiated this finding. However, several experimental studies have shown a difference in the slopes of the alveolar plateau for gases of different MW. Scheid and Piiper (13) recently reviewed these studies and concluded that there is a gas-phase diffusion limitation (stratified inhomogeneity) within the alveolar region.

Two possible conclusions can be reached by comparing the high pressure predictions from the 1 ATA data and actual high pressure data. One is that the 1 ATA data do not solely reflect the effects of gas-phase diffusion limitation. Other variables, such as alveolar-capillary membrane diffusion impairment, may contribute to the MW dependence of gas exchange under special conditions, such as exercise and alveolar hypoxia (16). The second possible conclusion is that other factors act at high pressure to alter $(A\text{-}a)Po_2$ which counteracts the limitation due to gas diffusion limitation. The $(A\text{-}a)Po_2$ at pressure is not caused by diffusion limitation alone. It has been shown that

breathing high density gas improves the $\dot{V}_A/\dot{Q}$ distribution (17-20) by a mechanism that is different from the alteration of $\dot{V}_A/\dot{Q}$ distribution (17).

It may very well be that several mechanisms are playing a role in determining (A-a)P_{O_2} at pressure. Future experiments need to analyze the elimination properties of gases of differing MW in animals exposed to hyperbaric environments to quantitate the importance of gas-phase diffusion limitation in affecting gas exchange in the diver.

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EFFECT OF COMPRESSION TO 5, 10, AND 30 BAR ON THE CONTRACTILE ACTIVITY OF ISOLATED ATRIAL PREPARATIONS FROM THE RAT HEART

J.A. Ask and I. Tyssebotn

Studies on intact animals and isolated cardiac tissue have shown an increase in developed force of contraction at high ambient pressure (70-200 bar) (1-4). Recent in vivo experiments have indicated that the contractility of the rat heart is enhanced when the animals are exposed to normoxic hyperbaric environments as low as 5 bar (5).

The present study aims at a characterization of pressure-sensitive changes in contractile activity in spontaneously beating auricular preparations of the rat heart compressed to 5, 10, and 30 bar, within the current pressure range of manned diving.

MATERIALS AND METHODS

Male Wistar rats (375-450 g) were anesthetized with pentobarbital i.p. (50 mg/kg body weight). The hearts were removed rapidly and immersed in an oxygenated Krebs-Henseleits solution consisting of: (in m*M*) NaCl 119.0, KCl 4.6, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 24.9, KH_2PO_4 1.2, and glucose 10.0. The right auricle with intact SA node was dissected free and mounted in an 8-ml, cylinder-shaped organ bath inside a hyperbaric pressure chamber (1, 3, 4). The solution described above was oxygenated (95% O_2:5% CO_2, final pH 7.45), preheated outside the pressure chamber, and pumped by a high-pressure pump (Bran & Luebbe, N-P 31) at a flow rate of 20 ml/min through the organ bath and out of the pressure chamber. This perfusion assured a constant temperature in the tissue bath of 37.0°C (range 36.9-37.1°C) throughout the experiments. To minimize the diffusion of inert gas from the chamber atmosphere to the saline solution, the organ bath surface was reduced to 40 mm^2 by a

Plexiglas lid.

Two spontaneously beating preparations from 2 rats were mounted in parallel in the tissue bath, and the contractile tension (T) was recorded isometrically via Grass Force Displacement Transducers (type FT03) and monitored via a Grass Polygraph (model 7P20) as described elsewhere (6). The preparations were stretched (0.25 g) to the length at which the maximal force of contraction was obtained and equilibrated for 30 min before the experiment. The peak tension (T_{max}) and maximal velocities of tension rise (T'_{max}) and fall (T'_{min}) were taken as expressions of contractility, whereas the number of beats per minute gave the chronotropy.

The pressure in the hyperbaric chamber was increased (1 bar/min) to 5, 10, and 30 bar in 3 series of experiments by adding He to the chamber atmosphere. Calculations show that the tension of He in the Kreps-Henseleits solution at the level of the cardiac preparations is less than 0.0002% of the actual chamber pressure. Thus, negligible amounts of He will be physically dissolved in the medium near the preparations, and changes in contractility are due to increased hydrostatic pressure. Recordings of the contractile activity were performed during compression at elevated stable pressure for 15 min, during decompression (1 bar/min), and in a period of 15 min after decompression was complete. In addition, control experiments were performed during an identical period.

In all experiments, propranolol, phentolamine, and atropine were added ($1/10^{-6}$ *M*) to inhibit neurotransmitters acting on cardiac α, β, and muscarinic receptors, respectively. The neuronal uptake mechanism of catecholamines was blocked by cocaine ($1/10^{-6}$ *M*).

Statistical analysis was performed with Wilcoxon's two-sample rank test. Means and SE of the mean were calculated for all data.

RESULTS

Chronotropic effects were not detected when the spontaneously beating rat auricles were compressed to 5, 10, or 30 bar (Table 1). On the other hand, significant increases ($P < 0.01$) in the force (T_{max}) and in the velocities of contraction (T'_{max}) and relaxation (T'_{min}) were apparent at 5 bar (Fig. 1, Table 1). The parameters describing contractility were maintained at the increased levels throughout the 15-min period at 5 bar and did not reach control values 15 min after completed decompression (Fig. 1).

Further significant increases in T'_{max} and T'_{min} but not in T_{max} ($P < 0.07$) were recorded at 30 bar, and findings at 10 and 5 bar were similar (Table 1). No changes in the tension that could be ascribed to changes in preloading of the preparations were detected at 5, 10, and 30 bar.

DISCUSSION

The present study has shown increased force and velocities of contraction

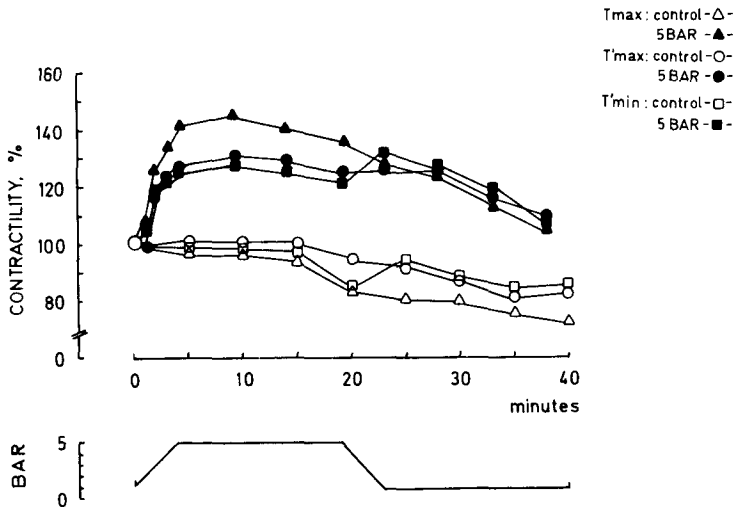

Fig. 1. Changes in the maximal force (T_{max}) and velocities of contraction (T'_{max}) and relaxation (T'_{min}) in rat auricular preparations compressed to 5, 10, and 30 bar for 15 min and in a 15-min period after decompression.

Table 1

Effects of compression to 5, 10, and 30 bar on the contractility (T_{max}, T'_{max}, and T'_{min}) and the chronotropy (beats/min) of spontaneously beating auricular preparations of the rat heart

	n	T_{max}, %	T'_{max}, %	T'_{min}, %	Beats/min, %
Control	7	97.0 ± 2.0**	99.0 ± 3.4**	99.6 ± 2.4**	98.4 ± 2.3 NS
5 Bar	7	140.9 ±12.2	126.4 ± 6.6	127.1 ± 8.8	99.5 ± 1.1
Control	7	93.7 ± 3.3** NS	100.0 ± 4.5** NS	99.6 ± 4.4** NS	99.1 ± 1.1 NS
10 Bar	8	136.6 ±16.4 NS	137.4 ± 8.1 *	132.8 ±11.0 **	104.7 ± 4.4 NS
Control	7	79.7 ± 5.1**	84.4 ± 6.0	89.7 ± 6.4**	97.2 ± 1.9 NS
30 Bar	6	179.0 ±16.6	190.5 ±25.7	175.5 ±18.5	97.2 ± 3.5

T_{max}: The maximal force of contraction: T'_{max} and T'_{min}: The maximal velocities of contraction and relaxation, respectively. The data given at 5, 10, and 30 bar represent values obtained immediately after stable pressures. Control values give changes at 1 bar in the same period. $^{**}P < 0.01$, $^{*}P < 0.05$, NS = $P > 0.05$. Mean ± SEM.

and relaxation, but unchanged chronotropy of spontaneously beating auricular preparations from the rat heart pressurized (1 bar/min) to 5, 10, and 30 bar.

Since the experiments were performed in the presence of adequate concentrations of α-, β-, and muscarinic receptor-blocking agents, a modulation of the contractile activity by neurotransmitters released from sympathetic or parasympathetic nerve terminals is not likely in the present study. Taken together with the findings of unchanged frequency of contraction and preloading (stretching) of the preparations, the enhanced contractility reflects a pressure-induced increase in the inotropic state of the atrial muscle. It is remarkable that this positive inotropic response is detected at pressure lower than 5 bar (Fig. 1).

The data from the in vivo experiments are in accordance with our in vivo recordings from rats exposed to 5 bar using identical rates of compression (5). In this investigation, the intraventricular pressure (Pv_{max}) and velocities of intraventricular pressure rise ($+dP/dt_{max}$) and fall ($-dP/dt_{max}$) are increased to approximately the same level as the force (T_{max}) and velocities of tension increase (T'_{max}) and decrease (T'_{min}) obtained in the present study at 5 bar. These corresponding results indicate that the observed changes in ventricular function at 5 bar may be explained as elevated inotropy of the ventricular myocardium. However, the pressure per se may not fully account for the enhanced inotropy detected at 5 bar because an increased density of breathing gas at 1 bar also increased the velocities of the intraventricular pressure development (dP/dt_{max}) in experiments with rats and cats (7).

Our findings on the rat myocardium are in agreement with other studies on isolated preparations from different vertebrate species demonstrating an increased developed force (1, 2, 4), although at much higher pressures (70-200 bar).

In conclusion, the present set of experiments has shown that the contractility of auricular preparations from the rat heart is considerably increased at pressures (5, 10, and 30 bar) highly relevant for current diving activity. Furthermore, increased contractile activity is explained by a pressure-induced, positive inotropic effect.

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BREATHING GAS DENSITY INFLUENCES MYOCARDIAL CONTRACTILITY

G. W. Bergø, J. A. Ask, and I. Tyssebotn

Recently published reports have shown increased myocardial perfusion (1-3) as well as increased contractility of the left ventricle (4) when rats are subjected to simulated dives at 5 bar containing normoxic nitrogen or He atmospheres. Since dives also imply an increased density of breathing gas, the described changes could possibly be affected by the changed breathing pattern and intrathoracic pressure changes per se. Bergø and Tyssebotn (5, 6) found that blood flow to the left ventricle increased in a dense normoxic sulfurhexafluoride (SF_6) atmosphere at 1 bar (approximately 5 times the density of air). In a light gas density atmosphere composed of normoxic He, the blood flow to the right ventricle was increased. Since in all known situations the metabolic demand of the myocardium is matched by the myocardial blood flow, these findings suggest increased workload of the heart.

In the present study we have measured the pressure development in the left ventricle with a Micro-Tip catheter pressure transducer in anesthetized rats, and a stiff saline-filled catheter connected to an AME physiologic pressure transducer in anesthetized cats. The breathing gas was 21% O_2 in SF_6.

METHODS

Male Wistar rats (330-360 g) and cats (3.4-4.2 kg) were anesthetized by pentobarbital in sufficient dose for surgery. Through small skin incisions, catheters were placed in both femoral arteries (for arterial pressure recordings and sampling of blood for acid-base measurements), and one femoral vein (where infusion of additional doses of anesthesia could be given), and in the left ventricle introduced through the right carotid artery. A PP-90 catheter filled with 10% Ficoll 70 (a nonionic synthetic sucrose polymer, mol wt 70,000,

Pharmacia Fine Chemicals, Uppsala, Sweden) in isotonic saline was introduced into the esophagus for indirect measurement of the intrapleural pressure. The ventricular pressure (VP) in rats was measured with a Micro-Tip catheter pressure transducer (Millar Instruments, Inc., Houston, TX), o.d. 0.78 mm. In the cats the VP was measured with a saline-filled stiff catheter connected to an AME physiologic pressure transducer (AME, Hoerten, Norway). In both cases the VP was recorded on a Hewlett Packard multichannel recorder. The maximal velocity of intraventricular pressure rise ($+dP/dt_{max}$) and fall ($-dP/dt_{max}$) was calculated from the VP curves.

Rat

Immediately after anesthesia was reached, the rat was placed on a heating pad controlling the core temperature to 37°C. After surgery was completed, the rat was placed in a small Plexiglas chamber, and control measurements were performed when the chamber was flushed with air at 3 times the chamber volume per minute (Fig. 1). The control period lasted for 15 min before the gas change to 21% O_2 in SF_6 was performed. The animals stayed in the heavy gas atmosphere for 25 min while the gas ventilation was 3 times the chamber volume per minute. Simultaneous measurements of VP, arterial pressure, intrapleural pressure change (dPip) ($Pip_{inspiration}$-$Pip_{expiration}$), heart rate (HR), and respiratory frequency (RF) were done.

The same measurements were undertaken during a 15-min control period in normal air at the end of the experiment.

Cat

When anesthetized, the cats were placed on a heating pad and the core temperature maintained at 37°C. They were placed in a Plexiglas chamber which was continuously ventilated at 10 liter/min with either normal air or 21% O_2 in SF_6. A 15-min control period on normal air was applied before the gas was shifted, and the experiment lasted for 20 min in the dense gas atmosphere. The dPip, VP, arterial pressures, $+dP/dt_{max}$ and $-dP/dt_{max}$ were measured as for the rats.

Statistical analysis was performed with Wilcoxon's two-sample rank test. Means and SEM were calculated for all data.

RESULTS

Rat

The principal findings are shown in Table 1 and Fig. 2. The arterial acid base chemistry remained at control values when the breathing gas was altered to O_2 in SF_6 [hemoglobin: 17.6 ± (SEM) 0.4 g%; Po_2: 93 ± 4 mmHg; O_2 saturation: 96 ± 1%; and O_2 content: 23.8 ± 0.3 ml/100ml] while the Pco_2 and HCO_3 increased by 14% to 39.3 ± 0.6 mmHg ($P < 0.02$) and 20.5 ± 0.2 mmol/liter ($P < 0.02$), respectively. The pH remained unchanged at 7.33 ± 0.02.

Immediately after the gas shift, RF fell by 11% ($P < 0.02$) and the dPip

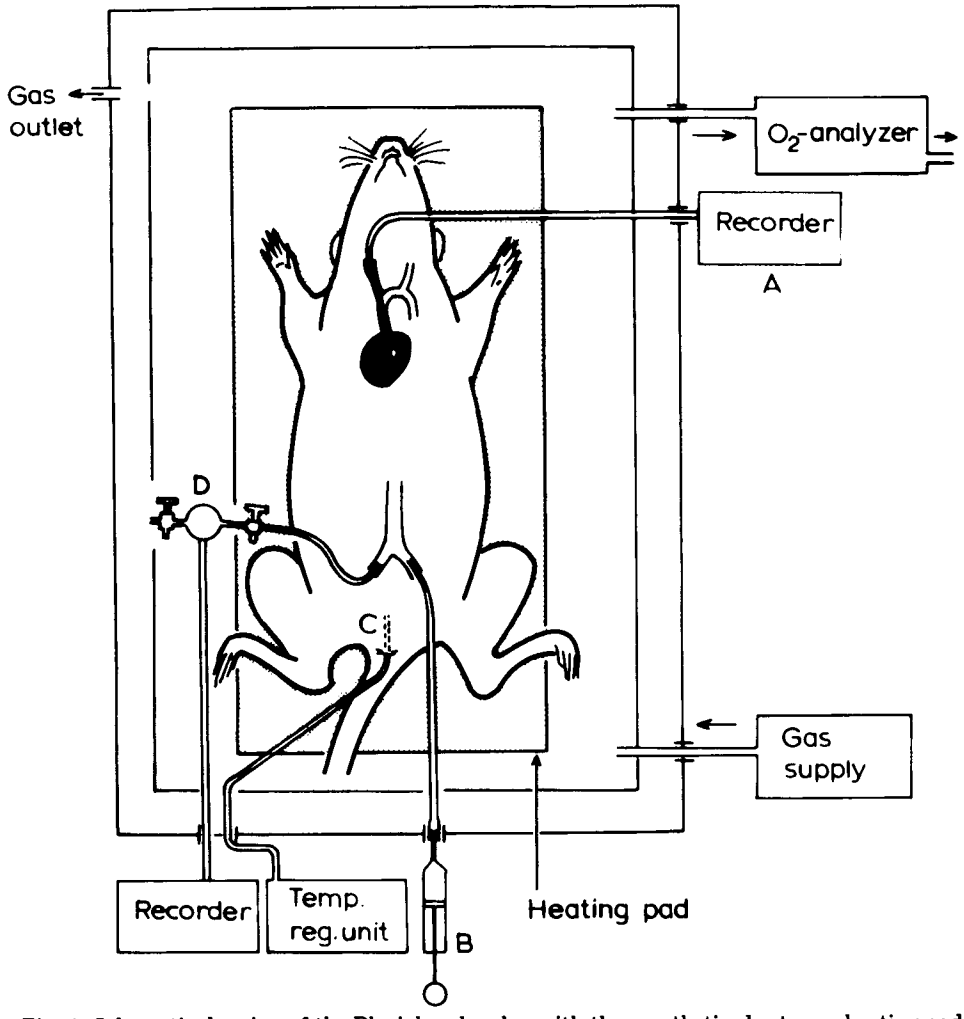

Fig. 1. Schematic drawing of the Plexiglas chamber with the anesthetized rat on a heating pad connected to a temperature regulatory unit. A, the cardiac catheter is connected to a recorder. B, catheter for arterial blood sampling. C, rectal temperature probe. D, arterial pressure transducer. Gas outlet and inlet are shown.

changed by 35% ($P < 0.02$). Concomitant to this change the VP increased slightly, but the $+dP/dt_{max}$ and $-dP/dt_{max}$ increased significantly (20-25%, $P < 0.02$) indicating an increased contractility. Similar changes were observed in all animals.

Figure 2 shows the relation between the percent change in $+dP/dt_{max}$ and the percent change in Pip which indicates a direct relation between these two parameters. The HR and the different arterial pressures were at the same time unchanged. The end-diastolic pressure was unchanged from control to dense gas atmosphere.

Table 1
The Effect of Dense Breathing Gas (21% O_2 in SF_6) on RF, dPip, Left VP, MAP, Left Maximal VP Increase ($+dP/dt_{max}$) and Decrease ($-dP/dt_{max}$) in Rats ($n = 6$) and Cats ($n = 6$)

	Rat		Cat	
	Control	O_2-SF_6	Control	O_2-SF_6
RF min^{-1}	91 ± 2	81 ± 1**	19 ± 1	14 ± 2*
dPip mmHg	3.7 ± 0.6	5.0 ± 0.9**	5.5 ± 1.1	6.6 ± 0.8*
HR min^{-1}	332 ± 15	331 ± 13	159 ± 15	165 ± 10
VP mmHg	98 ± 4	103 ± 5	120 ± 11	133 ± 12
MAP mmHg	79 ± 6	82 ± 4	107 ± 6	120 ± 9
$+dP/dt_{max}$	1680 ± 166	2160 ± 192**	3230 ± 442	4310 ± 932*
$-dP/dt_{max}$	1980 ± 227	2375 ± 260**	2730 ± 845	3500 ±1021*

All values are mean ± standard error of the mean (SEM). * $P < 0.05$ ** $P < 0.02$.

Cat

The findings are shown in Table 1 and Fig. 2. Since the gas composition in the inspired gas was maintained normoxic, only small and insignificant changes occurred in the arterial acid base chemistry during the experiment (hemoglobin: 10.8 g%; pH: 7.27; Pco_2: 45.7 mmHg; Po_2: 78.3 mmHg; HCO_3: 20.1 mmol/liter; O_2 saturation: 91.3%; O_2 content: 14.3 ml/100 ml).

Immediately after the introduction of the dense breathing gas, the RF fell ($P < 0.05$) and the dPip increased ($P < 0.05$) by 26 and 20%, respectively. Concomitant to these changes $+dP/dt_{max}$ and $-dP/dt_{max}$ increased by 33% ($P < 0.05$) and 28% ($P < 0.05$) respectively, whereas the VP only slightly increased. Similar changes occurred in all animals although great scatter of cardiac contractility was observed between animals during control. No change in the end-diastolic pressure was observed during the experiment. The HR and mean arterial pressure (MAP) were not changed significantly.

DISCUSSION

Previous studies have shown a possible relationship between a dense breathing gas and myocardial blood flow (5, 6), which might suggest increased workload of the heart. The changes in the breathing pattern may influence the myocardial work, whether the ambient pressure is increased or not. The present results show a close correlation between the changes breathing pattern in the dense atmosphere and the increase of cardiac contractility shown by a similar elevation of inotropic parameters as $+dP/dt_{max}$ and $-dP/dt_{max}$ in the order of 20-30% both in rats and cats. This can hardly be due to change in

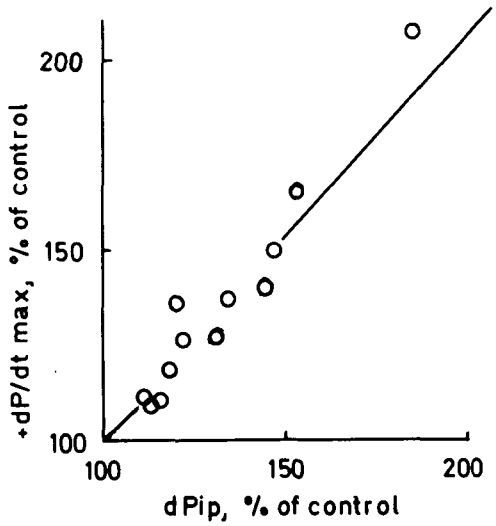

Fig. 2. The relationship between the maximal left VP rise ($+dP/dt_{max}$) in percent of control and the intrapleural pressure changes ($Pip_{inspiration}$-$Pip_{expiration}$ = dPip) in percent of control. Line of identity shown.

the Frank-Starling balance because the end-diastolic pressure is unchanged in the present study, and HR, cardiac output, and stroke volume were equal during control and during dense breathing gas exposure shown by Bergø and Tyssebotn (5, 6).

This study shows a close correlation between the $+dP/dt_{max}$ of the left ventricle and the intrapleural pressure changes in the dense gas atmosphere. The increased intrapleural pressure changes, dPip, must reflect an increased tidal volume. Alveolar ventilation, however, must be fairly constant because the arterial acid-base chemistry is negligibly changed. The increased tidal volume might influence stretch receptors on the lung surface or the response to the low pressure blood volume receptors in the atrias and the lung.

Since such alternations are shown both in rats and in cats it suggests that they may also occur in man.

CONCLUSION

The present study further supports a relationship between the respiratory pattern and cardiac performance in laboratory animals. The results indicate a direct influence of the RF and dPip on the inotropism of the cardiac muscle described by the increased $+dP/dt_{max}$ and $-dP/dt_{max}$. The mechanism for these changes are not yet known.

These and previous results indicate that partial pressure of oxygen, ambient pressure, and the breathing gas density change the cardiac performance during diving.

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FLUID SHIFT DURING HEAD-OUT WATER IMMERSION IN CONSCIOUS DOGS

K. Miki, G. Hajduczok, S. K. Hong, and J. A. Krasney

Head-out water immersion (WI) causes a diuresis in humans (1), subhuman primates (2), and dogs (3). During 3 h of WI in humans with no replacement of fluid losses, approximately 500 ml of the fluid, which was equivalent to approximately 25% of the initial plasma volume, was excreted via the urine. However, hematocrit had not changed significantly from control levels by 3 h of WI (4). During the early phase of WI, an increase in plasma volume, estimated indirectly from hematocrit and hemoglobin, has been reported in humans [McCally (5); +9% by 25 min of WI, Greenleaf (6); +4.3% by 30 min of WI, Khosla and DuBois (7); +8.3% by 20 min of WI]. In addition, Khosla and DuBois (8) have reported that a significant increase in plasma potassium and amino acid concentration occurs during WI in humans treated with vasopressin. They concluded that fluid entering the intravascular space might be derived from the intracellular fluid compartment. Therefore, WI seems to modify body fluid distribution among intracellular, interstitial, and plasma compartments as well as urinary excretion. However, dynamic aspects of changes in body fluid distribution which occur in concert with the diuresis during WI are not known.

The present study was designed to determine the dynamics of fluid movement among the intracellular, interstitial, and plasma compartments during WI. To achieve this, extracellular fluid volume, plasma volume, thoracic duct lymph flow, and urine flow were measured in chronically instrumented or anesthetized dogs during WI under thermoneutral conditions.

METHODS

Animal Preparation

This study utilized mongrel female dogs. Approximately 5 wk before the

experiment, the dogs were premedicated with morphine sulfate (1 mg/kg i.m.) and atropine sulfate (0.1 mg/kg i.m.) and anesthetized with thiamylal sodium (30 to 35 mg/kg i.v.). Subsequently, the spleen was removed to avoid splenic contracture and mobilization of erythrocytes during WI. After recovery from splenectomy, about 2 wk before WI experiments, the dogs were reanesthetized and catheters were positioned with their tips in the abdominal aorta and the interior vena cava via the right femoral artery and vein for measurement of systemic arterial and central venous pressures, respectively. Catheters were also placed in the left femoral artery and vein for subsequent establishment of an extracorporeal circuit. The extracorporeal circuit was used for measurement of circulating blood volume, hematocrit, plasma colloid osmotic pressure, and extracellular fluid volume. These catheters were routed to the interscapular space, exteriorized, and protected by a fitted jacket.

MEASUREMENTS

Plasma Volume

Circulating blood volume (BV) and hematocrit (Hct) were measured continuously in conscious dogs using the extracorporeal circuit (9). After the injection of ~350 μCi of ^{51}Cr-labeled erythrocytes, arterial blood from the femoral artery was withdrawn by an inlet pump at a constant flow rate (40 ml/min) and passed through a gamma-ray detector to determine the radioactivity in the shunted blood. The pulse height of radioactivity was analyzed with a multichannel analyzer (ND62, Nuclear Data, Shaumburg, IL). The win- dow was set for ^{51}Cr, and the total counts of the window were sent to a Horizon NorthStar microprocessor every 30 s. The blood volume at any given time, BV(t), was computed according to the general dilution principle: BV(t) = [total gamma counts of the injected tracer]/[gamma counts in blood]. A pair of platinum plates (conductive Hct cell) was placed in series with the gamma-ray detector and was used to measure Hct continuously. The Hct values obtained this way were compared periodically to Hct values obtained by microcentrifugation. Plasma volume was estimated in a continuous manner from the blood volume and Hct. The outflow from the conductivity cell drained into a small reservoir (dead space = 10 ml) and the blood was then returned to the animal via the venous catheter by adjusting the flow rate of the outlet pump using an analog servo system.

Extracellular Fluid Volume

Since a tracer which distributes to the extracellular fluid space is excreted very rapidly via the urine, it was necessary to carry out a nephrectomy in this series of experiments (10). Two weeks before the experiment, the left kidney was removed under anesthesia. After recovery, on the day of the study, the animals were reanesthetized with an intravenous injection of sodium thiamylal (30-35 mg/kg i.v.), and underwent removal of the other (right) kidney through a flank incision. The staged nephrectomy was used to minimize

bleeding. Anesthesia was maintained during the experiment with ketamine (1-5 mg/kg) following the nephrectomy. After 3 h to allow for hemostasis, 200 μCi of [^{125}I]iothalamate (GLOFIL-125, Iso-tex Diagnostics, Texas) and 350 μCi of ^{51}Cr-labeled erythrocytes were injected intravenously for the measurement of extracellular fluid and intravascular fluid volumes, respectively. Arterial blood from the femoral artery was withdrawn by an inlet pump at a constant rate (40 ml/min) and passed through a gamma-ray detector to determine the radioactivity of the shunted blood, as described above. The pulse height of radioactivity of the blood was analyzed with a multichannel analyzer (ND62, Nuclear Data Inc.). The total counts of the window, which were set before the experiment for ^{125}I and ^{51}Cr, were sent to a Northstar Horizon microprocessor every 30 s. The total counts of each radioisotope were determined according to the separation correction for a two-component mixture and then the counts were corrected for background. The counts of ^{125}I in plasma [CI(t)] were obtained as follows:

$$\text{CI(t)} = [\text{counts of } ^{125}\text{I in bypassed blood}]/[1-\text{Hct(t)}/100].$$

Extracellular fluid space at a given time [ECF(t)] was computed by the following equation:

$$\text{ECF(t)} = [\text{Total dose of } [^{125}\text{I}]\text{iothalamate}]/[\text{CI(t)}]. \quad (1)$$

Interstitial fluid volume was obtained by subtracting the plasma volume from the extracellular fluid volume, assuming that the observed changes in extracellular fluid volume were due to a net fluid shift occurring between the intracellular and extracellular spaces.

Thoracic Duct Lymph and Urine Flow

A chronic thoracic duct side-fistula was created according to the report of Girardet and Benninghoff (11) in another group of dogs. After the left cervical lymphatic duct and ampulla were identified, the cannula, filled with heparin solution, was introduced into the cervical lymphatic duct and advanced within it until the tip of the catheter was exactly flush with the inner wall of the ampulla and then ligatures were tied around the catheter. The catheter was then routed to interscapular space and exteriorized. Thoracic duct lymph was collected by applying a constant negative sampling pressure in conscious dogs at least 2 d after surgery. This technique allows for repetitive sampling of the lymph fluid in the awake animal.

Urine samples were collected every 20 min via a Foley catheter placed in the bladder. Urethral catheterization was performed with the aid of a duckbill speculum. Hct was determined every 20 min in triplicate by microcentrifugation to calibrate the conductivity cell for continuous determination of Hct. Raw Hct values were corrected for trapped plasma using a factor of 0.96.

Experimental Procedure

The WI experiments consisted of a 60-min control period in air, 100 min of water immersion, and then a 60-min recovery period in air. The dogs were immersed to the midcervical level in the quadruped position after the control period in air. Water in the immersion tank was held at a temperature of 37°C to keep the dog at the thermoneutral condition. The timed control experiments consisted of having the dogs stand for a 220-min period in air.

Animals used in this study were maintained in accordance with the guidelines of the Laboratory Animal Care Committee (SUNY-Buffalo), which approved this study, and those prepared by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. [NIH] 78-23, revised 1985).

Statistics

The mean values and the SE were calculated for all parameters. Comparisons between experimental values and individual control values were made by a one-way ANOVA and a modified least significant difference test (12). *P* values less than 0.05 were considered significant.

RESULTS

Water immersion caused an increase in plasma volume, which preceded the onset of the diuresis, while red cell volume remained unchanged throughout the experiment in the awake, intact dogs. Therefore, Hct decreased significantly during WI (Table 1). Urine flow increased significantly by 40 min of WI and reached a peak value of 1.77 ml/min relative to the control value at 60 min of WI, then it gradually decreased. Thoracic duct lymph flow tended to decrease immediately at the start of WI but the decrease did not prove to be statistically significant. In contrast to the dogs with intact kidneys, plasma volume increased linearly during the course of WI while red cell volume remained constant in the acutely nephrectomized dogs. Extracellular fluid volume also increased linearly during WI in nephrectomized dogs. The magnitude of the increase in plasma volume induced by WI was almost equal to that of the increase in extracellular fluid volume until 60 min of WI such that estimated interstitial fluid volume remained unchanged until 60 min of WI. However, after 80 min of WI, the magnitude of the increase in extracellular fluid volume was less than that of the increase in plasma volume. As a result, interstitial fluid volume tended to decrease from 80 min of WI. At 100 min of WI, 85% of the fluid that was shifted to the intravascular space was derived from the intracellular fluid space.

The time course of the changes in plasma volume before and during water immersion in intact, conscious dogs is shown in Fig. 1, compared with the changes occurring in acutely nephrectomized dogs. Plasma volume in intact dogs started to increase from the 5th min of WI. By 35 min of WI, plasma volume had increased by a maximum of 3.5 ± 0.5 ml/kg (7% of initial plasma

Table 1
Changes in Extracellular, Intercellular, and Plasma Volumes, Urine Flow, Thoracic Duct Lymph Flow, and Hematocrit

	Control, Absolute Values Before WI	Minutes of Water Immersion				
		20	40	60	80	100
Nonnephrectomized dogs ($n = 6$)						
Plasma volume, ml/kg	48.0 ± 2.0	2.0 ± 0.5*	3.4 ± 0.4*	3.0 ± 0.4*	2.8 ± 0.5*	2.8 ± 0.6*
Red cell volume, ml/kg	23.0 ± 1.0	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
Hematocrit, %	37.9 ± 1.2	-0.7 ± 0.4	-1.4 ± 0.3*	-1.1 ± 0.3*	-0.9 ± 0.3	-0.9 ± 0.4
Urine flow, ml/min	0.58 ± 0.10	0.26 ± 0.09	1.66 ± 0.76*	1.77 ± 0.80*	0.91 ± 0.33*	0.62 ± 0.21*
Lymph flow rate, ml/min	0.95 ± 0.13	-0.15 ± 0.17	-0.15 ± 0.17	-0.12 ± 0.16	-0.27 ± 0.12	-0.31 ± 0.13
Nephrectomized dogs ($n = 6$)						
Extracellular fluid volume, ml/kg	214.0 ± 14.0	2.8 ± 1.7	4.1 ± 1.4	5.7 ± 1.1*	7.4 ± 1.1*	8.7 ± 1.2*
Interstitial fluid volume, ml/kg	170.0 ± 10.0	-0.2 ± 2.0	-0.1 ± 1.0	-0.4 ± 1.5*	-0.5 ± 1.8	-1.5 ± 1.8
Plasma volume, ml/kg	40.0 ± 3.0	3.1 ± 0.5*	4.2 ± 0.4*	6.0 ± 0.9*	7.8 ± 1.2*	10.2 ± 1.6*
Hematocrit, %	36.0 ± 3.0	-1.6 ± 0.3*	-2.4 ± 0.4*	-3.1 ± 0.5*	-3.7 ± 0.6*	-4.6 ± 0.8*

Values are mean ± SE; values obtained during WI represent the difference from the preimmersion control level. Minus (-) denotes a decrease from the control Level. *Statistically significant difference from the control level.

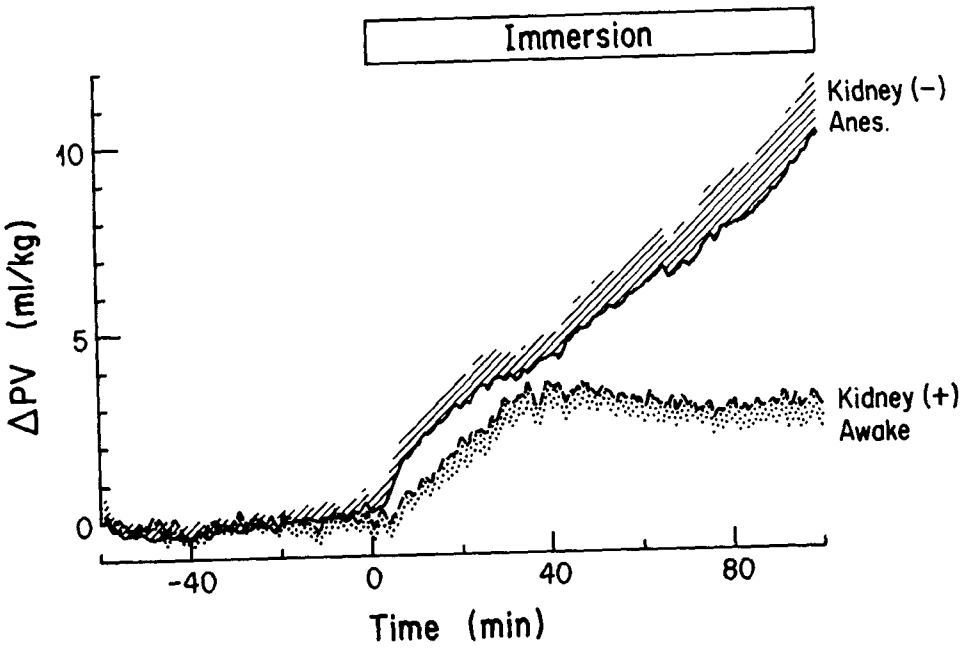

Fig. 1. Changes in plasma volume relative to control levels in conscious, intact dogs (broken line) are shown compared with the plasma volume in anesthetized and acutely nephrectomized dogs (solid line). Dotted and stippled areas above or below the mean values indicate SE envelope.

volume) and then it decreased slightly from the peak level. By contrast, plasma volume in the anesthetized and acutely nephrectomized dogs started to increase from the 3rd min of WI and then it increased almost linearly during the course of WI.

Figure 2 describes the calculated time courses of the fluid fluxes a) across the capillary wall (Jcap), b) cell wall (Jcell), c) thoracic duct lymph flow rate (JL), and d) urinary output (Ju). WI causes initially a negative net transcapillary fluid movement, which implies that the fluid moves from the extravascular space at a maximum rate of about 0.15 $ml \cdot kg^{-1} \cdot min^{-1}$. After 40 min of WI, Jcap maintained a negative value of around 0.1 $ml \cdot kg^{-1} \cdot min^{-1}$. A net negative fluid movement across the cell mem- brane, implying that the fluid moves from intracellular to extracellular spaces, was observed almost immediately after start of WI. Jcell reached a peak value of 1.1 $ml \cdot kg^{-1} \cdot min^{-1}$ at 20 min WI and then it was restored gradually to the control level. After the fluid movement across the capillary wall (Jcap) in association with the fluid movement across the cell wall (Jcell), the diuresis was noted at 30 min of WI. At 50 min of WI, Ju reached the peak value of 0.08 $ml \cdot kg^{-1} \cdot min^{-1}$ and then it gradually returned toward the control level. Thoracic duct lymph flow did not change significantly throughout the experiment.

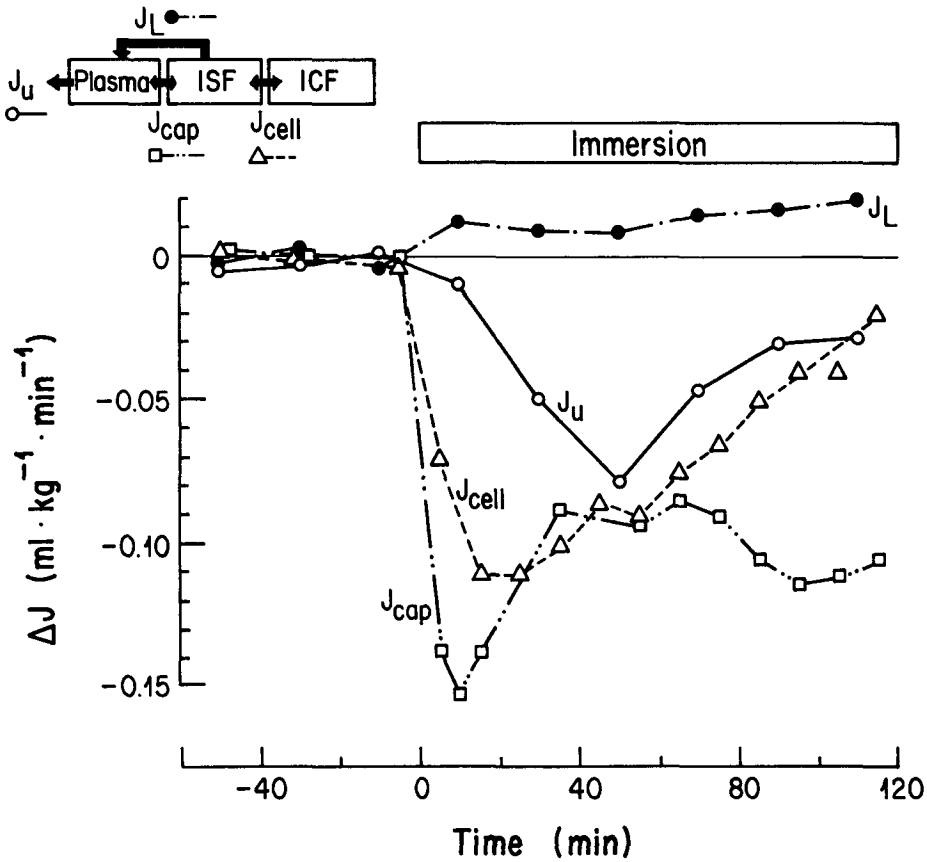

Fig. 2. Time course of the change in net fluid flux occurring between plasma and extravascular spare (Jcap), intracellular and extracellular fluid space (Jcell), thoracic duct lymph flow (JL), and urine flow (Ju) before and during water immersion. Mean values of six experiments are shown.

DISCUSSION

The present study clearly demonstrates that water immersion modifies the entire body fluid distribution among the intracellular, interstitial, and plasma compartments as well as the urine flow rate. The discussion will focus on the following three topics: The dynamic relationship of the time course of fluid movement between intracellular, interstitial, and plasma compartments relating to the diuresis during WI; the role of the diuresis during WI in body fluid regulation; and the mechanism underlying the sustained increase in plasma and extracellular fluid volume during WI.

Extracellular fluid volume, as measured by [^{125}I]iothalamate dilution method, increased significantly during WI. Since no fluid was administered during WI, the increase in extracellular fluid volume was caused by the intracellular fluid shift. Khosla and DuBois (8) suggested that intracellular fluid

was shifted to the extracellular fluid space during WI, based on data indicating increases in plasma amino acid and potassium concentration during WI. The observed changes in extracellular fluid volume in the present study support their view. It is of interest that intracellular fluid started to shift to the extracellular fluid space immediately after start of WI. In addition, the magnitude of the fluid flux out of the cell to the extracellular compartments (Jcell) was almost equal to that occurring across the capillary wall (Jcap). As a result, cell dehydration and hypervolemia occurred without changes in interstitial fluid volume until about 60 min of WI. The negative Jcell due to WI was gradually reversed toward the control level after about 30 min of WI, whereas Jcap maintained a negative value from 40 to 120 min of WI. Consequently, interstitial fluid volume tended to decrease after 60 min of WI. Therefore, it seems that intracellular fluid initially was mobilized to plasma. The volume of cell fluid which can be shifted to the extracellular space seems to be limited such that interstitial fluid tended to move instead to plasma after 60 min of WI.

As can be seen in Fig. 1, the diuresis seems to counteract the continuous tendency for plasma volume to increase in WI. it is likely that the diuresis is necessary to regulate plasma volume within a certain range during WI. Heretofore, the diuresis induced by WI has been considered to be primarily a reflex neurohormonal response to cephalad blood volume expansion caused by a translocation of blood (1). However, from the standpoint of plasma volume regulation, the increase in urine flow during WI seems to correct for an absolute increase in intravascular volume caused by fluid flowing in from the extravascular space. The diuresis due to WI might be triggered by the increase in absolute increase in plasma or extracellular fluid volume. However, the relative contributions of reflexes initiated by central volume expansion vs. the absolute increase in plasma volume to the diuresis are unknown at this time.

In summarizing the fluid shift among the intracellular, interstitial, and plasma compartments as well as the urine flow during WI, WI leads to an increase in plasma volume associated with the increase in extracellular fluid volume immediately after start of WI. Until 60 min of WI, the fluid flowing into the plasma was derived mainly from intracellular fluid space. After the increase in plasma and extracellular fluid volume, the diuresis occurred. Therefore, WI results in fluid movement in the direction of the intracellular to intravascular space and the fluid which is shifted to the intravascular space is excreted via the urine. The diuresis seems to counteract the absolute increase in plasma and extracellular fluid volume to regulate plasma or extracellular fluid volume within a certain range.

The possible mechanisms that lead to an increase in plasma volume due to WI include an increase in net transcapillary fluid absorption, or lymphatic fluid transport rate, or both. The thoracic duct lymph flow did not change significantly during WI. Therefore, transcapillary fluid movement, and not the lymph flow, plays a major role in the increase of plasma volume during WI. Transcapillary fluid movement is governed by balancing the hydrostatic and oncotic

pressures of the intravascular and interstitial fluid spaces and capillary filtration coefficient (9). In our previous studies, interstitial fluid hydrostatic pressure was measured by Guyton's capsule method (13) and capillary pressure was estimated indirectly from the arterial pressure, central venous pressure, and an estimated pre- to postcapillary resistance ratio of 5:12 (14). We found that the external water hydrostatic pressure did not transmit equally to interstitial and intravascular space (15). For example, the increase in mean capillary pressure relative to control level was 13 to 14 mmHg whereas interstitial fluid hydrostatic pressure measured by a tissue capsule method increased by 17 to 26 mmHg at upper hindlimb and lower forelimb, respectively. In addition, we estimated the magnitude of the changes in oncotic pressure gradient across the capillary wall to be less than 1 mmHg (14). This negative hydrostatic pressure gradient was sustained during WI exposure. Therefore, the sustained negative pressure gradient across the capillary wall is probably the cause of continuous fluid inflow from the extravascular space to the intravascular space, which in turn leads to the sustained increase in plasma volume observed in nephrectomized dogs. However, we are uncertain as to the mechanism responsible for the fluid shift out of the intracellular fluid space during water immersion. One possibility is that the negative hydrostatic pressure gradient across the capillary wall discussed above, in turn creates a hydrostatic pressure gradient between the intracellular and intravascular fluid space. Thus, intracellular fluid could move into the intravascular space as a result of the hydrostatic pressure gradient. Another possibility is that the shift of the interstitial fluid to intravascular space might cause an increase in interstitial fluid oncotic pressure if WI initially induces only a fluid inflow from interstitial to intravascular space. Consequently, intracellular fluid would be mobilized by an oncotic pressure gradient created across the cell wall. Clearly, more studies are needed to define the specific mechanisms that initiate and sustain fluid movement between the intracellular and extracellular spaces during WI.

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ROLE OF CARDIAC NERVES IN RENAL AND CIRCULATORY RESPONSES TO HEAD-OUT WATER IMMERSION

J. A. Krasney, G. Hajduczok, K. Miki, J. R. Claybaugh, and S. K. Hong

Head-out immersion in thermoneutral water (WI) leads to a redistribution of blood into the thorax which results in increases in cardiac filling pressures and cardiac output (1). It has been proposed that distention of thoracic structures in WI leads to reflex neural and hormonal adjustments which bring about a diuresis [Gauer-Henry reflex (1)]. In recent years we have developed and standardized a conscious, chronically instrumented canine model of WI which has allowed us to directly test the Gauer-Henry hypothesis for the first time.

In the present study, dogs were trained to undergo WI for periods of 100 min at the canine thermoneutral temperature of 37°C (2). One group of dogs was subjected to total extrinsic denervation of the heart by an intrapericardial approach while another group of dogs was subjected to a sham operation. After recovery from the surgery, the cardiovascular, renal, and hormonal responses to WI occurring in the sham-operated dogs were compared with those responses occurring in the cardiac-denervated dogs. The dogs were studied in the conscious state to avoid the effects of anesthesia and recent surgery.

METHODS

Female mongrel dogs weighing between 18 and 24 kg were trained over a period of 3 to 4 wk to undergo WI (2). The dogs stood in the water in the quadruped position in a sling-frame assembly. Subsequently, the dogs were divided into 2 groups: animals with cardiac nerves intact (INT) (n = 10), and animals with denervated hearts (CD) (n = 6). The dogs were fed a diet that provided 50 to 60 meq of sodium daily.

Two weeks before the experiments, the dogs were premedicated with

morphine (1 mg/kg i.m.) and anesthetized with sodium pentobarbital (30 mg/kg i.v.). A left thoracotomy was performed at the level of the fourth interspace using sterile procedures. The method used for the cardiac denervation was the one-stage intrapericardial technique (3). This technique involves the removal of neural tissue from the left superior pulmonary vein, common pulmonary artery and its left and right branches, and the superior vena cava with the ligation and section of the azygos vein. We added the modification of Fater et al. (3) which extends the denervation to include the remaining pulmonary veins and the inferior vena cava at the junction with the pericardium. In the INT dogs, the pericardium was opened but the heart was not denervated.

Tygon catheters were implanted in the left and right atria for measurement of pressures in these chambers, using Statham transducers. A Foley catheter with the central channel sealed with silicone rubber was also inserted in the left atrium to raise left atrial pressure selectively. An electromagnetic blood flow transducer was placed around the base of the aorta to measure phasic aortic blood flow, and a miniature solid-state pressure transducer (Konigsberg) was placed in the left ventricle via an apical stab wound. A balloon-tipped catheter was placed close to the left ventricle for recording pleural pressure. Cables and catheters were routed through the chest wall, the incision was closed in layers, the chest evacuated, and the exteriorized instruments were protected with a fitted jacket (Byron Medical Jackets, Buffalo, NY). Ampicillin was administered for 5 to 7 d postoperatively on the prophylactic basis. One week later, the dogs were reanesthetized with thiamylal sodium (35 mg/kg i.v.) and catheters were placed in the abdominal aorta and inferior vena cava via a femoral artery and vein to measure arterial pressure and to obtain venous blood samples. All catheters were kept filled with a mixture of heparin and chloramphenicol and flushed with heparin every other day between experiments.

The CD dogs failed to show positive inotropic or chronotropic responses to electrical stimulation of the stellate ganglia and intrathoracic vagi during surgery. In addition, the CD dogs failed to show significant changes in heart rate when arterial pressure was raised by injection of phenylephrine or lowered by injection of sodium nitroprusside when studied in the awake state a few days before the WI experiment. Further, these dogs failed to show positive chronotropic or inotropic responses to a bolus injection of tyramine (1 mg) into the left atrium. Last, the INT dogs showed a cardiac acceleration and increases in urine flow averaging 363 ± 24% (SE) and sodium excretion increased by 418 ± 16% in response to inflation of the left atrial balloon. Arterial pressure was unchanged during balloon inflation. By comparison, left atrial balloon inflation did not change heart rate, urine flow, or sodium excretion in the CD animals, indicating that certain afferent pathways from the heart had been eliminated (3).

Regional blood flow distribution was measured by the radioactive microsphere technique (4, 5). Fifteen-micron polystyrene microspheres (New England Nuclear, Boston, MA) labeled with one of five radionuclides were injected

into the left atrium. The five radionuclides were Gd-153, Sn-113, Ru-103, Nb-95, and Sc-46. The reference sample was withdrawn from the aorta at a rate of 7.64 ml/min. The five microsphere injections were made at -60 (prehydration), 0, 60, 120, and 180 min of the experiment. The animals were then anesthetized with pentobarbital sodium, and euthanasia was carried out by injecting KCl solution. The organs and tissues were removed, weighed, and fixed. Organ and tissue flows were determined by counting the tissues in a multichannel gamma analyzer. Tissue flows were expressed in $ml \cdot min^{-1} \cdot 100\ g^{-1}$ of tissue.

Before the experiment, the dogs were mildly hydrated with 0.45% NaCl solution in an amount equivalent to 2% of the dog's body weight. Urine flow was matched by an intravenous infusion of 0.9% NaCl solution throughout the experiment. The dogs were studied either in a timed control situation standing in air for 200 min, or standing in air for 100 min followed by 100 min of WI at 37°C. Pressure and flow data were recorded on a multichannel oscillograph and simultaneously digitized by a microprocessor.

Plasma catecholamines were determined by the CAT-A-KIT (Upjohn Co., Kalamazoo, MI). Plasma renin activity, plasma vasopressin, and plasma aldosterone levels were determined by radioimmunoassay (6).

The data were analyzed using a two-factor, completely randomized design analysis of variance (2). The Newman-Keuls test for multiple comparisons was used to determine differences between individual pairs of means when the *F* test was significant.

RESULTS

The dogs showed no change in rectal temperature, oxygen consumption, or plasma catecholamines during the WI experiments. While arterial pH and P_{CO_2} levels remained constant, the arterial P_{O_2} decreased from 97.6 ± 4.2 mmHg in the INT dogs to 88.3 ± 2.5 mmHg during WI and from 98.3 ± 5.4 to 88.6 ± 3.6 mmHg in the CD dogs during WI. The data reported herein were derived from 77 experiments performed using 16 dogs.

The cardiac output and heart rate responses occurring in the timed control (TC) and WI experiments in the INT and CD dogs are shown in Fig. 1. Cardiac output increased by a similar amount in the INT and CD dogs and the increases followed a similar time course. The elevated cardiac output was associated with a cardiac acceleration in the INT dogs. This response was abolished in the CD dogs. Preimmersion heart rate was higher in the CD dogs, probably reflecting the intrinsic discharge rate of the sinoatrial node.

The *upper panel* of Fig. 2 indicates that stroke volume was unchanged during WI in the INT dogs. However, the rise in cardiac output elicited by WI in the CD animals was associated with a rise in stroke volume. Figure 2 (*bottom*) shows that the INT dogs displayed an increase in left ventricular dP/dt_{max}, which is an indicator of ventricular contractile performance, during WI. The rise in dP/dt_{max} was abolished in the CD dogs. In addition, the level

of dP/dt_{max} before WI was lower in the CD dogs, suggesting that cardiac nerves make a tonic contribution to the level of dP/dt_{max} in the INT dogs.

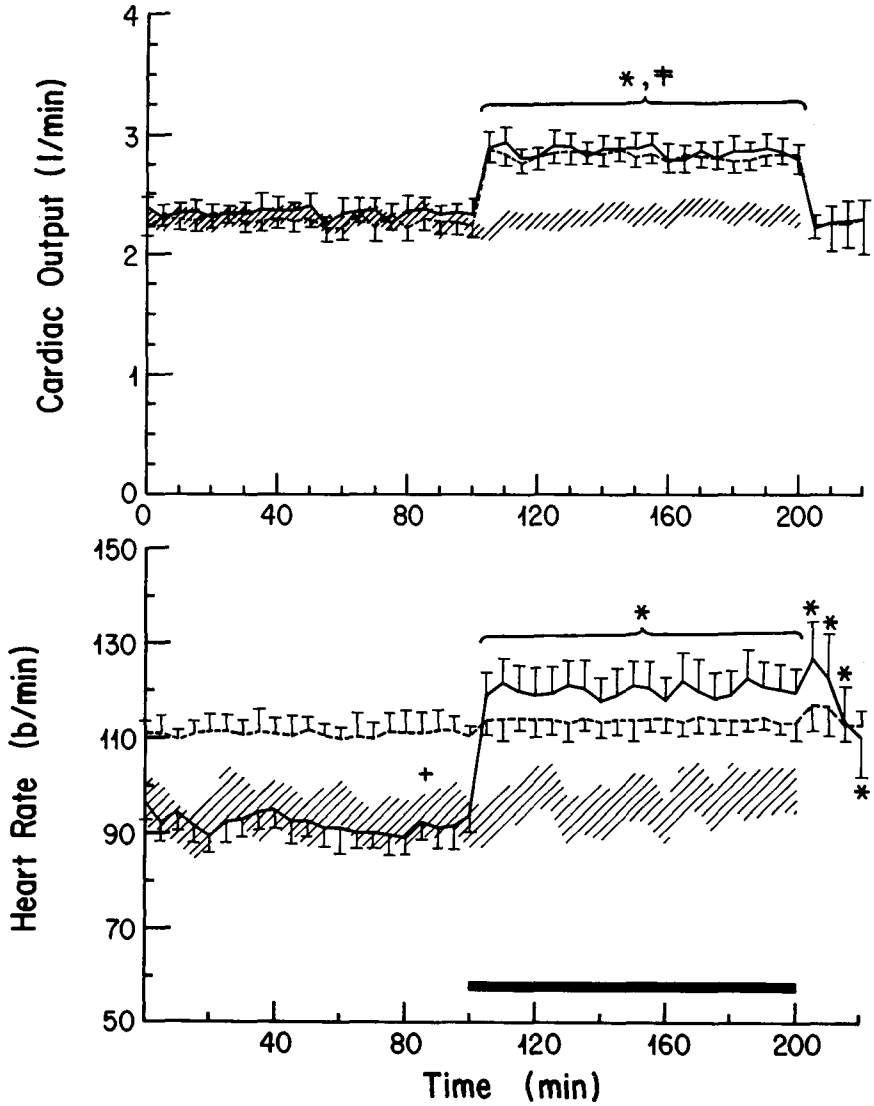

Fig. 1. Time course of the cardiac output (top) and heart rate (bottom) responses of INT and CD conscious dogs during head-out WI. Solid line indicates INT dogs; broken line indicates CD dogs. Values are presented in means ± 1 SE. Data were sampled by a microprocessor at 10-s intervals and averaged for 1-min periods every 5 min. WI period occurred between 100 and 200 min. Hatched line represents the INT dogs (± 1 SE) during a 200-min TC period in air. * INT values significantly different from control ($P < 0.05$ or $P < 0.01$). + CD values significantly different from control ($P < 0.05$ or $P < 0.01$).

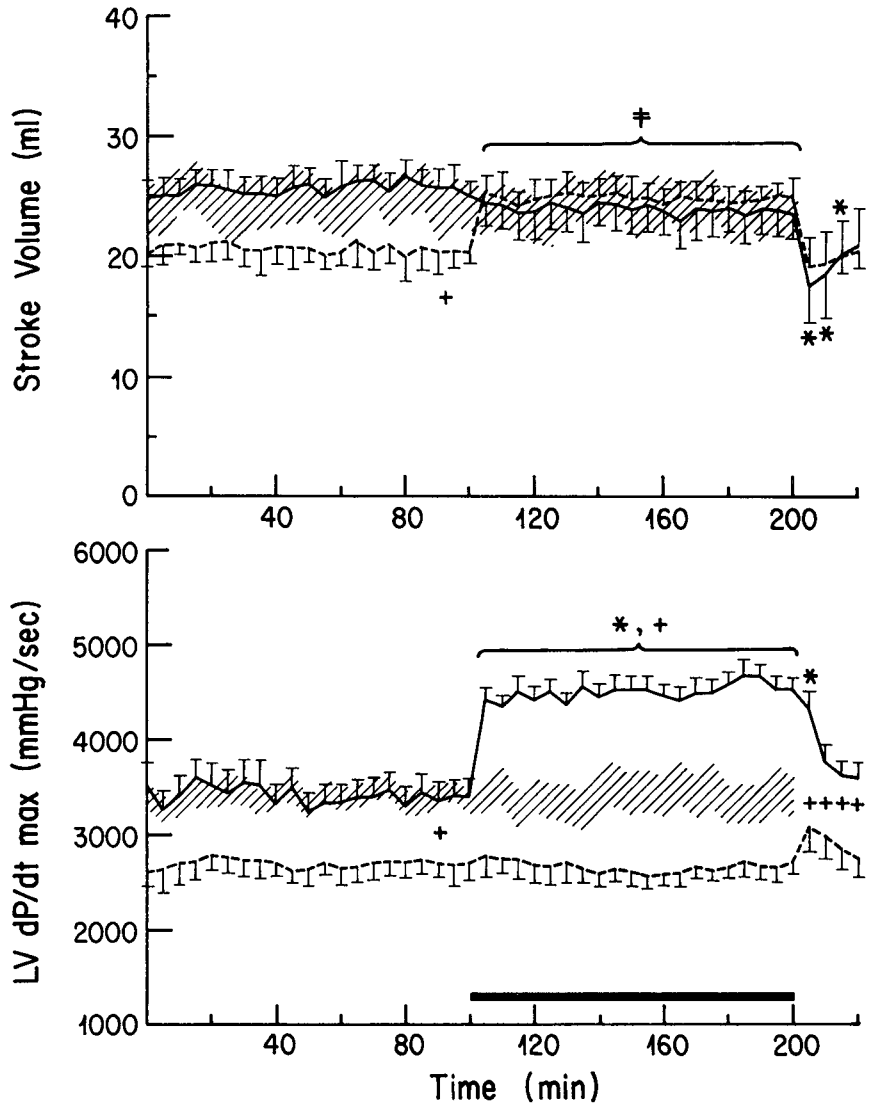

Fig. 2. Time course of the stroke volume (top) and left ventricular dP/dt_{max} (bottom) responses to WI in INT and CD dogs. Abbreviations and labeling of figure as in Fig. 1.

Figure 3 shows that WI elicited a substantial rise in pleural pressure as a result of hydrostatic compression of the chest wall in both groups of dogs. However, the central translocation of blood elicited an increase in cardiac filling pressure, as is indicated by the rise in left atrial transmural pressure determined by subtracting the pleural pressure from the absolute left atrial pressure. Similar increments in transmural distending pressures were also

observed in the right atrium and the left ventricle.

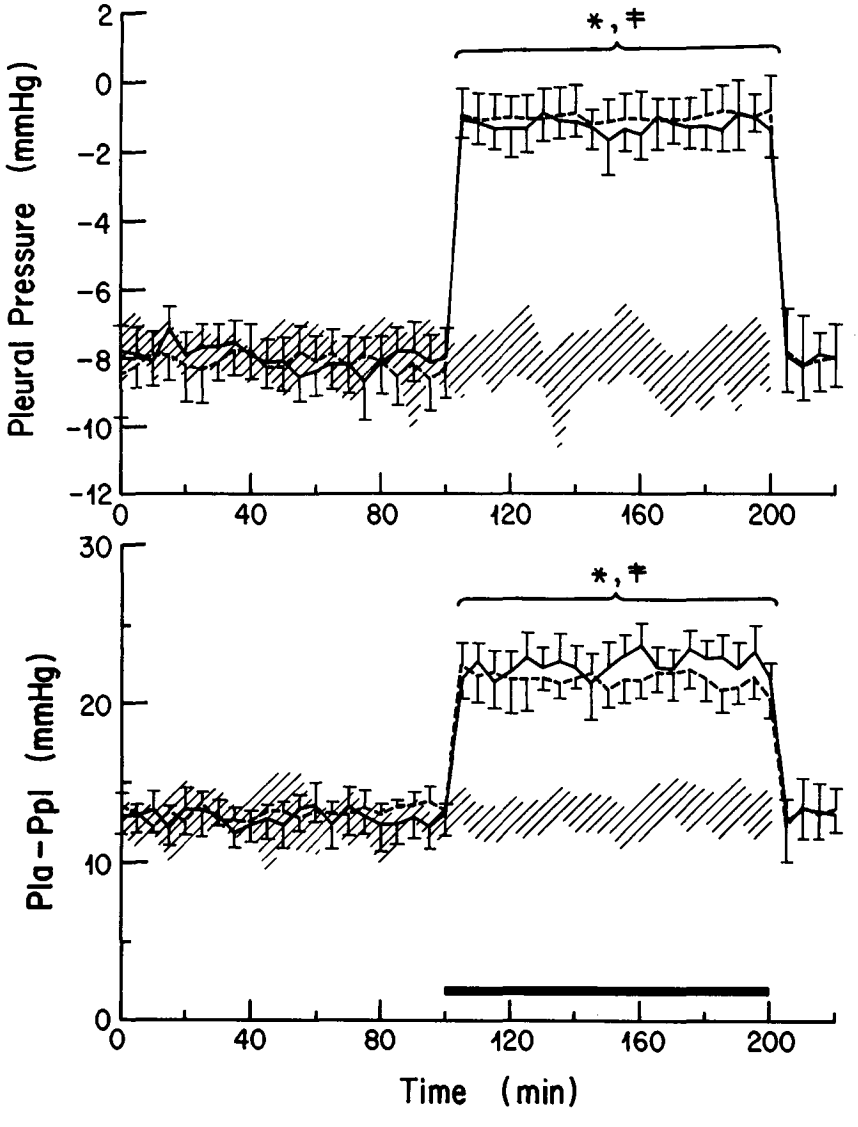

Fig. 3. Time course of absolute pleural pressure (top) and left atrial transmural pressure (PLA-Ppl) (bottom) responses to WI in INT and CD dogs. Abbreviation as in Fig. 1.

The increased cardiac filling pressure and the rise in cardiac output were associated with a rise in mean arterial pressure in both groups of dogs as is shown in Fig. 4. As the total peripheral resistance, calculated from the transmural mean aortic pressure minus the transmural right atrial pressure, was unchanged, the rise in mean aortic pressure was entirely due to the rise in

cardiac output. The aortic pulse pressure did not change significantly during WI in INT dogs. However, commensurate with the rise in stroke volume, CD dogs showed a significant elevation of aortic pulse pressure from 37 ± 2.8 to 44 ± 2.9 mmHg ($P < 0.05$) during WI.

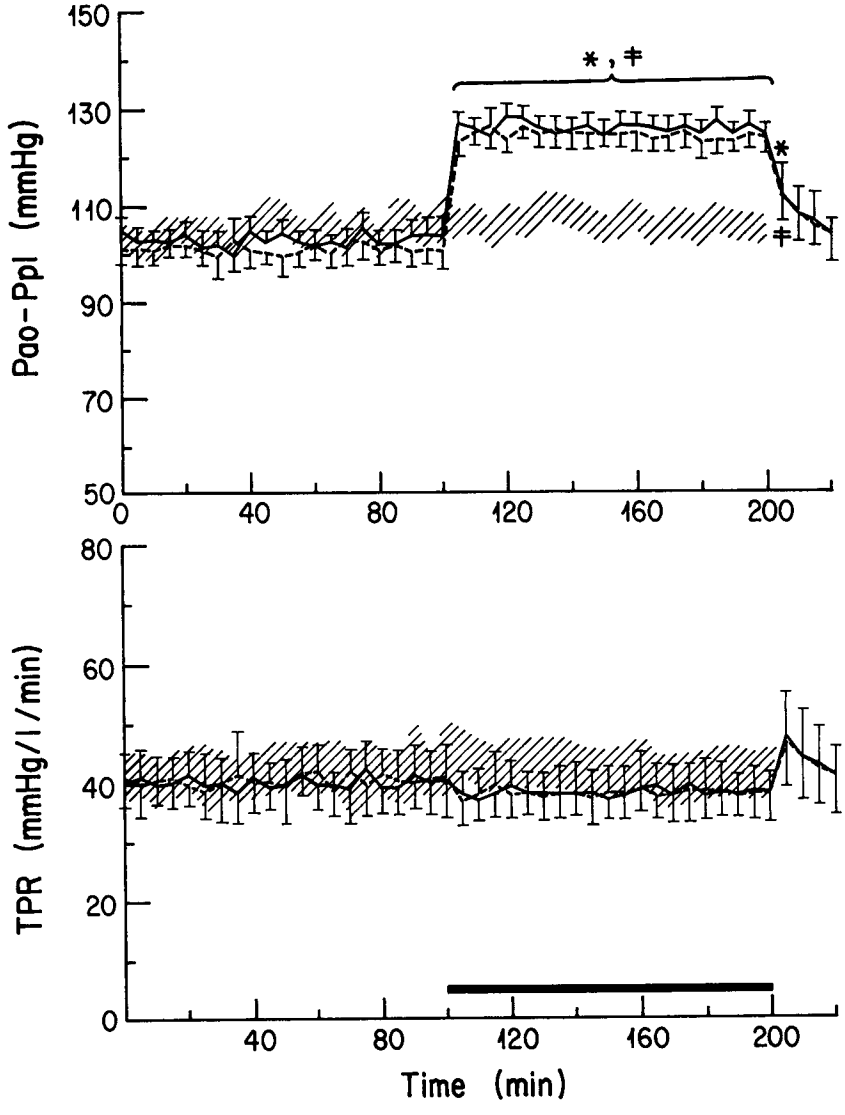

Fig. 4. Time course of aortic transmural pressure (Pao-Ppl) (top) and total peripheral resistance (TPR) (bottom) responses to WI in INT and CD dogs. Abbreviations as in Fig. 1.

Figure 5 shows that WI led to increases in urine flow which were similar in magnitude and time course. Indeed, the curves were virtually superimposable.

However, it will be developed that the mechanism of the diuresis was different between the 2 groups of dogs. For example, Fig. 5 (*bottom*) shows that diuresis was associated with an increase in osmolal clearance during WI in INT dogs, whereas no significant change occurred in osmolal clearance during WI in CD dogs.

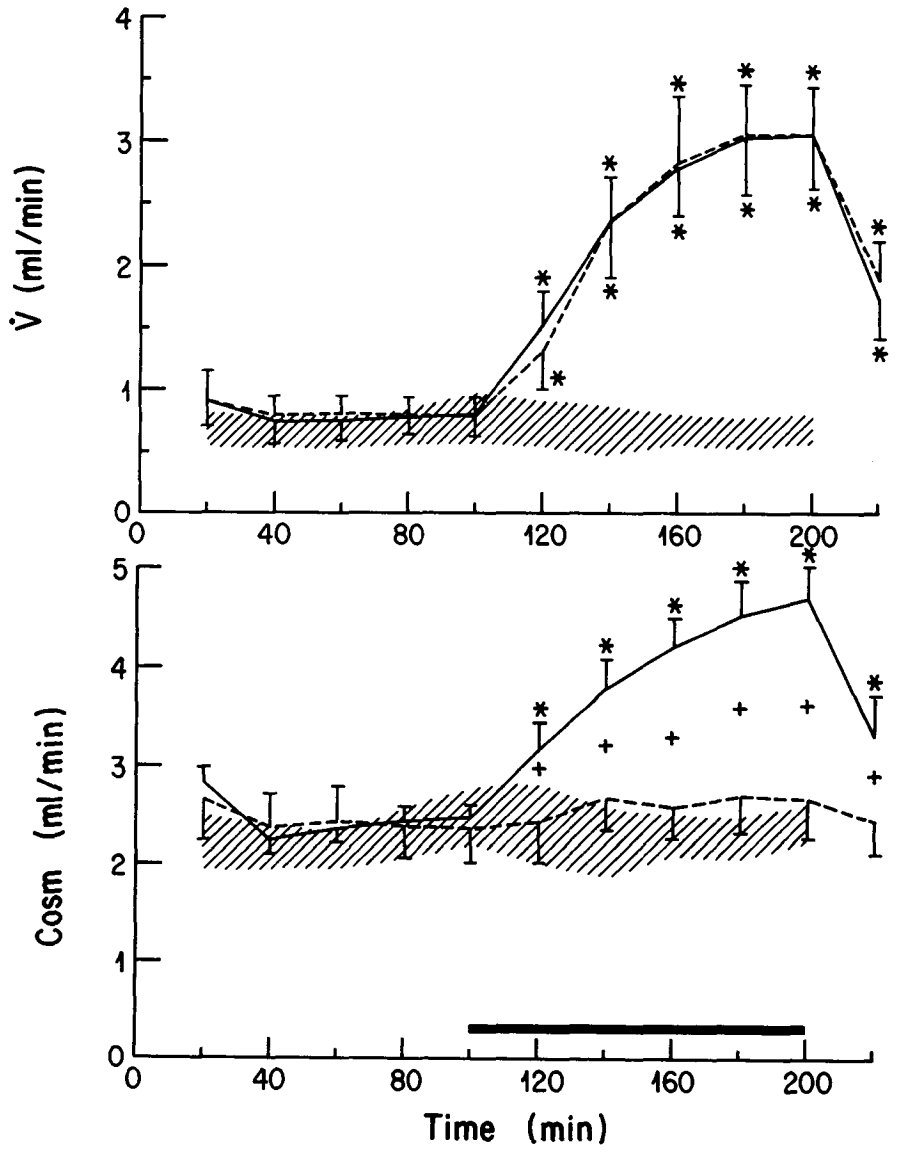

Fig. 5. Time course of urine flow ($\dot{V}$) (top) and osmolal clearance (Cosm) (bottom) responses to WI in INT and CD dogs. Abbreviations as in Fig. 1.

Increased osmolal clearance occurring in the INT dogs was primarily associated with an increase in sodium excretion, which is indicated in Fig. 6 (*top*). The increase in sodium excretion during WI was abolished in CD dogs. Elevated sodium excretion in INT dogs was manifest as an increase in fractional excretion of sodium, also shown in Fig. 6. The latter response indicates that elevated sodium excretion is related to increased tubular rejection of sodium. As expected, increased fractional excretion of sodium did not occur in CD dogs.

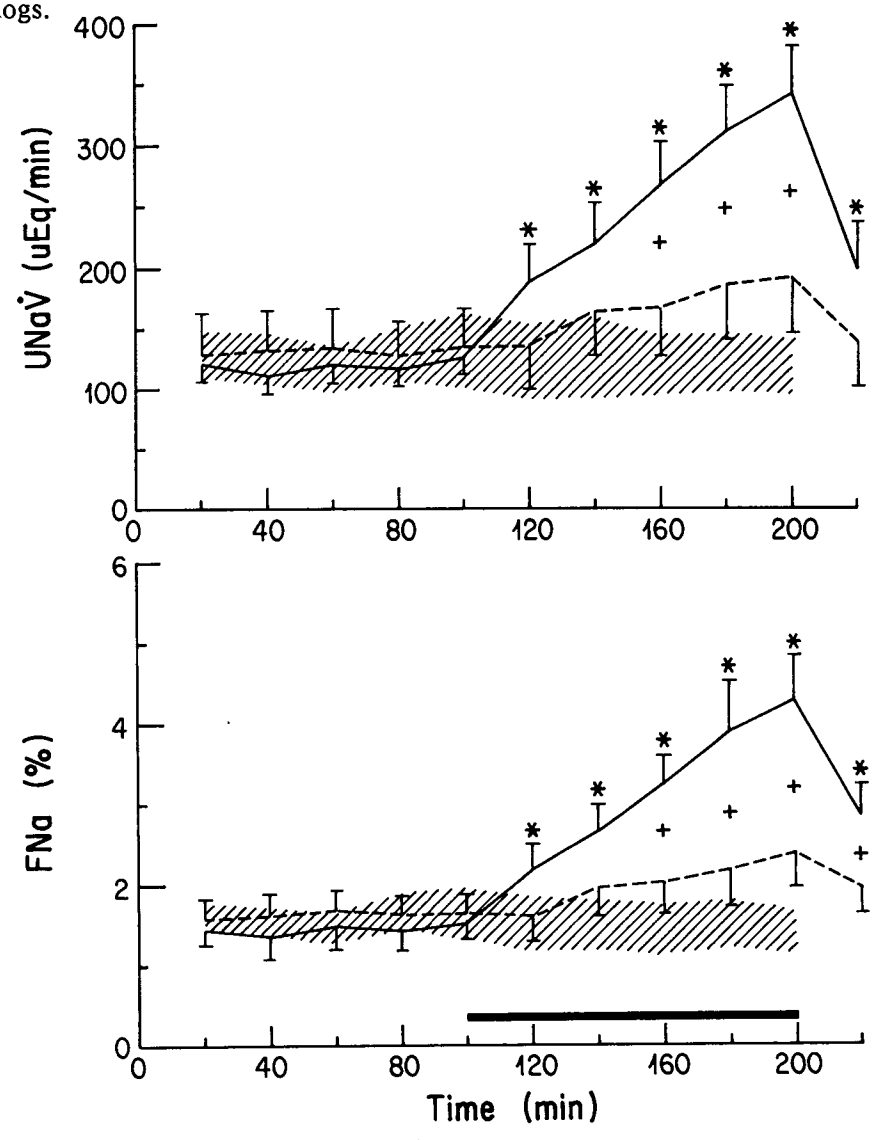

Fig. 6. Time course of sodium excretion ($U_{Na}\dot{V}$) (top) and fractional excretion of sodium (FE_{Na}) (bottom) responses to WI in INT and CD dogs. Abbreviations as in Fig. 1.

Diuresis elicited by WI in INT dogs was not associated with any change in free water clearance (Fig. 7). By contrast, the diuresis occurring in CD dogs was largely related to an increase in free water clearance. This response is reflected in the fact that urine osmolality decreased in the INT dogs, but the urine remained hypertonic to plasma. On the other hand, the urine became hypotonic to plasma in CD dogs (Fig. 7).

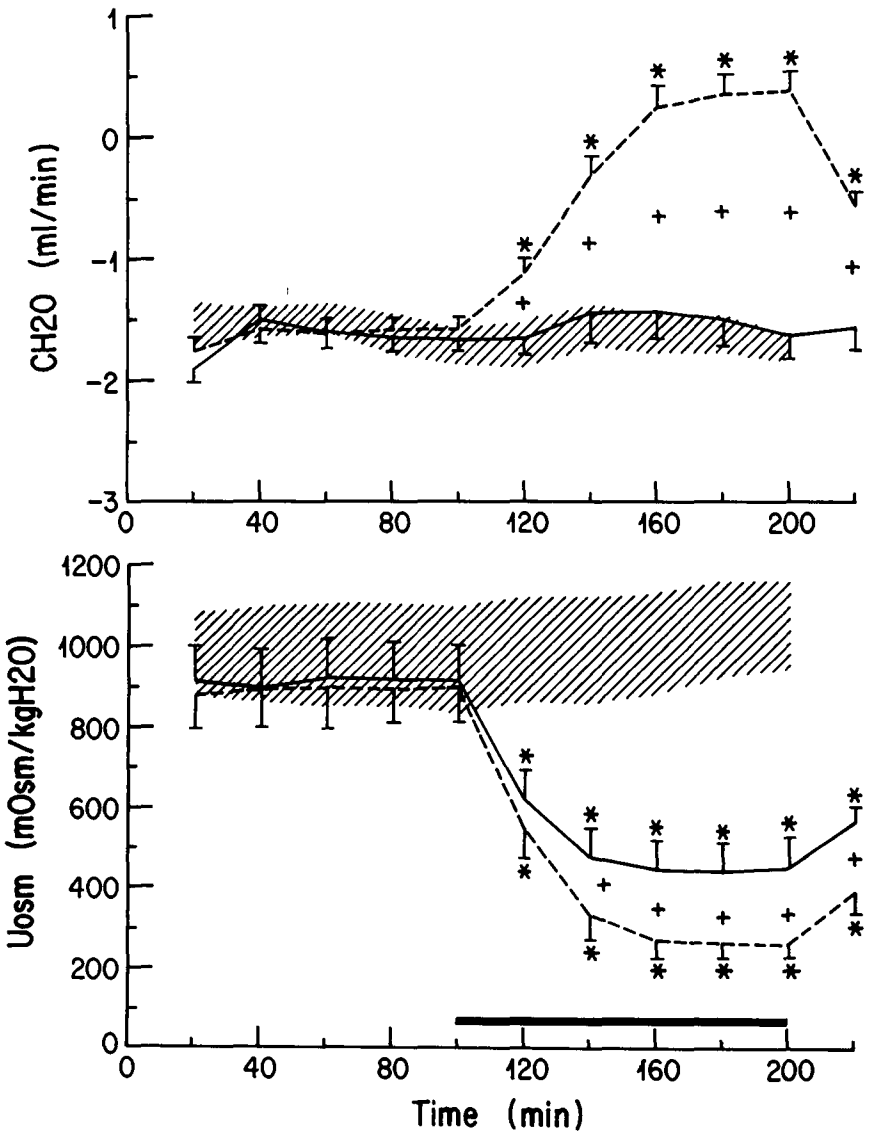

Fig. 7. Time course of free water clearance (CH_2O) (top) and urinary osmolal excretion (UOsm) (bottom) in INT and CD dogs. Abbreviations as in Fig. 1.

Figure 8 (*top*) shows that the renal responses to head-out WI in both groups of dogs were not associated with any significant renal hemodynamic effect in that creatinine clearance did not change. Figure 8 (*bottom*) shows that plasma levels of antidiuretic hormone did not change during WI in INT dogs. However, WI led to a significant reduction in plasma antidiuretic hormone levels in CD animals. Levels of plasma renin activity and plasma aldosterone levels did not change during WI in either group of dogs.

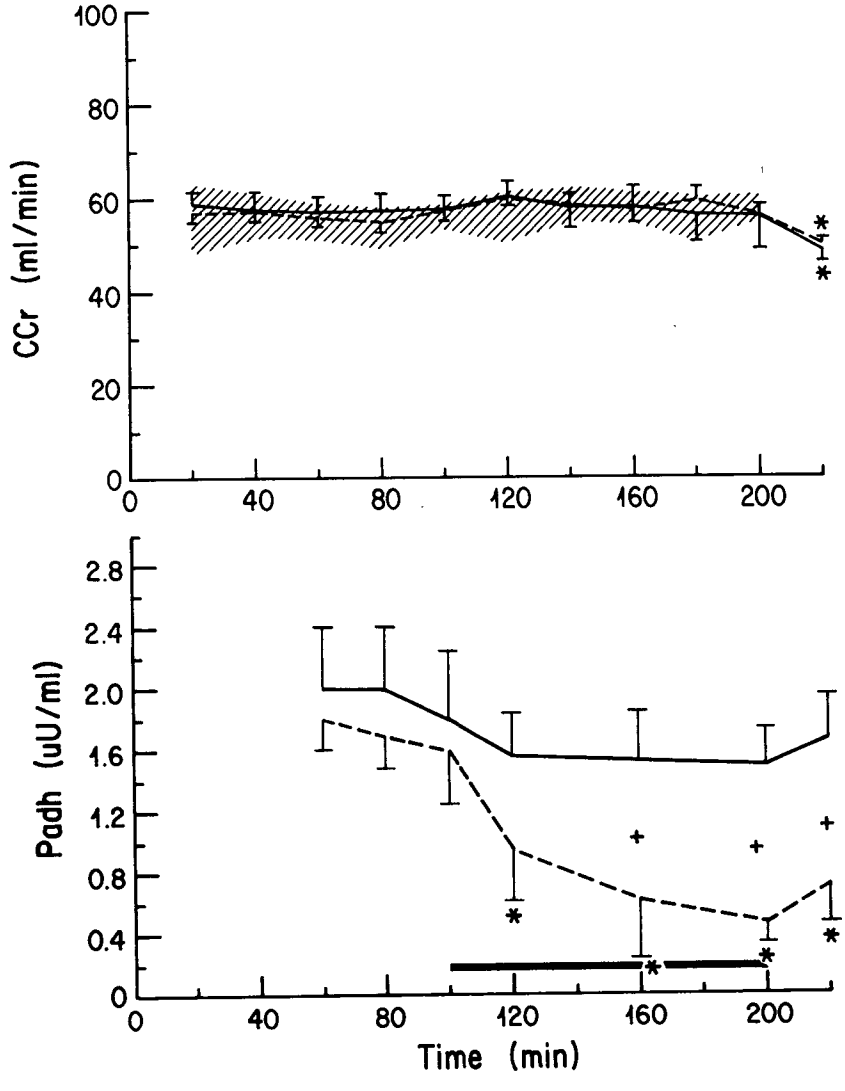

Fig. 8. Time course of creatinine clearance (Ccr) (top) and plasma antidiuretic hormone (Padh) levels (bottom) in INT and CD dogs. Abbreviations as in Fig. 1.

Regional blood flow responses determined by radiolabeled microspheres are shown in Figs. 9-11. Regional flow responses are presented as percent changes from pre-WI levels. It may be recalled from Fig. 1 that cardiac output and mean aortic pressure were elevated to a similar extent throughout WI in both INT and CD dogs. The regional blood flow responses to WI were similar in the INT and CD dogs, with the exception of the flow responses of the hypophysis which are discussed below.

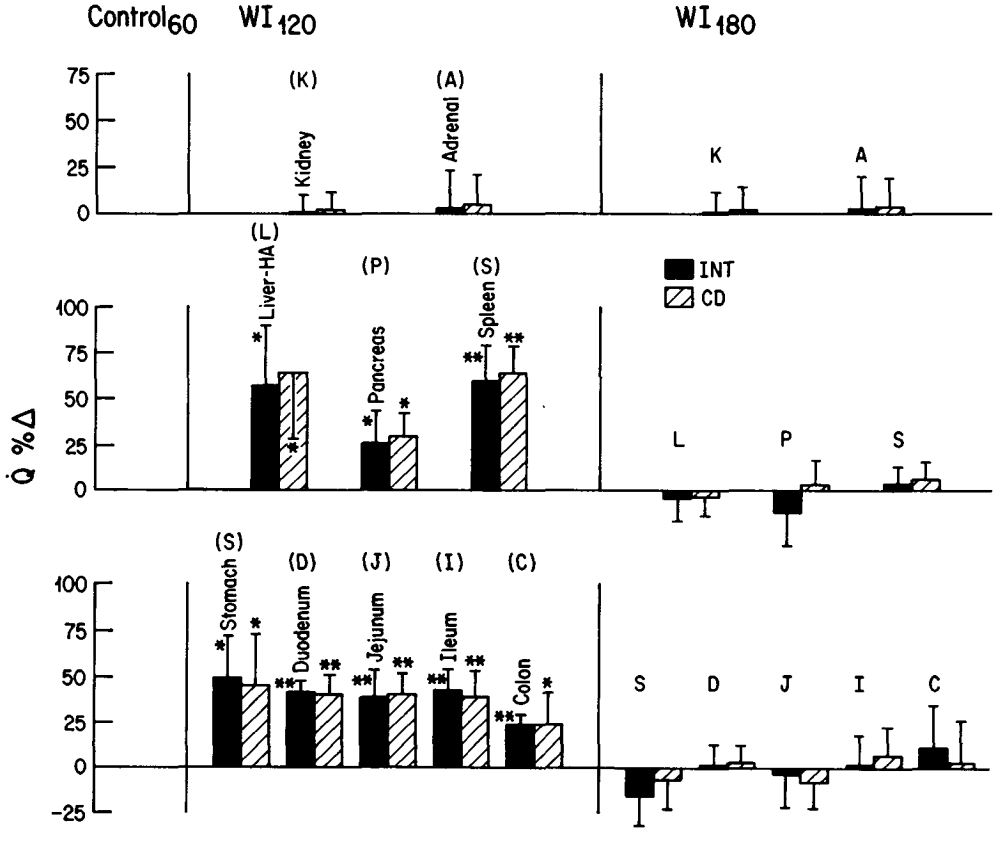

Fig. 9. Present change from control values (60 min) in regional blood flows in abdominal viscera during WI which began at 100 min. Liver flow is for hepatic artery. Solid bars indicate INT dogs, hatched bars indicate CD animals. * Significantly different from control value ($P < 0.05$) or ** = $P < 0.01$).

Figure 9 (*top*) shows that neither renal cortical or adrenal blood flows changed during WI. Since arterial pressure rose, this observation indicates that a vasoconstrictor autoregulatory response occurred in these tissues. On the other hand, interesting regional flow patterns evolved in the other abdominal viscera as seen in the other panel of this figure. Regional blood flows to the pancreas and to the gastrointestinal tract were elevated early in WI to levels

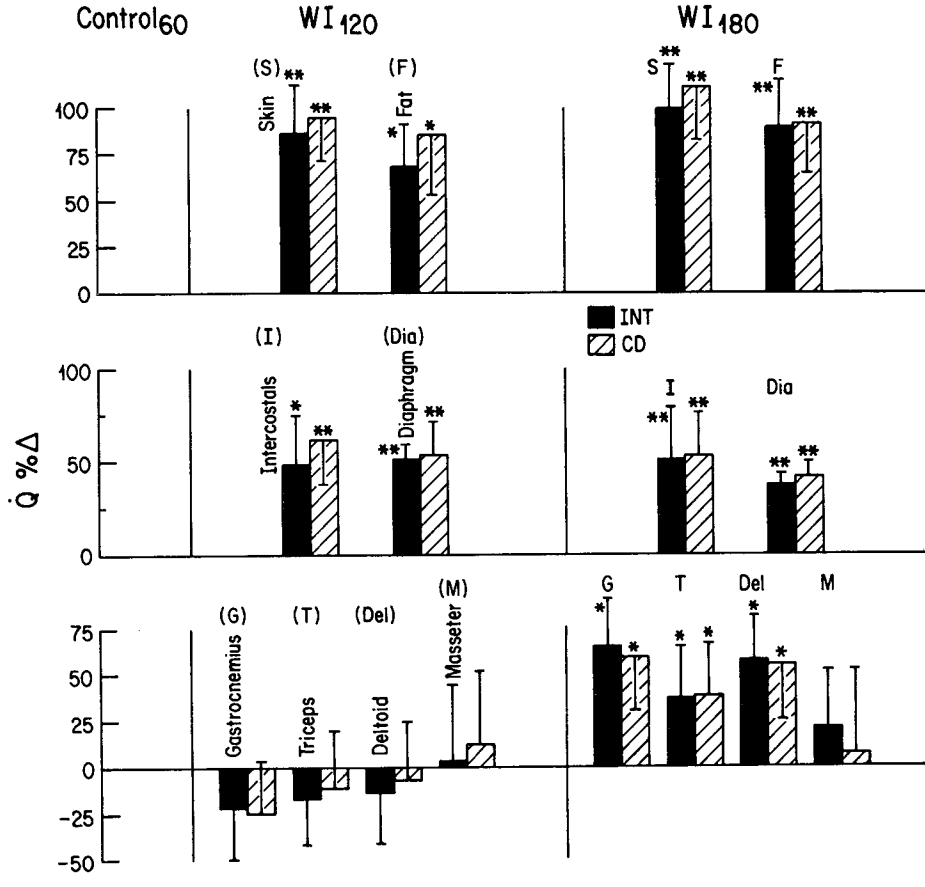

Fig. 10. Percent change from control values for regional blood flows for skin, fat, and muscle in WI. Abbreviations as in Fig. 9.

that were in proportion to the increase in cardiac output. However, blood flows to the spleen and hepatic arterial circuit of the liver were elevated to levels that were disproportionately greater than the increase in cardiac output. These visceral flow responses were time dependent, however, in that a vasoconstriction occurred in these vascular beds later in WI and these regional flows returned to pre-WI levels.

Figure 10 (*bottom*) shows that blood flows to nonrespiratory skeletal muscles did not change significantly during the early phase of the WI response. However, blood flow to these nonrespiratory skeletal muscles increased late in WI, with the exception of blood flow to the masseter which was not immersed. These data indicate that cardiac output is distributed to abdominal visceral organs early in WI and then cardiac output is redistributed in nonrespiratory skeletal muscle later in the WI response. On the other hand, regional blood flows to respiratory skeletal muscles were elevated in a sustained fashion throughout WI as were blood flows to the skin and to adipose tissue.

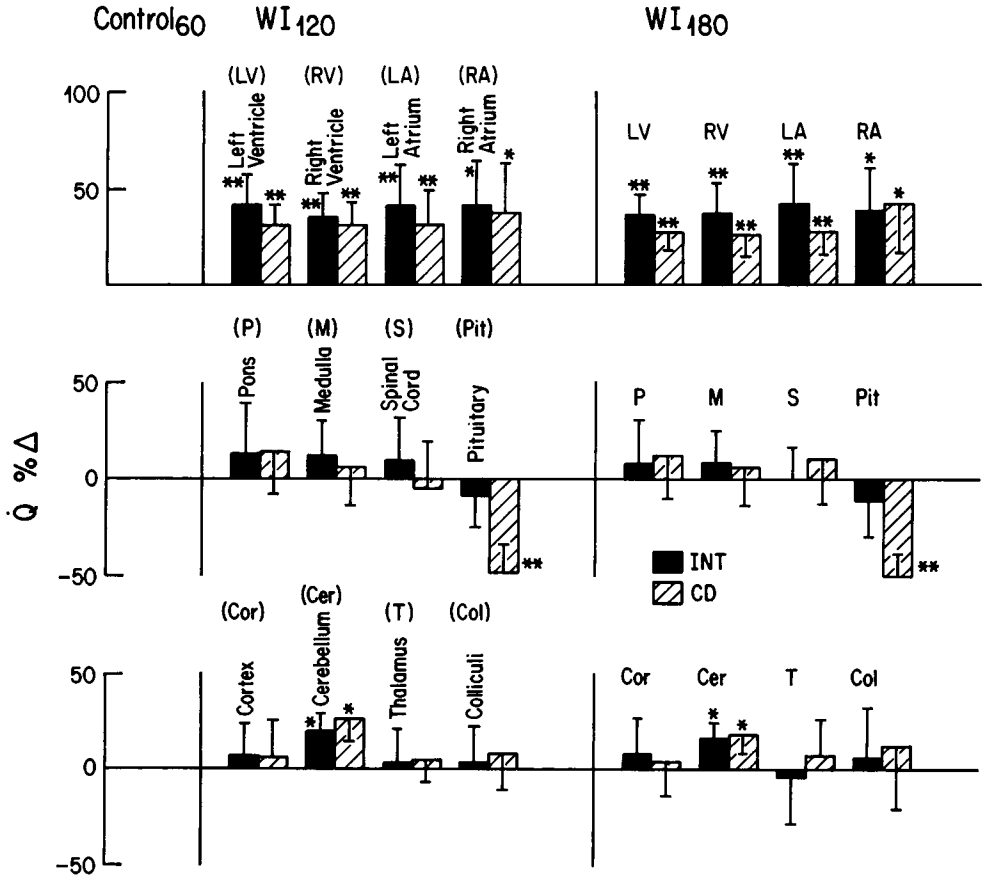

Fig. 11. Percent change from control values for regional blood flows for coronary and cerebral circulation in WI. Abbreviations an in Fig. 9.

Figure 11 (*top*) shows that regional coronary blood flows increased in both the INT and the CD dogs during WI throughout the period of exposure. However, increases in regional coronary flows were significantly greater in INT dogs as compared to CD dogs. The transmural distribution of coronary blood flow across the left ventricular myocardium (endocardial:epicardial flow ratio) was unchanged in either group of dogs during WI.

Regional cerebral flow responses to WI are shown for both groups of dogs in Fig. 11 (*bottom*). Regional blood flows to most parts of the brain were unchanged during WI, indicating that vasoconstrictor autoregulatory responses occurred in these regions. However, blood flow to the cerebellum increased in both groups of dogs during WI. In addition, blood flow to the hypophysis was unchanged during WI in INT dogs. By contrast, however, blood flow to the hypophysis declined significantly in the CD dogs during WI.

As the oxygen consumption remained constant in these conscious dogs during head-out water immersion (WI), the increase in systemic blood flow was well in excess of metabolic requirements. The arterial oxygen tension fell by about 10 Torr during WI. However, arterial blood was still 90% saturated with oxygen and the drop in oxygen tension was not enough to stimulate the arterial chemoreflexes as ventilation and the arterial CO_2 tension were unchanged.

Elevation of cardiac output was similar in magnitude to what we had observed previously in conscious, intact dogs during WI. Since the magnitude of the rise in cardiac output and the time course of the response was similar in sham-operated dogs (INT dogs) as compared to cardiac-denervated dogs (CD dogs), cardiac nerves are not required for an elevation of cardiac output to occur during WI.

However, the mechanism of the rise in cardiac output occurring in INT dogs differs from the mechanism of the rise in cardiac output during WI in CD dogs. The rise in cardiac output in INT dogs was associated with a cardiac acceleration and a rise in left ventricular dP/dt_{max} which indicates a positive inotropic response in the left ventricle. Although a rise in cardiac filling pressures occurred consequent to a rise in venous return of blood to the heart, the rise in heart rate and improved inotropic state of the ventricle presumably prevented an increase in stroke volume from becoming manifest. Inasmuch as cardiac acceleration and positive inotropic response were absent in CD dogs, increased cardiac rate and contractility are dependent on cardiac nerves. In the absence of cardiac nerves, the stroke volume increased and this was entirely responsible for the increase in cardiac output in this situation. The rise in stroke volume occurring in CD dogs was most likely due to a Frank-Starling response elicited by cardiac distention. The rise in heart rate occurring in INT dogs represents the only major difference between dog and man in terms of the physiologic response to WI because the heart rate generally is unchanged or slows during WI in man (7). The rise in heart rate in the dog presumably reflects the existence of a prominent Bainbridge response in the dog. The reason as to why the dog increases its heart rate in WI and man does not is not entirely clear, however. At present, no information is available as to whether left ventricular performance changes in man during WI. In any case, positive cardiac chronotropic and inotropic responses during WI in the dog are dependent on intact cardiac nerves.

Inasmuch as the arterial pressure increased to similar levels in both groups of dogs in the absence of a change in calculated total peripheral resistance, the rise in cardiac output was entirely responsible for the rise in aortic pressure. Hence, WI elicits the translocation of a volume of blood from the venous side of the circulation into the arterial compartment of the circulation. It should be emphasized that the rise in arterial pressure was associated with an elevated heart rate and an unchanged total peripheral resistance in INT dogs, and an unchanged total peripheral resistance in CD dogs. This implies that the operating point of the high-pressure sinoaortic

baroreflex must be changed in WI, because normally an elevation of arterial pressure of this magnitude would lead to reflex slowing of the heart rate and systemic vasodilation. Since the total peripheral resistance was not reflexly altered in the expected direction in CD dogs it is probable that a central input from the cardiac receptors is not responsible for changing the operating point for this component of the baroreflex.

Urine flow responses were similar in terms of the magnitude and time course of diuresis responses for both INT and CD dogs. The observation that diuresis curves can be superimposed is rather striking. However, mechanisms for diuresis responses were quite different for the 2 groups of dogs.

It should be emphasized that renal responses were not associated with any changes in blood flow to the kidneys because there were no changes in glomerular filtration rate as measured by creatinine clearance and no changes in renal cortical blood flow as measured by radiolabeled microspheres. On the other hand, renal vasoconstriction occurred because arterial pressure increased in both groups of animals and this response probably reflects renal vascular autoregulation. Since renal blood flow and glomerular filtration rate were constant, renal responses probably reflect primarily alterations in renal tubular function.

Osmolal clearance increased in INT dogs, which was primarily related to increased sodium excretion and fractional excretion of sodium, because potassium excretion did not change significantly. By contrast, a diuresis of equivalent magnitude and time course was achieved in CD dogs by an increase of free water clearance, whereas osmolal clearance and sodium excretion did not change significantly in these animals. Therefore, cardiac nerves play a major role in the natriuretic response of the kidney in the conscious dog during WI. The natriuretic response may be mediated largely via a reflex reduction in renal sympathetic nerve activity caused by mechanical loading of cardiac receptors during WI. Prosnitz and DiBona (8) have shown that renal sympathetic nerves can have significant influences on renal sodium reabsorption when stimulated at levels that do not alter renal hemodynamics. In addition to a cardiac receptor-dependent neurogenic natriuresis occurring, it is also possible that other factors, such as the release of atrial natriuretic peptides, may contribute on the humoral basis to the natriuresis. However, although we have demonstrated that atrial natriuretic factor levels increase in plasma during WI in the conscious, intact dog, the time course of the increase in atrial natriuretic factor is not well correlated with the time course of the WI natriuretic response (9). In addition, Goetz et al. (10) have demonstrated that, while an increase in plasma levels of atrial natriuretic factor along with a natriuresis can be elicited in the conscious dog during left atrial balloon inflation, the natriuretic response to left atrial balloon inflation is abolished in CD dogs, yet plasma levels of atrial natriuretic factor still increase. Therefore, a clear role for atrial natriuretic factor in mediating the natriuresis of WI remains to be demonstrated.

The persistence of a diuresis of comparable magnitude and time course in

CD animals indicates that redundant mechanisms exist which allow for elimination of fluid from the body during WI. Conversion of the diuretic response from an osmolar diuresis to a water diuresis in CD animals was associated with a significant decrease in plasma levels of antidiuretic hormone (ADH). Plasma ADH levels were unchanged during WI in intact animals along with plasma renin activity and aldosterone levels. The unmasking of a significant decrease of plasma ADH after CD implies that, contrary to several views (1, 3), input from cardiac nerves actually acts to promote the release of ADH during WI in intact animals. Conventionally, it is generally thought that left atrial distention during volume expansion usually leads to a reflex depression of ADH.

The fall of plasma ADH in CD dogs was probably primarily responsible for the water diuresis occurring during WI. Our measurements of regional blood flow to the hypophysis indicate that hypophyseal blood flow was unchanged during WI in INT dogs, whereas hypophyseal blood flow fell significantly during WI in CD dogs as did plasma levels of ADH. Indeed, in 2 CD dogs we were able to determine that the fall in hypophyseal blood flow was specifically due to a decline in neural lobe blood flow. As the neural lobe is the major source of arterial blood supply for the hypophysis, this observation suggests the possibility that ADH secretion may be coupled to blood flow in this situation. It is additionally possible that a decline in metabolic activity of the ADH secreting neurons may lead to a decline of neural lobe flow.

With the exception of the aforementioned hypophyseal flow responses, regional blood flow responses to WI were similar in both intact and CD dogs. Therefore, cardiac nerves play little role in determining these regional flow response patterns.

Blood flows to skin and adipose tissue were elevated by the largest amount as compared to other tissues during WI, and flow elevations were sustained throughout the exposure. As there was no change in core temperature in these dogs, the increased flows to skin and fat probably represent responses to superficial heating of these tissues with the dogs going from air at 26°C to water at 37°C (5).

In addition, there were sustained elevations of blood flows to respiratory muscles during WI. These responses are similar to those we observed previously in anesthetized dogs (5) and are related to the increased work of breathing associated with hydrostatic compression of the chest wall.

Regional coronary blood flows increased in a sustained fashion in both groups of dogs during WI. These responses reflect the increased work of the heart associated with central translocation of blood in WI. However, elevation of regional coronary flow was less in CD dogs than in INT dogs. This attenuated response is probably related to the difference in the cardiac response occurring in the 2 groups of dogs. The oxygen demand of the heart in INT dogs was probably greater because of increased heart rate and time-tension work in these animals as compared to CD dogs in which the work of the heart was increased primarily by increasing stroke volume (2).

Increased cardiac output was initially directed to the abdominal viscera

early in WI. Subsequently, later in WI, the elevated cardiac output was redistributed in a time-dependent fashion toward nonrespiratory skeletal muscles. Therefore, skeletal muscle was initially vasoconstricted during WI and this was followed later by a muscle vasodilation. Conversely, a vasoconstrictor response occurred in the abdominal viscera later in WI. The mechanisms involved in this interesting time-dependent shift in the distribution of cardiac output are unclear at present. One possibility is that time-dependent changes in the activity of sympathetic vasoconstrictor nerves to these vascular beds could occur. Another possibility could be related to local changes in vasoactivity involving regional autoregulatory mechanisms. For example, delayed vasoconstriction in the viscera may represent a time-dependent response to a wash-out of vasodilator metabolites in association with the initial hyperemic response. On the other hand, delayed vasodilation in skeletal muscle may represent a local response of muscle vessels to warming of the tissue in the thermoneutral state. In addition, there is a rise of interstitial fluid pressure in muscle during WI which could lead to relaxation of muscle arterioles on a myogenic basis (11). Indeed, this delayed muscle vasodilation may act to "steal" blood flow away from the viscera and visceral vasoconstriction may simply be compensatory. Further experiments are required to sort out these hypotheses.

Regional cerebral blood flows were largely unchanged during WI. Therefore, autoregulation of brain-blood flow occurred. However, we observed selective increases in blood flow to the cerebellum during WI in both groups of dogs. The cerebellum receives somatic afferent inputs from many peripheral receptor groups, including muscle spindles and tendon and joint receptors, along with the vestibular input. Because of the buoyant effect of WI, which leads to a relief of postural stress in a situation that simulates weightlessness, it is probable that changed somatic input profile in this situation may alter the activity of cerebellar neurons which may in turn be responsible for this interesting blood flow response. It has been known for some time that the cerebellar fastigial nucleus plays an important integrative role in mediating arterial pressure regulation in orthostasis and postural adjustments. In addition, it has been shown that stimulation of the fastigial nucleus can bring about a resetting of the baroreceptor reflex (12). Therefore, the selective cerebellar blood flow response may reflect changed local activity in this neural region in response to altered somatic, vestibular, and postural inputs during WI.

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SIMULTANEOUS OBSERVATION IN RESPIRATORY AND CIRCULATORY RESPONSES IN MAN AFTER FACIAL APPLICATION OF ICE BAG

Y. Honda, Y. Sakakibara, T. Morikawa, Y. Tanaka, and W. Nakamura

When the trigeminal area of the face, particularly the regions innervated by its ophthalmic branch, comes into contact with water, the diving response is known to be elicited (1). A number of studies have been made on cardiovascular alterations with this condition. To our knowledge, however, no detailed simultaneous measurements of respiratory as well as circulatory responses to a cold compress with water were conducted in man. Both respiratory and circulatory variables were noninvasively and continuously measured in the present study.

MATERIALS AND METHODS

Experiment A: Ice-bag Application

Six healthy males, 28 to 59 yr old were examined. Diving response was induced by applying an ice bag, previously soaked in ice water, on the forehead and cheeks for 1 min. The temperature of the ice bag was 1 to 3°C, and the trial was repeated 3 times for each subject. Throughout the experiment, the subjects were blindfolded by covering the orbital region with an elastic hairband.

Breath-by-breath measurements in tidal volume (V_T, respiratory frequency (f), minute ventilation ($\dot{V}$), and end-tidal P_{CO_2} and P_{O_2} ($P_{ET_{CO_2}}$ and $P_{ET_{O_2}}$, respectively) were conducted by using a 9-liter, closed-circuit respirometer with a CO_2 absorber and a continuous supply of O_2 from a cylinder. $P_{ET_{O_2}}$ was maintained at approximately 120 to 150 mmHg, i.e., a hyperoxic condition.

The circulatory variables were continuously measured using a computer-based, on-line system developed by Miyamoto et al. (2). Four stainless steel

strips were arranged around the chest wall and the neck. Stroke volume (SV), heart rate (HR), and cardiac output ($\dot{Q}$) were automatically determined by signals from these electrodes. The computer sampled the first derivative and the basic values of the transthoracic impedance, dZ/dt and Zo, at a rate of 200 times/s when triggered by the R wave of ECG. The sampling was repeated for a number of cardiac cycles during spontaneous breathing. Usually, repetition for 6 to 12 cycles was sufficient to obtain an averaged wave form with a favorable signal-to-noise ratio for computer processing.

Experiment B: Wet-cloth Application

To ensure the more complete response, the whole facial surface was covered by a wet cotton sheet for 2 min. In addition, cold water previously kept in a refrigerator was infiltrated by dripping from the reservoir. The water temperature of the wet cloth was 11 to 13°C, which was about 10°C higher than in experiment A. In this experiment, the effect of breath holding lasted for 30 s and was examined during room-air breathing as well as face immersion. Fourteen healthy male subjects aged 19 to 39 participated in this experiment.

Breath-by-breath measurement of the respiratory variables was obtained from the signal of a hot-wire flowmeter (Sanei 1H 21A, Tokyo) which was inserted between a mouthpiece and a respiratory valve. The subjects breathed room air by open circuit during the test. Oxygen uptake ($\dot{V}O_2$) and carbon dioxide output ($\dot{V}CO_2$) were also measured by collecting expired air in the control as well as the face-immersion period.

Continuous on-line measurements of the circulatory variables were conducted, as in experiment A.

RESULTS

Experiment A

The observed changes in response to ice-bag application are illustrated in Figs. 1 and 2. Differences from the control values were plotted at 10-s intervals.

As to the circulatory parameters seen in Fig. 1, HR consistently decreased throughout the test period whereas SV was markedly depressed in the initial 10 s, then significantly increased during the next 20 to 30 s, showing a biphasic response. Accordingly, $\dot{Q}$ was initially depressed, then leveled off at nearly the control magnitude.

Contrary to the circulatory variables, V_T, f, and $\dot{V}$ showed random variation. Significant change was seen only in f at the 20-s value. However, $P_{ET}CO_2$ significantly lowered at 10, 20, and 40 s. This result seems to indicate that alveolar ventilation was stimulated rather than inhibited.

Experiment B

Figures 3 and 4 show the ventilatory and circulatory variables observed.

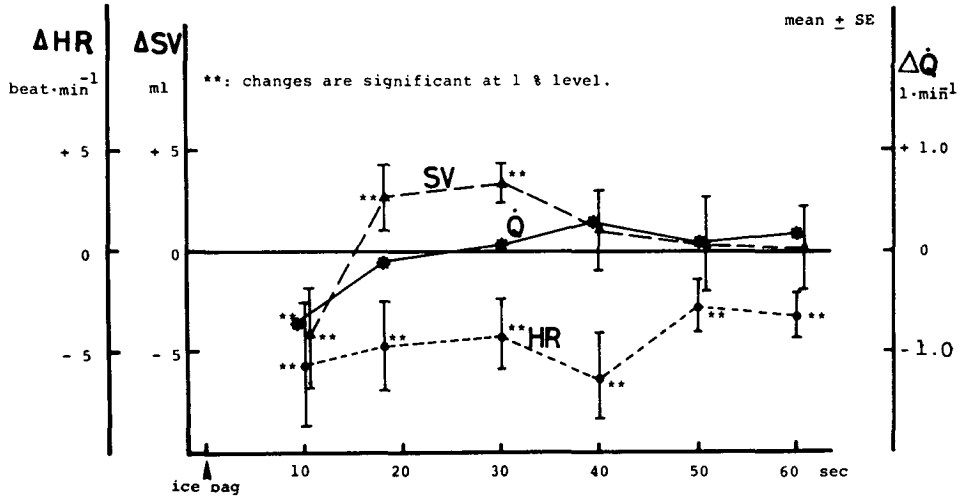

Fig. 1. Mean circulatory responses to facial application of ice bag in 6 normal males. Values are expressed as changes from the pre-diving level. Vertical bar: mean ± SE.

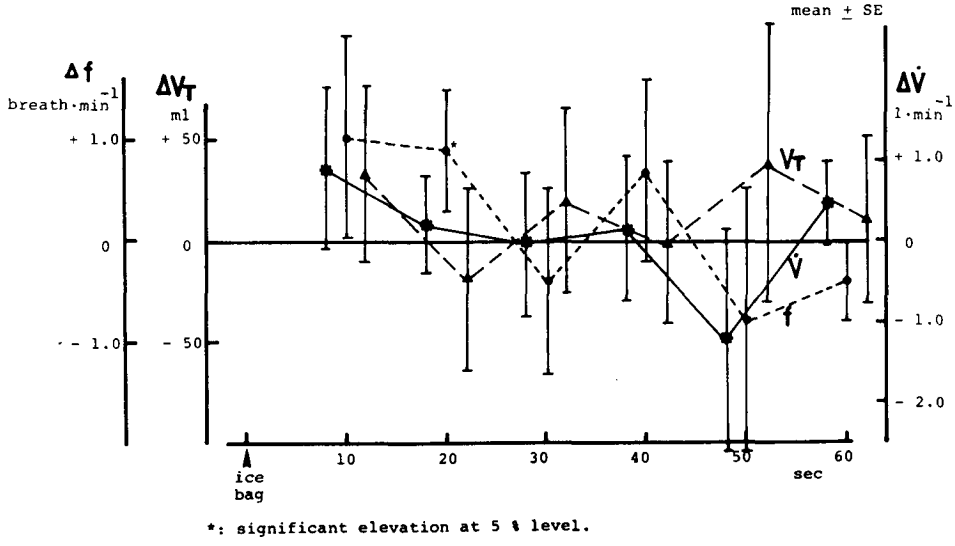

Fig. 2. Mean respiratory responses to facial application of ice bag in 6 normal males.

As in experiment A, HR was decreased in all subjects except 1, whereas the change in SV was not consistent. Due to HR depression, $\dot{Q}$ was significantly decreased. No significant change was observed in the ventilatory parameters

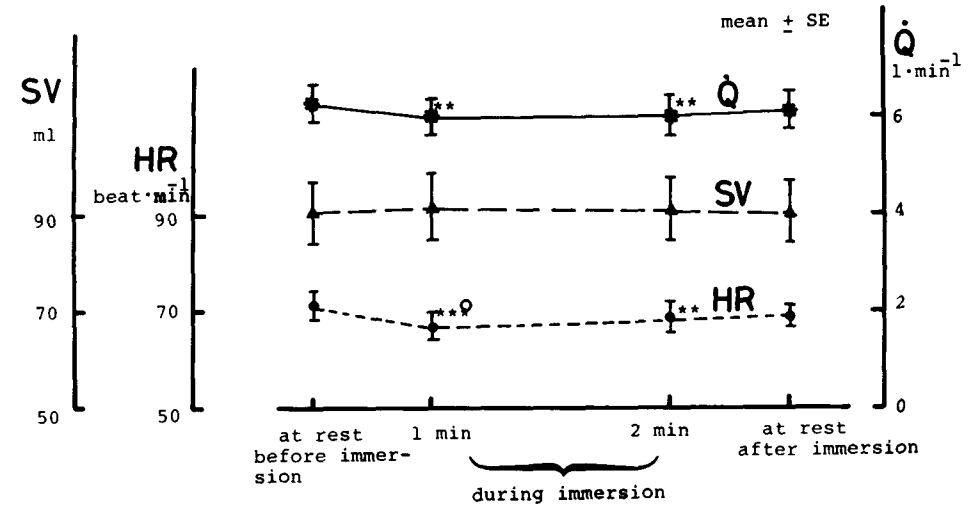

Fig. 3. Mean circulatory responses to facial immersion of 14 normal males. Nonapneic diving response was elicited for 2 min by covering the whole facial area with a wet cotton cloth.

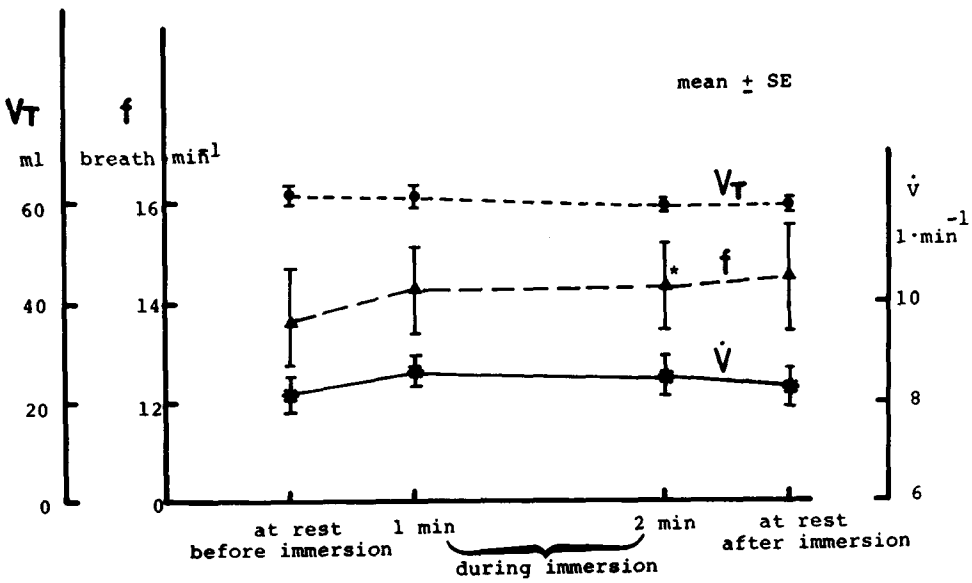

Fig. 4. Mean ventilatory responses to facial immersion of 14 normal males.

except in f at the 2nd-min value, as indicated in Fig. 4. $P_{ET_{CO_2}}$ was also not changed significantly. On the whole, respiratory data agree with those obtained in experiment A.

No significant differences in $\dot{V}O_2$ and $\dot{V}CO_2$ were seen before, during, or after face immersion. In Fig. 5, effects of breath holding on the circulatory variables were compared with those of face immersion. The *right columns* (Fig. 5) indicated the total response to breath-hold plus face immersion. The magnitudes of breath-hold response were almost the same between air and during face immersion and 4 to 5 times the nonapneic face immersion.

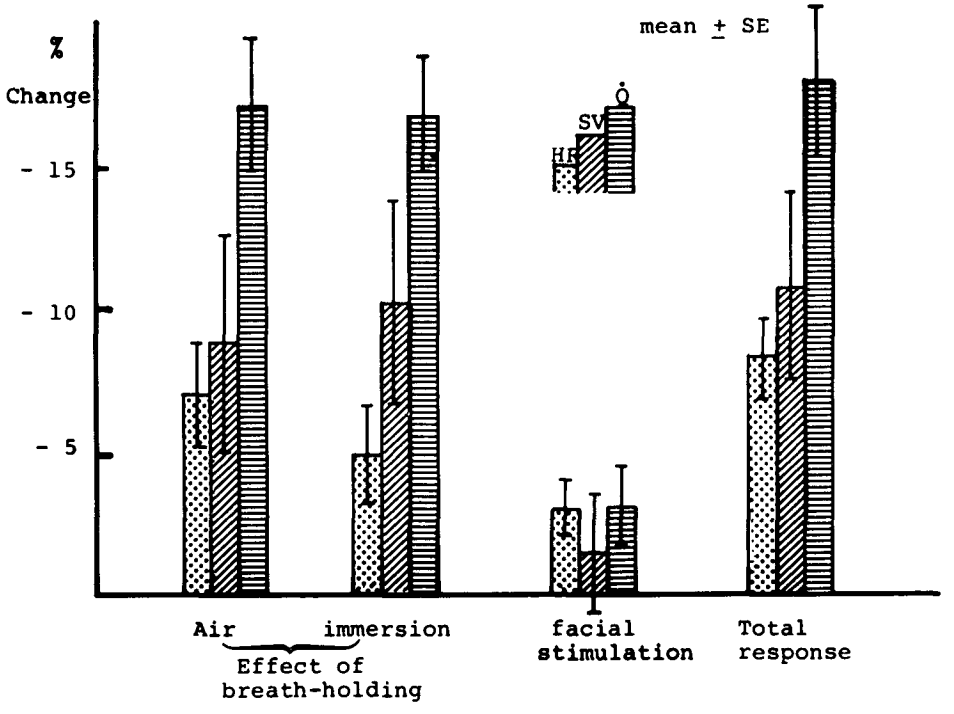

Fig. 5. Changes in circulatory parameters in response to breath holding and face immersion. Two groups in left column illustrate the effect of breath holding in air and in face immersion, respectively. Second-right-column group indicates the effect of nonapneic diving response. Right-column group indicates total diving response (face immersion + breath holding).

DISCUSSION

Nonapneic Diving Responses by Ice-bag and Wet-cloth Application

Although the magnitude of HR depression in response to both face-immersion procedures with spontaneous breathing in the present experiment was smaller than those seen in breath-hold diving, its decrement was always consistent. The degree of HR depression from the ice-bag stimulation was about twice the wet-cloth application. Since the applied temperature in the

former was about 10°C higher than the latter, this result was considered to be a temperature-dependent effect as reported in the case of breath-hold diving in humans (3-5). The same finding was reported by Kobayashi and Ogawa (6). Therefore, it seems certain that the diving response was elicited in our experiment. The present data agree with those of Kobayashi and Ogawa (6), Kawakami et al. (7), Paulev (4), Heistad and Wheeler (8), Whayne et al. (9), Folgering and Olivier (10), and Mukhtar and Patrick (11). On the other hand, Sasamoto (12) and Lin et al. (13) claimed that simple face immersion did not elicit a bradycardia unless accompanied by breath holding. Recently, Bjertness et al. (14) reported that a subject exercising on the bicycle ergometer, who was breathing via a snorkel with face immersion, exhibited no bradycardia. Contrary to HR, SV tended to increase throughout the test period except immediately after ice-bag application. It increased significantly at the 20th and 30th s in experiment A. In experiment B, SV was also slightly increased during immersion, although not significantly.

We found no significant depression of the ventilatory parameters in the nonapneic diving response. Instead, f increased significantly in a few observations. This result is at variance with the data reported by Folgering and Olivier (10) and by Mukhtar and Patrick (11). Although the depression of ventilation found by those authors is transient and fluctuated with time, an inhibitory ventilatory drive seems to exist, because breath-holding time was significantly increased during face immersion (11). Perhaps face immersion into water would have elicited a better response (Elsner RW, personal discussion). We may have failed to obtain depressed ventilation because the nonspecific afferent information from the cutaneous cold receptors stimulated ventilation and thus counteracted the diving response (11, 15). At any rate, the present study demonstrates that nonapneic diving responses do not elicit parallel changes between circulatory and ventilatory variables.

Relative Contribution of Nonapneic Face Immersion and Breath Holding in the Diving Response

As shown in Fig. 5, the magnitude of circulatory depression due to breath holding is 4 to 5 times that of nonapneic face immersion. Therefore, depression of circulatory activity during breath-hold diving was assumed to be mainly derived from cessation of breathing, which causes inhibition of pulmonary vagal afferent discharge to the cardiovascular regulation centers (16). The relative contribution of these two mechanisms in humans is controversial. Sasamoto (12), Lin et al. (13), and Bjertness et al. (14) claim that during face immersion the bradycardia appeared only when accompanied by breath holding, whereas Kawakami et al. (7) and Whayne et al. (9) observed the same degree of HR depression in both conditions. In agreement with the present study, Paulev (4) and Mukhtar and Patrick (11) found that breath holding played a dominant role in diving response. It must be noted that in natural diving with whole-body immersion, the central blood shift due to hydrostatic effects elicits other important alterations in cardiovascular activities (17).

Comparison of Diving Response Elicited by Breath Holding in Air and During Face Immersion

We have demonstrated that the effects of breath holding on the circulatory parameters were almost the same in air breathing as well as during spontaneous breathing with face immersion (Fig. 5). Variable observations on this matter have been reported in the literature. Most of them (3, 4, 8, 9, 12, 18, 19, 26) found stronger HR depression in response to breath holding in water than in air. On the other hand, Paulev (4) obtained almost the same results as our study. No clear explanation for this discrepancy is possible at present.

In summary, we concluded that:

1. The degree of nonapneic diving response in healthy males appears to be different when comparing ventilatory and circulatory parameters. The changes were generally consistent in the latter and random in the former.
2. Among the circulatory variables, depression of HR was seen most clearly, whereas SV changes were for the most part insignificant.
3. Significant increases were seen in the respiratory frequency in a few observations during the diving period.
4. The magnitude of depression in the circulatory parameters by breath holding was found to be 4 to 5 times the depression by nonapneic face immersion.

Thus, an oxygen conservation response, well documented in the diving species (1, 5), was not demonstrated in the present experiment in humans.

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SESSION 8: POSTER PRESENTATIONS
HEALTH AND SAFETY OF DIVERS

9th International Symposium on Underwater and Hyperbaric Physiology
Undersea and Hyperbaric Medical Society, Bethesda, MD, 1987

DOPAMINE RELEASE IN A HELIUM-OXYGEN HYPERBARIC ENVIRONMENT

J. C. Rostain and C. Forni

During exposure to high pressure of a helium-oxygen mixture, men and animals developed a high pressure nervous syndrome (HPNS) (1-5). Characteristics of this syndrome suggested that this hyperbaric environment induces some disturbances in neurotransmission. Several authors have suggested the role of monoamines in the occurrence of some of the symptoms of HPNS (6-8). We have studied the effects of high pressures of a helium-oxygen mixture on the release of electrochemically monitored dopamine in the striatum of free-moving golden hamsters.

METHODS

Differential pulse voltametry was used to detect the electrochemical current produced by the oxidizable molecules according to the method developed by Adams (9), Gonon et al. (10), and Marsden (11). The experimental device consisted of a reference electrode and an auxiliary electrode consisting of a small stainless steel screw, and a working electrode made of a rigid rod containing 10,000 carbon fibers built according to the techniques described by Forni (12) and Forni and Nieoullon (13). The working electrodes were calibrated in vitro before each implantation, according to the method of Forni and Nieoullon (13).

In vivo experiments were performed on golden hamsters (100-150 g), with the working electrode chronically implanted under general anesthesia (pentobarbital 90 mg/kg i.p.) in the striatum on both sides of the brain and the reference and auxiliary electrodes fixed in the bone.

Initially, D-amphetamine (3 mg/kg i.p.) or pargyline (90 mg/kg i.p.), which is known to increase the extracellular level of dopamine, was

administered to verify the electrochemical response corresponding to the increase of dopamine.

Subsequently, the free-moving hamsters (n = 8) were compressed in a pressure chamber to 81 bar (equivalent to 800 msw) with helium at a rate of 0.5 bar/min (duration 2 h 40 min). Oxygen was maintained at 0.4 bar. Temperature was progressively increased from 25 to 33°C to ensure the thermal comfort of the animals. The stay at 81 bar lasted 4 h, and decompression, 14 h. Control measurements were performed before and after each experiment at pressure, for a time equivalent to the hyperbaric experiment.

In addition, the effect of the changes of temperature (increase from 22 to 35°C and decrease from 22 to 12°C) and partial pressure of oxygen (increase to 0.4-0.5 bar and decrease to 0.14 bar) on dopamine release were examined (n = 5 in each situation).

RESULTS

Control measurements during 24-h periods indicated that the electrochemical responses had a variability of ± 10%.

D-Amphetamine or pargyline administration elicited an increase in the amplitude of the electrochemical response detected in vivo in the caudate nucleus of free-moving hamsters (D-amphetamine: mean increase 46.6% ± 16.3% from control values; n = 6. Pargyline: mean increase 67 ± 10%; n = 6).

Helium-oxygen hyperbaric environments induced an increase in the amplitude of the electrochemical response detected in the striatum of the hamsters (n = 8). This increase occurred during the compression between 11 and 50 bar before the appearance of HPNS clinical symptoms, such as tremor (68 ± 9 bar; n = 7) and myoclonia (81 bar; n = 6). We did not observe convulsions with our experimental conditions (compression up to 81 bar).

Increase of the electrochemical response (which was 30-150% according to the animals) persisted during the 4-h stay. During the decompression, a new increase appeared from 61 bar onward, in some animals it was larger than during compression (up to 370%). The return to control values was recorded at the end of decompression but often several hours after.

The experiments without pressure, in which temperature increased from 22 to 35°C or decreased from 22 to 12°C, did not induce significant changes in the electrochemical responses recorded in the striatum. The increase of partial pressure of oxygen to 0.4 to 0.5 bar did not change significantly the electrochemical response. Only the decrease of partial pressure of oxygen to 0.14 bar seemed to reduce the dopamine release in the striatum of some animals.

DISCUSSION

The high pressure of helium-oxygen mixtures (up to 81 bar) induced an increase in the electrochemical responses recorded in the striatum of the golden hamsters. The experiments carried out at the surface with drugs that

enhance the extracellular level of dopamine indicated that the electrochemical responses recorded by the electrochemical device are related to the increase of this neurotransmitter. The work of Forni and Nieoullon (13) with this type of indwelling electrode demonstrates that it is sensitive to dopamine. Differential pulse voltamograms recorded during the experiments at pressure show an analogous signal at the same applied potential. Consequently, the increase in amplitude of the electrochemical response to the hyperbaric environment might be related to an increase in dopaminergic activity. In some animals this increase would be higher than those recorded with D-amphetamine and pargyline. The results obtained at the surface in hypothermic or hyperthermic environments and those recorded in hyperoxic or hypoxic environments indicate that changes in temperature or partial oxygen pressure in the range used during hyperbaric experiments do not affect electrochemical responses.

In the golden hamsters, the increase in dopamine release in the striatum occurs before the first clinical symptoms of HPNS, such as tremor and myoclonia. Consequently, it seems that high pressures of helium-oxygen up to 81 bar induce changes in dopaminergic activity and these changes may be related directly or indirectly to HPNS.

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EFFECT OF NITROGEN-BASED, FIRE-RETARDANT ATMOSPHERES ON VISUAL AND MENTAL PERFORMANCE

D. R. Knight, S. M. Luria, J. F. Socks, and W. Rogers

Low concentrations of oxygen create fire-retardant atmospheres that do not support flaming combustion. For example, 13% oxygen in nitrogen retards the burning of paper (1) and extinguishes liquid hydrocarbon fires (2). In inhabited spaces, the greatest protection from fire theoretically exists at the lowest concentration of oxygen tolerated by man.

Human life can be supported by very low concentrations of oxygen if the atmosphere is sufficiently pressurized to set the partial pressure of oxygen (Po_2) at 160-300 Torr (3). The reason is that it is the partial pressure, not molar fraction of atmospheric oxygen that determines the amount of oxygen transported by blood to the cells (4). The lowest feasible concentration of oxygen would then be the minimum Po_2 that can be tolerated divided by the maximum barometric pressure (PB) that does not produce decompression sickness. What are these values?

Visitors and residents are not adversely affected by hypoxia at the altitude of Denver, where the Po_2 is 130 Torr. Nor do divers develop decompression sickness when exposed to compressed air at PB as high as 1194 Torr (5). If these are considered the limiting values, then the lowest tolerable oxygen level in a nitrogen-based atmosphere that is safe for extended exposure is 11%, their quotient.

Reports that a Po_2 of less than 130 Torr impairs visual sensitivity have been published (6, 7). According to the above reasoning, it may be that 11-13% oxygen degrades vision at sea level, where the Po_2 is 87 to 99 Torr, but would have no effect when hyperbaric pressure maintains the Po_2 above 129 Torr. Therefore, we exposed men to 21, 17, and 13% oxygen at normal atmospheric pressure and 13% oxygen in hyperbaric air (970 PB). We postulated that the 17% oxygen and hyperbaric 13% oxygen would lead to the same degree of hypoxia because they resulted in the same oxygen partial pressure (approxi-

mately 130 Torr), but that 13% oxygen would lead to greater hypoxia because of its lower partial pressure (99 Torr). Based on the available evidence, we expected to find decrements in visual and mental performance at Po_2 99 Torr.

In a separate study, humans were exposed to 21 and 11.5% oxygen at normal atmospheric pressure and 11.5% oxygen in hyperbaric air (1141 PB). Again, since it is believed that oxygen pressure is responsible for life support, we expected that hyperbaric, 11.5% oxygen (Po_2 131 Torr) would not degrade visual and mental performance. The 11.5% oxygen at normal pressure (Po_2 88 Torr) was expected to lead to decrements in visual and mental performance.

The lowest concentration of oxygen tolerated by man seems to be influenced not only by total barometric pressure but also by the concentration of carbon dioxide in the breathing mixture. Studies indicate that 3.0% carbon dioxide protects against the effects of hypoxia or reduces the concentration of oxygen that can be safely tolerated (8, 9). A group of subjects was exposed to 21 and 13% oxygen under normal atmospheric pressure, both with and without 3.1% CO_2, to see if the decrements expected at 13% oxygen might be diminished or eliminated by the addition of CO_2 to the breathing mixture.

METHODS

Subjects

Fifteen staff members of this laboratory, both civilian and military, ranging in age from 20 to 57 yr (mean = 35), volunteered to participate. One was a woman, 5 were smokers, and 2 had defective color vision. Their mean height and weight were 176.6 cm and 81.2 kg, respectively. Two men participated in 3 studies and 3, 1 a smoker, participated in 2 studies. The remaining subjects participated in only 1 of the 3 studies.

Administration of the Gas

The experiment was conducted in a hyperbaric chamber in which 4 subjects could be tested at the same time. The gas was administered through oral-nasal masks (Scott, 140 ml dead space), permitting each subject to breathe a different gas during the same session. During the experiments, samples of the expired breath were taken in front of the mouth and analyzed by a mass spectrometer (Perkin-Elmer 1100 Medical Gas Analyzer). The performance of the mass spectrometer was checked with standard gases analyzed by the Scholander technique (10). The end-tidal partial pressures of oxygen-carbon dioxide were the products of barometric pressure multiplied by the end-tidal concentrations of oxygen-carbon dioxide. Maximal oxygen concentrations in the mask were compared with those of the breathing gas. Three exposures were repeated because of suspected gas leaks, as suggested by sudden increases in oxygen concentration to 21% on the recordings of tidal gas composition. Correlation and regression analyses showed that the concentration of the breathing gas accounted for 94% of the variability in the peak concentration of oxygen in the mask. (The peak percentage of oxygen in the mask ($\%O_2$) was

related to the molar fraction of oxygen in the gas tank (F_{O_2}) by the following linear regression equation: $\%O_2 = 0.89\ (F_{O_2}) + 1.33$, $r = 0.9$, $r^2 = 0.94$, SE of estimate = 0.86, $n = 78$.) The slope and origin of the regression line virtually coincided with the slope and origin of a hypothetical line-of-identity, indicating that there were no leaks during the tests. The oxygen saturation of arterial blood Sa_{O_2} was measured noninvasively with a pulse oximeter (Novametrix Medical Systems, Inc., Model 500).

Procedure

Three double blind studies were done, with the exposure conditions (Table 1) counterbalanced for order of presentation.

Table 1
Conditions of Exposure

	Condition	Breathing Gas
Study A: 24.4 ° C db, 63% rh	P_{O_2} 162:	20.9% O_2, balance N_2
	P_{O_2} 130:	16.8% O_2, 0.6% CO_2, balance N_2
	PB 970 - P_{O_2} 126:	13.4% O_2, 0.8% CO_2, balance N_2
	P_{O_2} 99:	13.0% O_2, balance N_2
Study B: 24.3 ° C db, 52% rh	P_{O_2} 162:	20.9% O_2, balance N_2
	P_{CO_2} 24 - P_{O_2} 157:	20.4% O_2, 3.1% CO_2, balance N_2
	P_{O_2} 100:	13.0% O_2, balance N_2
	P_{CO_2} 24 - P_{O_2} 99:	12.9% O_2, 3.1% CO_2, balance N_2
Study C: 23.5 ° C db, 53% rh	P_{O_2} 160:	20.9% O_2, balance N_2
	PB 1141 - P_{O_2} 131:	11.5% O_2, balance N_2
	P_{O_2} 88:	11.5% O_2, balance N_2

db = dry bulb temperature of the chamber atmosphere; rh = relative humidity of the chamber atmosphere; P_{O_2} = partial pressure of oxygen, Torr; P CO_2 = partial pressure of carbon dioxide, Torr; PB = barometric pressure, Torr. Unless stated otherwise, P_{CO_2} was < 1 Torr and the mean PB was 770 Torr.

Studies A and B (n = 7 each study)

These 3.7-h sessions began at 0800 with the adjustment of barometric pressure in the occupied chamber. Scotopic thresholds were measured at 0844 after 25 min in the dark. At 0920, the chamber was illuminated to 0.85 milli-Lamberts to begin the testing of contrast sensitivity, field of view, and mental arithmetic. The same schedule of tests was repeated at 1020, after a second 25-min period of darkness. An environmental-symptoms questionnaire was administered only once, after the contrast sensitivity.

Study C (n = 8)

These 1.7-h sessions began at 0730 or 0930. The same procedures and schedules were used, except that the tests were administered only once. The math test was given after the questionnaire.

TESTS

Scotopic Sensitivity

The test stimulus was a 0.5° circle of light projected from the 200-w bulb of a slide projector. The stimulus flickered at 2 Hz and was presented 10° to the left of a pinpoint, fixation light. The intensity of the stimulus was attenuated with neutral density filters. All observers viewed the stimulus with their right eye from a distance of 50 cm.

The thresholds were measured with the method of constant stimuli. After determining the subject's range of sensitivity, a set of intensities in 0.1 neutral density steps was chosen. These were presented to the subject in random order, usually 4 times each, but as many as 8 times if the subject's responses were inconsistent. A frequency-of-seeing curve was calculated and plotted on cumulative probability paper. The 50% point of this curve was taken as the threshold. To reduce the amount of guessing, the subjects were warned that the light would not be presented on every trial, and in fact about 10% of the trials were catch trials. There was very little guessing.

Contrast Sensitivity

Detection thresholds for sine-wave gratings were measured at 3 spatial frequencies, 0.5, 2, and 7 Hz. These were presented on the green phosphor screen of a cathode ray tube, which had a mean luminance of 1.5 cd/m^2. One of the frequencies was chosen at random and presented below threshold. Its amplitude of modulation was then increased by reducing the attenuation in 2-dB steps until the observer identified the grating as either large, medium, or small. Then another frequency was chosen and the procedure repeated. If the subject identified the frequency incorrectly, the trial was discarded. Typically, 3 thresholds were obtained for each frequency, although as many as 6 might be obtained if the subject was erratic. The contrast was as;

$$\frac{\text{luminance of the bright bar - luminance of dark bar}}{\text{luminance of the bright bar + luminance of dark bar}}$$

All luminances were measured with a Pritchard Spectra Photometer, Model 1985.

Visual Field

The field of view in the subject's right eye was measured with a Goldmann perimeter. Measurements were taken along 24 meridians at 15° intervals from 0 to 345°. As the subject fixated the center of the hemisphere, a 0.25 mm test stimulus was slowly moved toward the center, along one of the

meridians, until it was seen.

Math Test

The test used was an automated version of one used by Adams (11), which has been shown to be sensitive to the effects of nitrogen narcosis (12, 13). The subject calculated arithmetic problems projected on a television screen and typed his answers into the VAX 11/730 computer with a numerical keyboard. The test comprised 30 problems, each consisting of three, 3-digit numbers arranged as follows:

723
438
270

The subject was required to add the first two numbers and subtract the third, proceeding column by column from right to left. The time allowed for response to each problem was adjusted from session to session during training, to force each person to continue working near the limit of his or her ability, as his or her skill improved. The score was the number of correct responses. The time allowed for each subject during the test session was fixed on the final training session, and varied from 4.3 to 9.5 s. The interval between problems was 800 ms. The number of training sessions, which continued until the subject had reached an asymptote, varied from four to seven per subject.

Health Questionnaire

An environmental-symptoms questionnaire was administered by computer (ESQ-III software package) to ascertain the subjects' perceptions of their health in nine categories (14). Heart rate and respiratory rate were also measured.

RESULTS

Health Questionnaire and Oxygen Levels

None of the subjects showed any signs of hypoxia or decompression sickness, although they frequently reported that the oral-nasal masks were uncomfortable. A factor analysis of the questionnaire indicated that each group of subjects was alert for every exposure condition (Table 2). No palpitations or dyspnea were reported, nor were the heart rates and respiratory rates unusually rapid (Table 2).

The end-tidal partial pressure of oxygen (PET_{O_2}) was reduced in proportion to the P_{O_2} of the breathing gases (Eq. *1*; Fig. 1).

$$PET_{O_2} = 0.81\ (P_{O_2}) - 19.94$$

($n = 0.94$, SE of the estimate = 7.93, $n = 78$). (*1*)

Table 2
Indices of Health

	Condition of Exposure										
P_{O_2}:	162	162	160	157	131	130	126	100	99	99	88
P_{CO_2}:	0	0	0	24	0	5	8	0	24	0	0
PB	775	775	766	770	1141	774	970	769	767	762	765
	Indices of Health										
AMS-c	0.2	0.1	0.0	0.1	0.0	0.0	0.1	0.2	0.1	0.1	0.2
AMS-r	0.1	0.1	0.0	0.2	0.1	0.1	0.2	0.1	0.2	0.1	0.1
ENT	0.2	0.2	0.1	0.2	0.1	0.1	0.4	0.1	0.2	0.2	0.1
Cold	0.0	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.0	0.0	0.1
Stress	0.3	0.2	0.1	0.3	0.2	0.2	0.4	0.2	0.3	0.3	0.3
Alert	3.9	4.0	4.2	4.3	3.8	3.9	3.4	3.6	3.8	3.7	3.3
Exert	0.1	0.0	0.1	0.2	0.1	0.0	0.1	0.2	0.2	0.2	0.2
Ache	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.2	0.1	0.1
Tired	0.4	0.3	0.2	0.3	0.3	0.2	0.6	0.5	0.4	0.5	0.5
	Cardiorespiratory Responses										
1st Heart Rate	74	69	66	72	65	77	69	82	71	77	73
2nd Heart Rate	65	60	—	58	—	65	60	70	65	68	—
1st Resp Rate	13	12	12	13	12	12	13	14	12	13	11
2nd Resp Rate	14	13	—	14	—	11	13	10	15	12	—

Rows 1-3 describe the conditions of exposure, as defined in Table 1. The next 9 rows tabulate group mean scores for relative severities of nine health conditions. Abbreviations: AMS-c = cerebral symptoms of acute mountain sickness; AMS-r = respiratory symptoms of acute mountain sickness; ENT = ear, nose, and throat discomfort; Cold = cold stress; Stress = cardiorespiratory and mood symptoms; Alert = alertness; Exert = effects of exercise; Ache = muscular discomfort; Tired = fatigue. The first heart rate (beats/min) and respiratory rate (breaths/min) were measured at 5 to 15 min. Repeated measurements were made at 2 h of exposure.

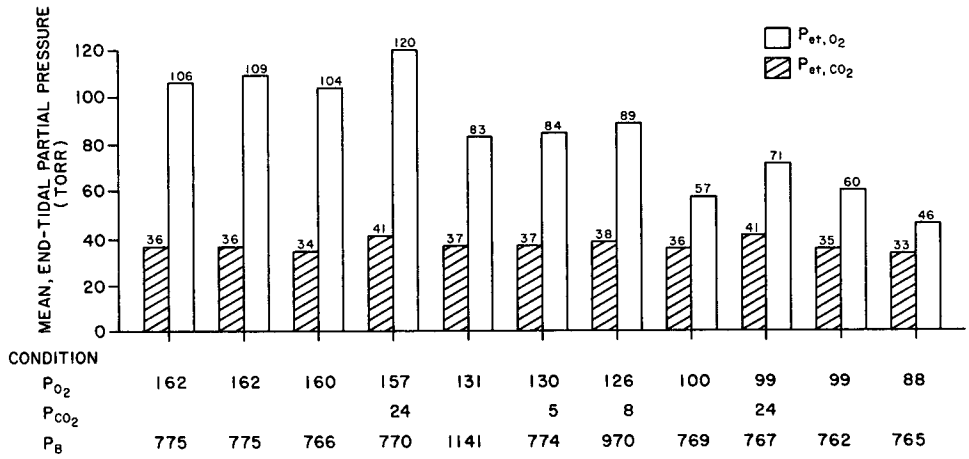

Fig. 1. Alveolar gas partial pressures. The mean end-tidal partial pressures (vertical bars) indicate the alveolar partial pressures of oxygen and carbon dioxide. The horizontal axis specifies the P_{O_2}, P_{CO_2}, and PB in each condition of exposure, in units of Torr. The percentage oxygen in the breathing gas is the quotient, $P_{O_2}/PB \times 100$.

During study C, the values of Sa_{O_2} were lower when 11.5% oxygen was inhaled at PB 765 Torr than at PB 1141 Torr (Fig. 2). This indicated that the Sa_{O_2} was reduced in proportion to the drop of ambient P_{O_2}, rather than in relationship to the drop of oxygen concentration from 20.9 to 11.5%.

Scotopic Sensitivity

There were no significant effects of P_{O_2} on the scotopic thresholds, according to the Friedman Analysis of Variance by ranks. Nor did P_{CO_2} of 5 to 24 Torr affect the threshold for scotopic vision. There was a tendency in study C for the thresholds to be worse at P_{O_2} 88 Torr compared to 131 to 160 Torr (Table 3).

Contrast Sensitivity

In studies A and B, contrast sensitivity was significantly better for 2 and 7 Hz during the repeat testing in P_{O_2} 130 Torr and for 0.5 Hz in P_{O_2} 99 Torr, according to the Friedman Analysis of Variance by ranks. P_{CO_2} of 5 to 24 Torr did not significantly change contrast sensitivity. In study C, there was a tendency for contrast sensitivity to be better at P_{O_2} 88 Torr than at 160 Torr (Table 3).

Visual Field

The mean threshold was calculated for all measurements at each meridian for each condition. The reductions of Po_2 from 157 to 162 Torr to 99 to 131 Torr did not systematically change the thresholds; nor did Pco_2 5 to 24 Torr affect the field of vision. Only in study C did the lowest Po_2 (88 Torr) seem to narrow the field of view (Fig. 3).

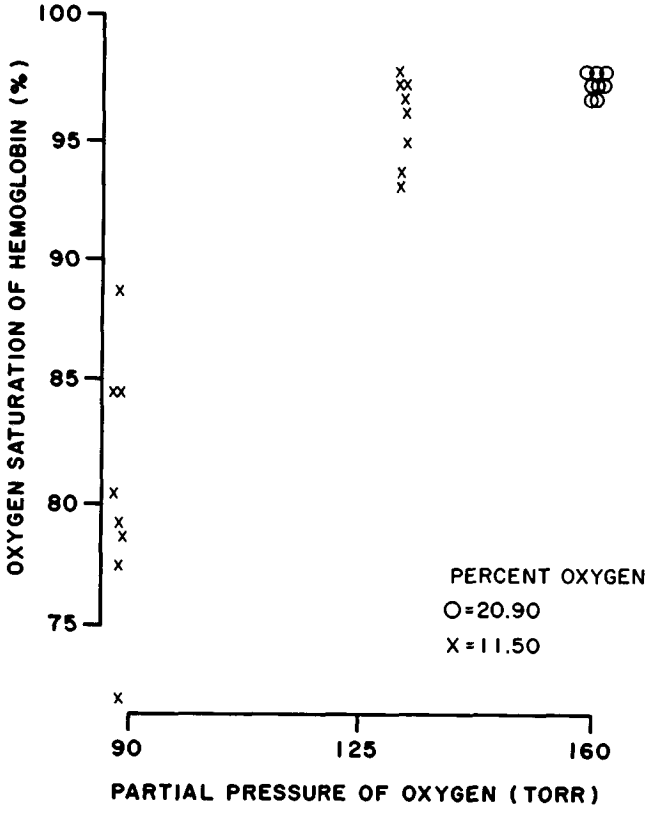

Fig. 2. Environmental effect on hemoglobin saturation. The paired data (Po_2, Sa_{O_2}) were obtained after 45 min of exposure, during testing of the subjects' scotopic vision.

Math Test

Neither the reduction of Po_2, elevation of Pco_2, nor elevation of P_B had a significant effect on mean scores for the math test. The mean number of correct answers decreased under the hypoxic conditions of study C, but the differences were not significant according to an analysis of covariance (Fig. 4).

Table 3
Visual Response to Hypoxia

	Exposure		
Po_2:	160	131	88
	Visual Threshold		
Scoptopic:	4.16	4.05	4.28
	Contrast Discrimination		
0.5 Hz:	0.087	0.071	0.088
2.0 Hz:	0.088	0.078	0.079
7.0 Hz:	0.159	0.176	0.155

The group mean values for each condition of study C are tabulated as threshold sensitivities of scotopic vision ($cd/m^2 \times 10^{-4}$) and contrast discrimination (unitless). Lower test scores represent better visual sensitivity. Testing of scotopic vision began after 0.9 h of exposure and contrast sensitivity after 1.3 h.

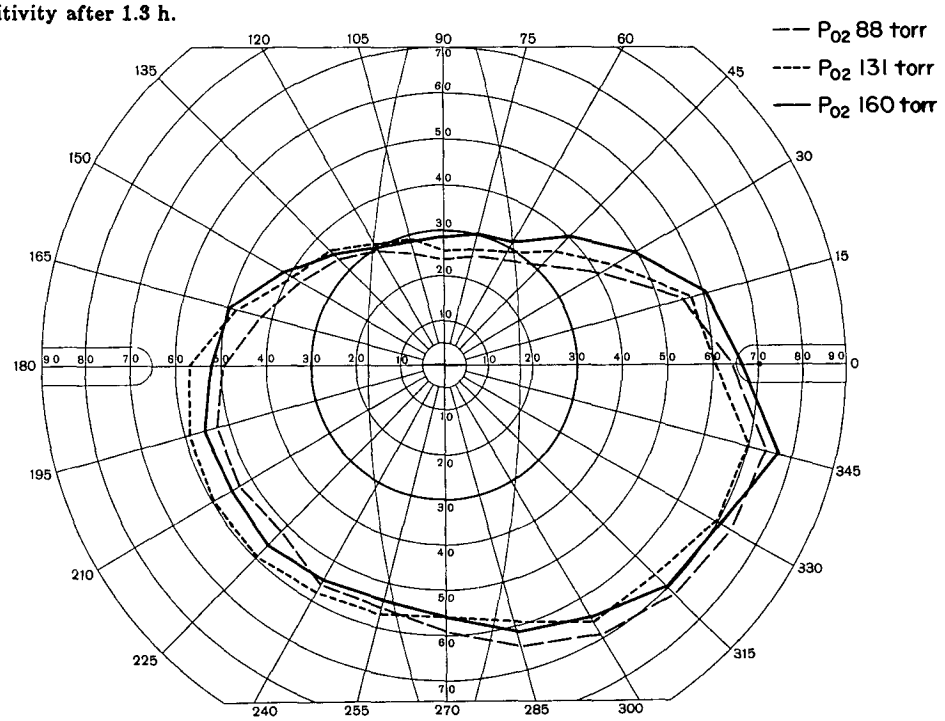

Fig. 3. Field of view in the right eye. The subjects' mean visual angle at each meridian is plotted for the exposures in study C. The measurement began at 1.3 h of exposure.

DISCUSSION

Visual Sensitivity

Visual sensitivity has been reported to be impaired during the 1st h of exposure to hypoxia (15). McFarland and Evans (7), McFarland (6, 16) and Hecht et al. (17) found that it deteriorated when the ambient Po_2 was less than 130 Torr. The impairments were proportional to the reduction of Po_2, with significant deterioration occurring at 119 Torr. Twice as much light was required to see a given stimulus when breathing 87 Torr oxygen than when breathing 160 Torr oxygen. Sensitivity was quickly restored when the subjects breathed 760 Torr oxygen (7, 16). Both photopic and scotopic thresholds were equally affected by low oxygen concentrations, indicating that hypoxia exerted no differential effect on the rods and cones (17). Contrast sensitivity was similarly degraded by 30 min of exposure to an altitude of 12,000 ft [Po_2 100 Torr (18)]. Field of view was reduced to an altitude of 10,000 ft (Po_2 109 Torr)(15, 19-21). In summarizing the relationship between Sa_{O_2}, Po_2, and sensory-mental performance, McFarland (16) claimed that visual sensitivity decrements occurred at $Sa_{O_2} < 95\%$. These findings led us to expect deteriorations in visual performance when our subjects breathed nitrogen-based gases containing 88 to 99 Torr O_2, but this did not occur.

In the present experiments, no degradations of vision occurred with reduced oxygen. Either our subjects were not hypoxic or their vision was more resistant to hypoxia than previously reported. There was little doubt that our subjects were hypoxic, since the measurements of PET_{O_2} indicated that the alveolar Po_2 were reduced in proportion to the Po_2 of the breathing gases (Eq. *1*; Fig. 1). The reduction of alveolar Po_2 presumably diminished the quantity of oxygen contained in the alveolar space and arterial blood. This was supported by the observed reduction of Sa_{O_2} in proportion to the lowering of Po_2 (Fig. 2). The levels of Sa_{O_2} must have depended on Po_2 rather than oxygen concentration, because 11.5% oxygen failed to reduce the Sa_{O_2} during exposures to hyperbaric pressure.

Why, then, were there no impairments of visual and mental performance with reductions of Po_2 below 130 Torr? First, Gellhorn (22) found no significant decrement in intensity discrimination until subjects breathed 9% (68 Torr) oxygen for 5 min. Moreover, 6% carbon dioxide improved the discrimination thresholds. This suggested that visual sensitivity was preserved when Po_2 were reduced to 88 to 130 Torr. Second, the retinal blood vessels were observed to expand during exposures to 76 to 89 Torr oxygen (15, 23), indicating that autoregulatory mechanisms increase nutritional blood flow to the visual system during the levels of hypoxia experienced by our subjects. But more important, we believe, is that we used a double-blind procedure, minimizing the possibility of biased results. We therefore conclude that the human visual system is more resistant to hypoxia than previously reported.

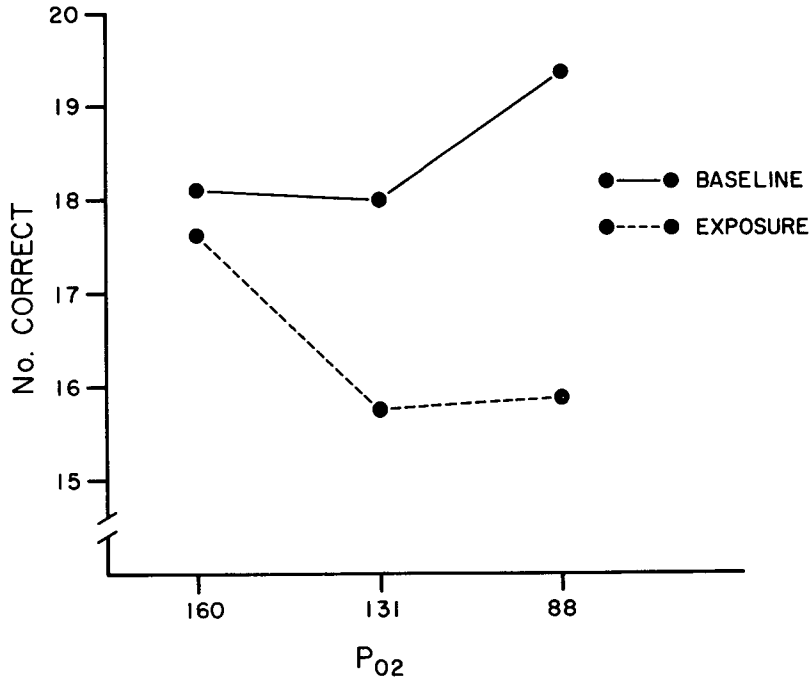

Fig. 4. Math test. Timed computations were performed by the subjects of study C. The mean number of correct answers in each condition was computed from measurements taken before exposure (baseline) and after 1.3 h of exposure.

Mental Performance

As with visual sensitivity, we did not confirm previous reports of decrements in mental performance under hypoxia. Banderet and Burse (24) reported decrements on an addition task when subjects were at a simulated altitude of 4500 m, the equivalent of approximately 12% oxygen at sea-level pressure. Since our results tended to be in the expected direction, it is possible that they were not significant because of the small number of subjects. Banderet and Burse used 23 subjects and hence would be more likely to demonstrate an effect than we would with 8 subjects.

It is also possible that our test was not sensitive to hypoxic effects, but this is doubtful in view of its demonstrated sensitivity to other stressors. In fact, we believe that our procedure of determining a critical exposure time for the test problems for each subject made our test more sensitive, because we ensured that the subjects worked at the limit of their abilities before exposure to the environmental stressors.

It seems most likely that Banderet and Burse's results (24) were due to an effect other than hypoxia, because the decrements were found early in the exposure but disappeared within 5 or 6 h. There is no theoretical reason to expect adaptation to hypoxia in such a short period. Quite the contrary, the

evidence suggests that the effects of hypoxia became progressively worse over time. Banderet and Burse's results may thus be due to the initial stress and apprehension of being a subject in such an experiment. The double-blind procedure and the counterbalancing of conditions that we used controlled for this, and may explain why our subjects performed well when breathing 88 Torr oxygen.

Effect of Carbon Dioxide

It was postulated that supplemental carbon dioxide improved the visual performance of hypoxic individuals by enhancing oxygen transport to the brain (8, 9). Our measurements of expired-gas composition tended to support this hypothesis. The elevation of PET_{O_2} by 3% carbon dioxide (Fig. 1) indicated that alveolar partial pressure of oxygen were raised 14 Torr above levels occurring during exposures to 99 Torr oxygen without carbon dioxide. An increase in tidal volume may explain why 3% carbon dioxide raised the PET_{O_2} in the absence of an increased ventilatory rate. We could not determine if supplemental carbon dioxide protected visual performance because 99 Torr oxygen did not exert a detrimental effect. However, intentional contamination of the breathing gas with 0.6 to 3.0% carbon dioxide did not impair vision or mental arithmetic.

SUMMARY

The results of this study supported the strategy of reducing oxygen concentration to suppress fires, as long as the Po_2 is maintained at physiologically satisfactory levels. Because our subjects performed better than expected, we did not observe deterioration of performance at Po_2 88 to 130 Torr. Either longer exposures or higher levels of metabolic activity, such as with exercise or industrial activity, may cause a deterioration of human performance at Po_2 88 to 130 Torr. Further work is needed to evaluate the differential responses of visual sensitivity to Po_2 and oxygen's molar concentration.

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The Nature and purpose of the study and the risks involved were explained verbally and given in writing to each subject before his voluntary consent to participate. The protocol and procedures for the study were approved by the Committee for Protection of Human Subjects, Naval Submarine Medical Research Laboratory, New London, CT.

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EEG CHANGES CORRELATED TO PERFORMANCE DECREMENT IN MAN BREATHING COMPRESSED AIR

K. Ozawa, H. Ohiwa, J. Tatsuno, and S. Kosugi

When men are exposed to compressed air they show performance decrements as symptoms of nitrogen narcosis. Many researchers have studied brain electrical activities of man in the compressed air environment to investigate the mechanism of the performance decrements. As for averaged evoked potentials, there is a general agreement that amplitudes of auditory, visual, and somatosensory evoked potentials are depressed at depth as compared with the surface (1-6).

In contrast with the averaged evoked potentials, there are considerable differences as to the changes in background or spontaneous EEG among previous studies. Roger et al. (7) and Townsend et al. (8) reported the changes that could be interpreted as an increase in EEG arousal level, although Kinney et al. (6) reported no EEG change even at 7 ATA. In addition to this discrepancy, there is some evidence that the increase in nitrogen partial pressure causes a decrease in EEG arousal level (9, 10).

Since the amplitude and even the shape of averaged evoked potential are dependent on background EEG (11, 12), the change in background EEG in the compressed air environment must be explained before interpretations of the depression of average evoked potential are made. Therefore this study was designed to reveal the changes in both background EEG and auditory evoked potential in the compressed air environment. Furthermore, the parameter of brain electrical activity, which was closely correlated to performance change, was investigated to analyze the cause of performance decrements in this environment.

METHODS

Subjects were 8 professional male divers (mean age, 34.8 yr) with normal EEG. All of them showed dominant alpha rhythms in parietal and occipital

lobes when eyes were closed, and typical alpha attenuation or blocking when eyes were opened. Depth conditions were 1 (surface control), 3, and 7 ATA. Breathing medium was air. All subjects were tested in all depth conditions separated by 3 or 4 d. The order of depth condition was counterbalanced among the subjects.

Subjects were attached to silver-cup electrodes for EEG (monopolar recording from Cz referenced to linked mastoids) and electrooculogram (EOG) (bipolar recording from left and right outer canthus), then compressed to the desired depth. After arrival at the bottom, the subjects' bodies and recording apparatus were checked for 4 min. Then the subjects were tested in the rest and task sessions. In the rest session they were required to stay quietly for 4 min and in the task session they were ordered to perform a simple reaction time task for 6 min. They were required to close their eyes throughout both sessions. The order of the sessions was changed randomly among subjects.

For the simple reaction time task, tone bursts (1000 Hz, 70 dB sound pressure level, 50-ms duration with 10-ms rise-decay time) were presented through binaural headphone speakers. Subjects had to respond to these stimuli as quickly as possible by a microswitch. About 100 trials were performed in the task session. Interstimulus intervals were altered between 2 and 4 s to avoid the formation of temporal conditioning. The control of stimulus presentation and the measurement of the reaction time to the nearest millisecond were performed by a personal computer (Apple II Plus, Apple Computer, Inc.).

The EEG measurement was done throughout both sessions. EEG signals were amplified on a polygraph (RM-6000, Nihon Koden, Inc.) with type AB-620G amplifiers using a time constant of 0.3 s and a limit frequency setting of 300 Hz. EEG signals were simultaneously stored on an FM data recorder (Model A-814, Sony Magnescale, Inc.) for the off-line analysis by a medical computer (Signal Processor 7T07A, San'ei Sokki, Inc.).

For the analysis of background EEG, mean power spectra in the rest and task sessions were calculated. Reproduced EEG signals from the FM data recorder were passed through a low-pass filter (cutoff frequency, 50 Hz) and A/D conversions were performed (sampling period, 10 ms; sampling points, 1024; frequency resolution, 0.0977 Hz). The Hanning Window was used as a window function. Then a power spectrum was calculated by Fast Fournier Transform. After 16 repetitions of this sequence of processing, the mean power spectrum and EEG energy percentage between 2 and 20 Hz were obtained. Frequency ranges less than 2 Hz and more than 20 Hz were excluded in calculation of EEG energy percentage because these ranges were easily contaminated with various artifacts.

The auditory evoked potential by stimuli used for the reaction time task were obtained from reproduced EEG signals passed through a low-pass filter (cutoff frequency, 100 Hz). Eighty trials, which were free from any artifact such as large eye movement or body movement, were selectively averaged (sampling period, 5 ms; sampling points, 256). Amplitudes of N1 and P2 component from the prestimulus level (mean of 50 ms before stimulus) and

latencies of them were measured.

RESULTS

The simple reaction time showed a progressive delay as the depth increased. Mean reaction time in milliseconds at 1, 3, and 7 ATA were 233.6 (± 26.27), 242.6 (± 35.12), and 255.4 (± 37.31), respectively. An analysis of variance revealed a statistically significant difference among depth conditions (F [2:14] = 4.722, $0.01 < P < 0.05$). There was virtually linear correlation between simple reaction time and depth. A regression line calculated by least square method was $y = 334x + 231.6$ (x: depth, y: simple reaction time).

By inspection of original data, no change was found in background EEG throughout the depth conditions. However there were slight differences in power spectra among depth conditions. Figure 1 shows the changes in power spectra of 1 subject. As the depth increased the peak frequency moved toward a slow frequency region, especially in the rest session. Changes in mean peak frequency of all subjects are shown in Fig. 2. In the rest session, mean frequencies in hertz at 1, 3, and 7 ATA were 10.0 (± 0.51), 9.9 (± 0.45), and 9.7 (± 0.46), respectively. It showed a progressive decrease as the depth increased. There was a statistically significant difference among depth conditions (F [2:14] = 4.924, $0.01 < P < 0.05$). In the task session, mean peak frequencies at 1, 3, and 7 ATA were 10.1 (± 0.58), 10.0 (± 0.47), and 10.0 (± 0.41), respectively. In contrast to the rest session, no statistically significant difference occurred among depth conditions in the task session, although mean peak frequency in this session was higher than that in the rest session in all depth conditions.

Mean EEG energy percentage revealed another difference in background EEG in the rest session (Fig. 3). A large difference was found in frequency range of alpha wave (8 to 13 Hz). The activity in 10 to 11 Hz decreased at 7 ATA as compared to 1 ATA, whereas the activity in 9 to 10 Hz increased at 7 ATA as compared to 1 ATA. However a statistically significant difference among depth conditions was found only in 10 to 11 Hz activity (F [2:14] = 9.167, $P < 0.001$). In the task session there was no statistically significant difference in EEG energy percentage among depth conditions. This was due to the increase in 10 to 11 Hz activity and the decrease in 9 to 10 Hz activity at 7 ATA.

Examples of the averaged evoked potentials by tone bursts are shown in Fig. 4. In all subjects, two peaks, namely, N1 component about 80 to 100 ms after stimulus and P2 component about 170 to 190 ms after stimulus, were recognized clearly. There was no change in the shape of auditory evoked potentials throughout the depth conditions.

Changes in evoked potential parameters are summarized in Table 1. No consistent change occurred in mean latency of either N1 and P2 components throughout the depth conditions. An analysis of variance revealed no significant difference in latency among depth conditions. The mean amplitude of

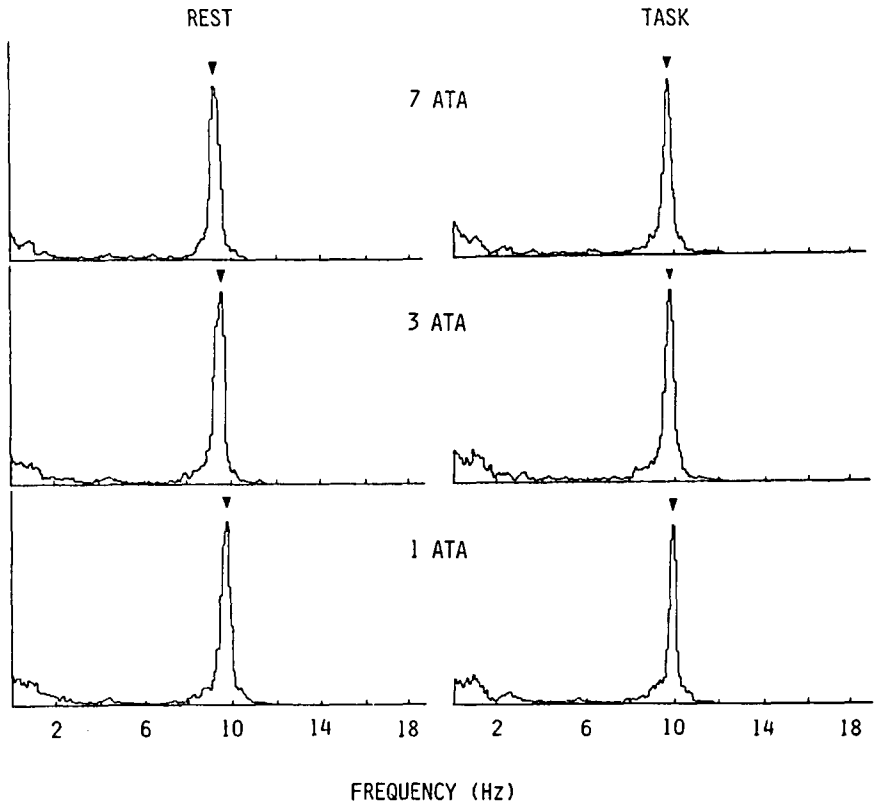

Fig. 1. Examples of power spectra of EEG (Cz referenced to linked mastoids) at each depth condition from one subject. (▽ = peak frequency).

N1-P2 complex showed a progressive decrease as the depth increased. It was also found that the decrease was mainly caused by N1 component. An analysis of variance revealed a significant difference in amplitude among depth conditions except for P2 component.

To investigate the relationship between background EEG and auditory evoked potential, Pearson product-moment correlation coefficient between percentage change in background EEG from 1 ATA and percentage change in amplitude of the evoked potential from 1 ATA was calculated. There was no statistically significant correlation between these parameters (Table 2).

The relationship between percentage increment in simple reaction time from 1 ATA and percentage decrement in amplitude of the evoked potential from 1 ATA is shown in Fig. 5. Pearson product-moment correlation coefficients between these parameters were also calculated. The decrease in N1-P2 complex showed a statistically significant correlation with the increase in simple reaction time in both 3 and 7 ATA, although the correlation coefficient was higher in 7 ATA than in 3 ATA.

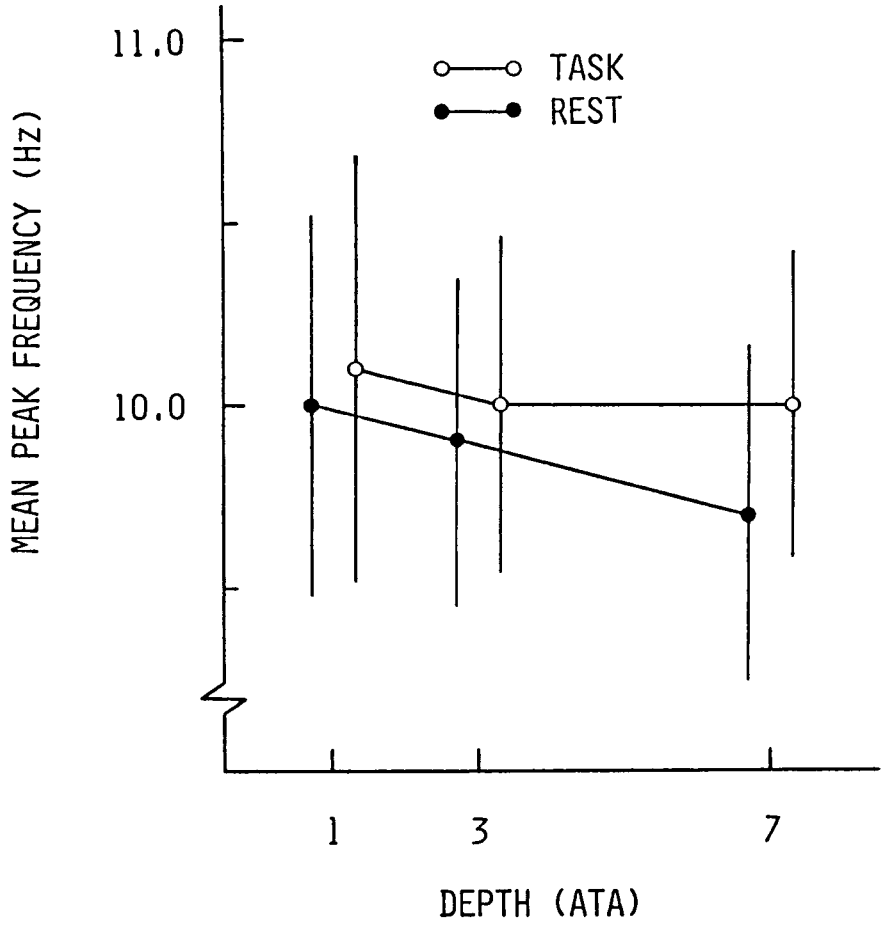

Fig. 2. Changes in mean peak frequency of power spectrum of EEG at rest and task session.

DISCUSSION

Although Kinney et al. (6) reported no EEG change even at 7 ATA, our results showed that some changes, such as the decrease in alpha-wave frequency, the decrease in fast-alpha activity, and the increase in slow-alpha activity, occurred as the depth increased in the rest session. These changes indicate that the increase in partial pressure of nitrogen caused a slight decrease in EEG arousal level. Inasmuch as Kinney et al. (6) analyzed the EEG in 0.25-Hz steps, they may not have been able to detect these slight changes in EEG (0.3 Hz decrease in peak frequency at 7 ATA). McKay et al. (10) reported not only the decrease in alpha-wave frequency but also the increase in theta activity during a 7-ATA normoxic nitrogen-oxygen saturation dive. Their results suggest that the further increase in partial pressure of nitrogen

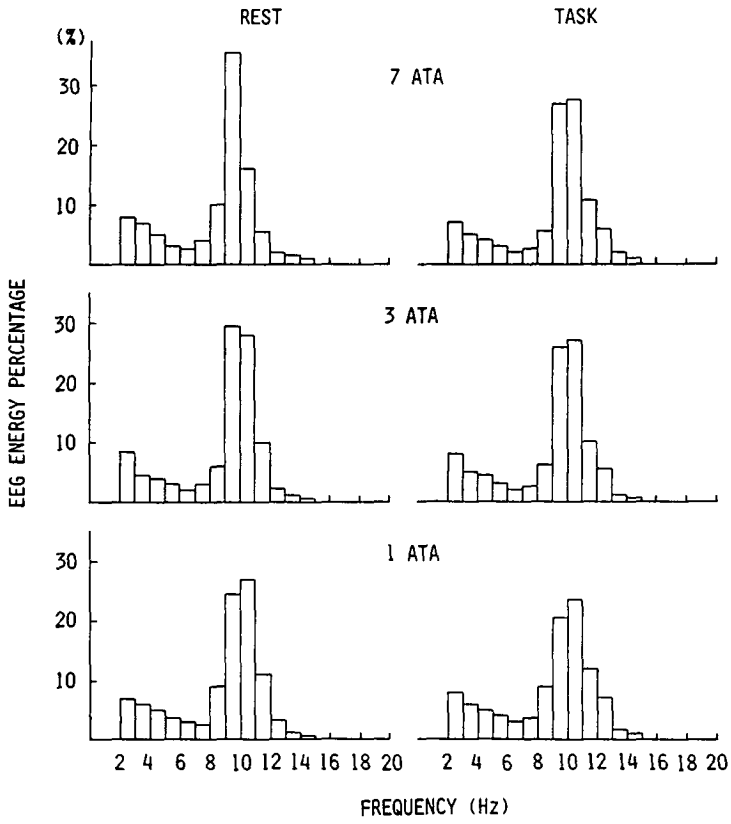

Fig. 3. Mean EEG energy percentage (2-20 Hz) at each depth condition.

causes the synchronization in EEG.

In the task session, a slight increase in EEG arousal level was found in all depth conditions as compared with the rest session. This change caused the disappearance in EEG difference among depth conditions found in the rest session. The EEG changes in the task session suggest that performing the simple reaction time task prevented the decrease in EEG arousal level. This phenomenon indicates that nitrogen narcosis can be overcome to some extent by willful effort, as Behnke et al. (13) have already suggested. We conclude that the delay in simple reaction time at the depth was not due to the change in background EEG, because there was no difference in background EEG among depth conditions in the task session.

Contrary to our results, some previous studies (7, 8) reported the increase in EEG arousal level at depth. One of the causes of this discrepancy may be the individual difference in susceptibility to nitrogen narcosis and oxygen toxicity. Criscuoli and Albano (9) pointed out that the decrease in EEG arousal level (prenarcotic electrical pattern in their terms) due to an elevated nitrogen

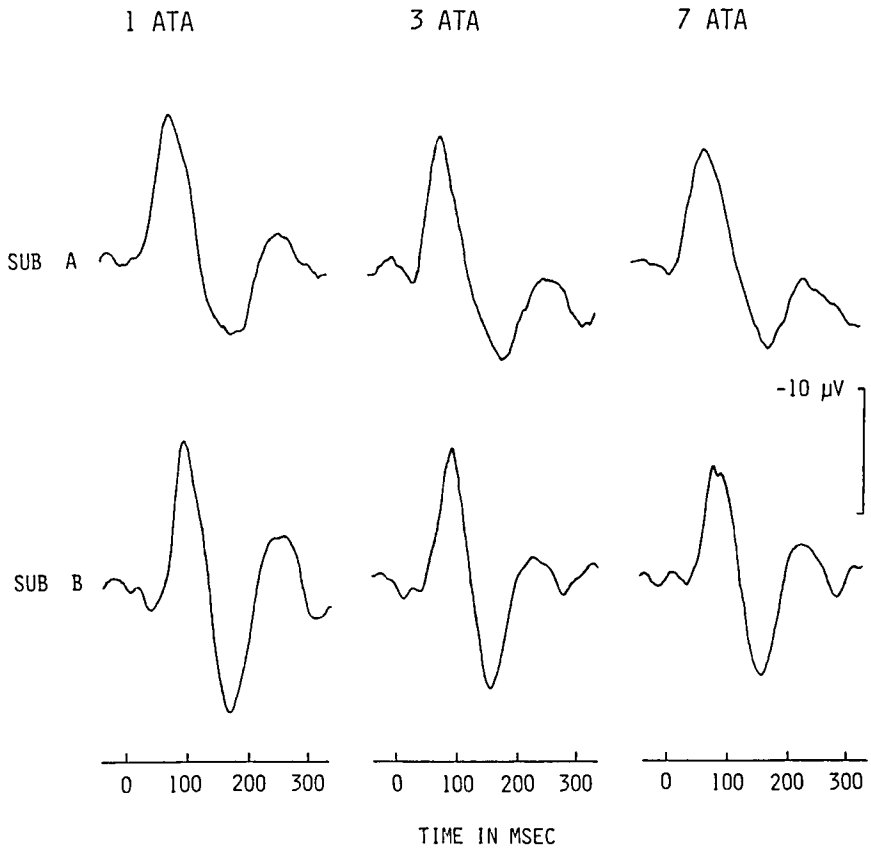

Fig. 4. Examples of auditory evoked potentials at each depth condition from 2 subjects.

partial pressure appeared first, then the increase in EEG arousal level (neuronic hyperexcitability in their terms) due to a high oxygen partial pressure tended to appear as depth increased. Therefore, the possibility exists that EEG arousal level increases at 7 ATA in the subject who has high susceptibility to oxygen toxicity. Another cause of this discrepancy may be the period of EEG measurement. Criscuoli and Albano (9) also reported that EEG desynchronization (a sign of the increase in EEG arousal level) due to hypercapnia was found during compression and a few minutes after arrival at the bottom. In future research, EEG measurements must be performed and careful consideration must be given to these factors. Moreover, it is desirable that EEGs are recorded from various sites of the scalp, because the regional difference is one of the characteristics of the EEG and, therefore, it is dangerous to infer functional states of the brain by the EEG recorded from a particular site of the scalp (14).

No change occurred in latency of the auditory evoked potential as Bennett et al. (1) reported. Kinney et al. (6) inferred that the major sensory

Table 1
Mean and Standard Deviation of Auditory Evoked Potential Measures at Each Depth Condition

AEP Parameter	Depth (ATA) 1	3	7	$F(2:14)$
Latency, ms				
N1	89.8 (± 11.03)	93.5 (± 11.56)	94.3 (± 10.60)	2.955
P2	181.1 (± 10.69)	180.2 (± 9.72)	186.8 (± 13.81)	3.684
Amplitude μV				
N1	10.6 (± 0.83)	10.2 (± 0.91)	8.9 (± 1.14)	12.877*
P2	6.7 (± 0.76)	6.5 (± 1.01)	6.2 (± 1.15)	1.620
N1-P2	17.2 (± 1.36)	16.7 (± 1.70)	15.2 (± 2.14)	9.280*

* $P < 0.01$.

pathways were neither hypo- nor hyperexcitable in the compressed air environment, because the latency of the visual evoked potential showed no change. As for the auditory mechanisms, Stockard et al. (15) suggested that impulses were transmitted to medial geniculate bodies in the thalamus within 10 ms after stimuli, then projected to the primary auditory cortex of the brain. Therefore, the latency of N1 or P2 component may occur mostly in the central nervous system. The effect of compression on auditory pathways must be studied by the analysis of auditory brainstem responses.

Like previous studies (1-3, 5) the amplitude of auditory evoked potential, especially N1 component, showed the significant decrease in the compressed air environment. Because the decrease in amplitude was not related to background EEG, it must be caused by the direct effect of the increase in nitrogen partial pressure. The analysis of the relation between auditory evoked potential amplitude and simple reaction time revealed the close correlation between these two parameters. Consequently, the decrease in amplitude of evoked potential must reflect the mechanism of performance deterioration caused by the increase in nitrogen partial pressure.

The origin and meaning of N1-P2 complex were widely discussed by Picton et al. (12) and Hillyard et al. (16). They suggested that this complex might reflect the stimulus-evaluating mechanism of the central nervous system. In addition, Shimokohchi (17) suggested that this complex had the characteristic of the orienting reflex, which is closely related to the mechanism of attention. As symptoms of nitrogen narcosis, delays in responses to sensory stimuli, slowing of mental activities, limitations of powers of association and fixation of ideas, and some emotional changes such as euphoria and a feeling of excitement are reported (18). These symptoms indicate not only the decrease

Table 2
Pearson Product-Moment Correlation Coefficients
Between Percentage Change in Background EEG From 1 ATA
and Percentage Change in Auditory Evoked Potential Amplitude from 1 ATA

	3 ATA			7 ATA		
	N1	P2	N1-P2	N1	P2	N1-P2
EEG peak frequency	0.26	-0.24	-0.02	0.25	-0.05	0.13
EEG energy percentage						
δ(2-4)	-0.05	0.40	-0.10	-0.06	-0.31	-0.51
θ(4-8)	-0.48	-0.17	-0.46	0.03	-0.63	-0.46
α(8-13)	0.51	-0.28	0.39	0.42	0.66	0.66
β(13-20)	0.02	0.57	0.56	0.13	0.48	0.29
2-3	0.11	0.44	0.19	0.33	0.38	0.20
3-4	-0.24	0.29	-0.14	-0.10	-0.45	-0.51
4-5	-0.64	0.17	-0.58	-0.08	-0.56	-0.45
5-6	-0.65	-0.18	-0.30	0.43	-0.51	-0.19
6-7	-0.32	-0.32	-0.31	0.30	-0.52	-0.36
7-8	0.09	-0.02	-0.12	0.15	-0.48	-0.35
8-9	0.16	-0.37	-0.23	0.19	-0.61	-0.38
9-10	0.02	-0.07	-0.04	-0.14	-0.41	-0.37
10-11	0.21	-0.11	0.39	0.12	0.48	0.41
11-12	0.40	-0.10	0.44	0.23	0.33	0.62
12-13	-0.25	0.11	-0.27	-0.09	0.49	0.07
13-14	0.49	-0.01	0.10	-0.21	0.03	0.08
14-15	0.03	0.24	0.18	0.04	0.45	0.43
15-16	0.28	0.64	0.52	0.25	0.43	0.47

No significant correlation was found.

in the level of attention but a change in direction of attention from outside to inside. It is inferred that the decrease in amplitude of N1-P2 complex reflected these changes in the mechanism of attention.

Some problems in using auditory stimuli in the hyperbaric environment have been noted in previous studies (2, 4, 5). The possibility existed that some changes in performance characteristics of the auditory system, namely, the stimulus-producing system (headphone) and stimulus-receiving system (human ear), occurred at 7 ATA. Nevertheless, we used auditory stimuli for two

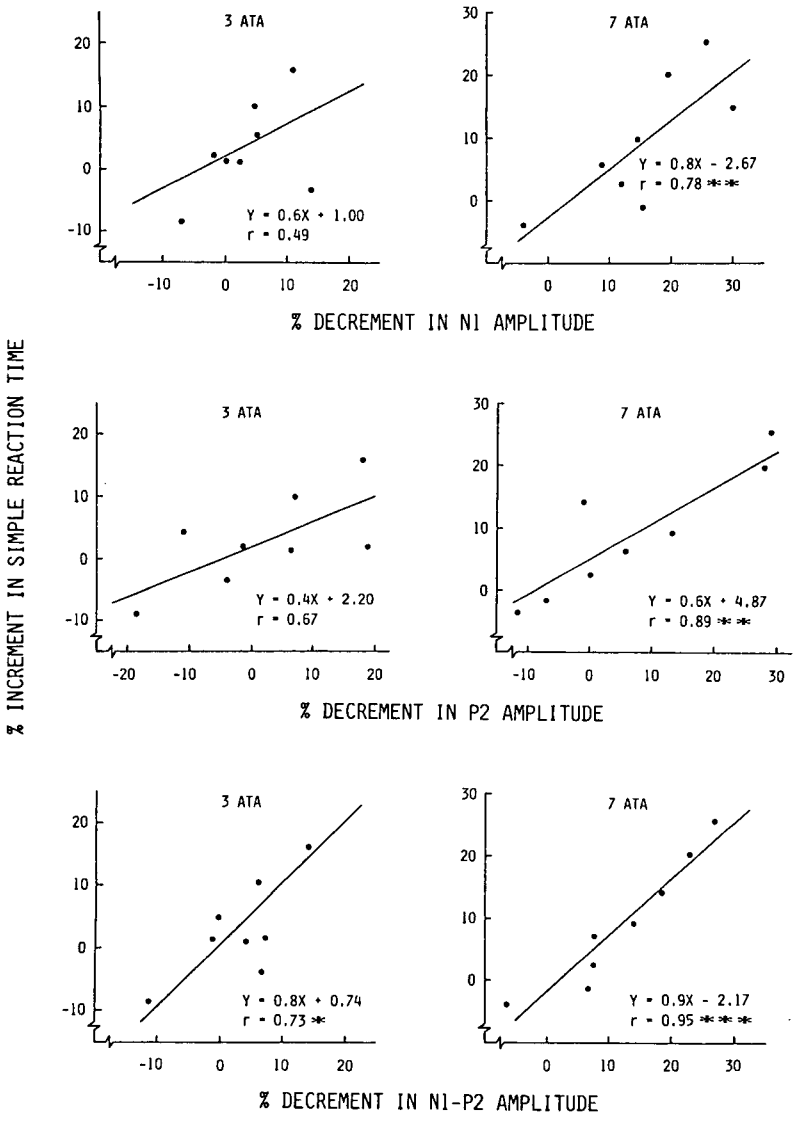

Fig. 5. The relation between percentage decrement in amplitude of auditory evoked potential from 1 ATA and percentage increment in simple reaction time from 1 ATA.

reasons. One is that the averaged evoked potential is most easily and constantly obtained by auditory stimuli; the second is that the origin and meaning of averaged evoked potential have been most widely investigated in auditory evoked potential. Indeed, the amplitude of auditory evoked potential is affected by tone intensity, especially in the low-intensity range. However, the amplitude

approaches its maximum level at the intensity of 60 to 70 dB, if the interstimulus interval is altered within 10 s (19). Because we used a rather high-intensity tone (70 dB) with an interstimulus interval of 2 to 4 s, some changes in tone intensity caused by the raised pressure must have had no practical effect on the amplitude of evoked potential in this experiment.

From the methodologic point of view, the somatosensory evoked potential by electrical stimuli is most desirable. However, the recording procedure of the somatosensory evoked potential is rather complicated, and sometimes difficulties occur in identifying components because stable responses are not always obtained in somatosensory evoked potentials, as Langley and Hamilton (4) have reported.

From the theoretical point of view, auditory or visual evoked potentials are desirable as the method for studying brain functions, because human behaviors are largely dependent on auditory or visual stimuli and the brain functions must be evaluated in the sequence of stimulus-input, stimulus-processing, and response-output. Kinney et al. (6) reported that the visual evoked potential could be used as a sensitive indicator of nitrogen narcosis. They presented visual stimuli through the porthole of the chamber. Because the structure of our chamber, such as the position of porthole and test place for subject, was not suited for the Kinney procedure, we could not confirm the effectiveness of the visual evoked potential. This circumstance may be true in other chambers. Some improvements in instruments which enable the fine control of visual stimuli inside the chamber are needed for the application of the visual evoked potential as a routine test.

Therefore, from the practical point of view, if careful consideration is paid to stimulus control, the auditory evoked potential can be used as a convenient and reliable test for nitrogen narcosis, as Bennett et al. (1) have already pointed out.

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VENTILATORY PARAMETERS INFLUENCES ON EFFICIENCY OF CO_2 SCRUBBING

S. Radić, P. Denoble, S. Gošovic, and M. Živković

The importance of efficient scrubbing of carbon dioxide in closed and semiclosed circuit scuba is well known. In general, carbon dioxide scrubber duration is inversely dependent on workload and carbon dioxide output, respectively. In manned testing by the same carbon dioxide divers' output, differences in scrubber efficiencies emerged. We suppose that various ventilatory patterns of divers wearing rebreathers could explain this phenomenon. Diver respiratory pattern is influenced by respiratory resistance, high oxygen partial pressure, and individual diver performances. Hyperbaric environments favor a moderate hypoventilation with increased tidal volume and decreased breathing frequencies. It is more accentuated in experienced divers (carbon dioxide retainers). Hyperventilation can also be seen in diving situations. This series of unmanned tests was designed to check the above mentioned relationships.

METHODS

Two series of unmanned tests were conducted to examine the effects of ventilation, carbon dioxide input, and tidal volume on carbon dioxide scrubber efficiency. A 2.2-liter, cylindrically shaped canister filled with 2 kg of soda lime "Dräger-Sorb 800," enclosed in a breathing loop and submerged in 20°C water bath, was exercised by a breathing machine (Fig. 1). At ventilatory rates of 26.8 and 53.0 liter STPD/min, with tidal volumes of 2 liter BTPS/breath, carbon dioxide injection varied from 0.75 to 3.0 liter STPD/min, in the first series. In the second one, for tidal volumes of 1, 2, and 3 liter and carbon dioxide input of 1.5 liter/min, ventilation varied from 10 to 50 liter/min. Sample gas was drawn off at the inhalation hose and analyzed for carbon dioxide content by an infrared analyzer. The scrubber breakthrough was preset

at 1% carbon dioxide in sample gas. At that point scrubber duration and capability were established.

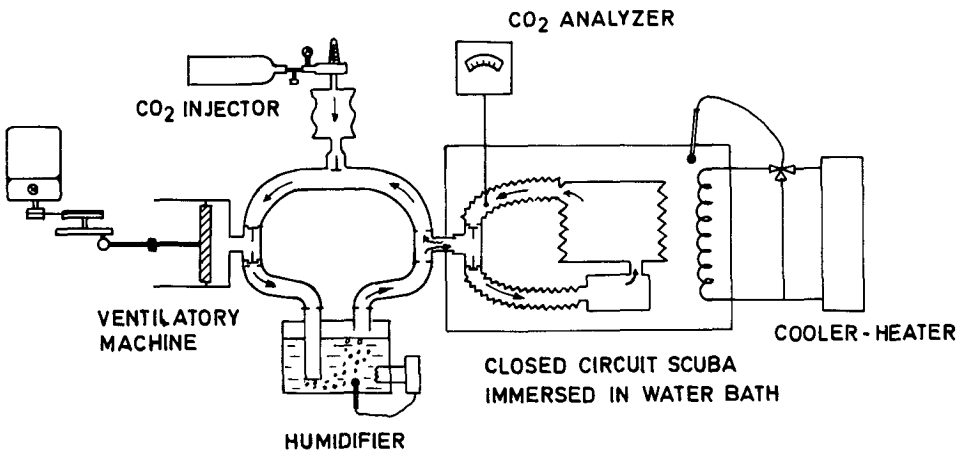

Fig. 1. Experimental set up to study the ventilatory parameter influences on CO_2 scrubber efficiency.

RESULTS

Carbon dioxide scrubber efficiency vs. carbon dioxide input at ventilation rates of 26.8 and 53.0 liter/min is presented in Table 1 and Figs. 2-5.

Effects of carbon dioxide injection in liter per minute on scrubber efficiency at ventilatory rates of 26.8 and 53.0 liter/min are shown in Figs. 2 and 3. Figures 4 and 5 represent the relation between carbon dioxide removal efficiency and carbon dioxide percentage in input gas. It is obvious that capability and duration of scrubber are inversely proportional to carbon dioxide input. With greater ventilation the scrubber broke through earlier.

Scrubber efficiency in relation to ventilation rate at 3 different tidal volumes is presented in Table 2. Figure 6 only shows the effect of tidal volume on scrubber capability. It is obvious that by increasing the tidal volume at a constant ventilation rate and minute carbon dioxide injection in physiologic range of carbon dioxide concentration, scrubber efficiency decreased.

DISCUSSION

Among numerous factors affecting carbon dioxide absorbtion in closed and semiclosed circuit scuba, we examined only the dependence of carbon dioxide input and gas flow dynamics on carbon dioxide absorbtion. Reviewing data on this subject, we found no systematic research on ventilatory parameter's influences on carbon dioxide absorbent efficiency. Although the variation of ventilation in diving can markedly change carbon dioxide percentage in expiratory gas at constant surrounding pressure, there are no data

Table 1
Effect of Ventilation and CO_2 Input on Absorbent Efficiency*

Ventilation, liter/min	CO_2 Input		Absorbent Efficiency	
	Injection, liter/min	Content, %	Duration, min	Capability, liter/kg
26.83	0.741	2.8	343	127.1
	0.983	3.7	223	109.6
	1.237	4.6	166	102.7
	1.444	5.5	137	98.9
	1.715	6.3	105	90.0
	1.963	7.4	84	82.4
	2.264	8.2	69	78.1
	2.521	9.2	58	73.1
	2.957	11.2	46	68.0
53.00	0.727	1.4	308	111.9
	0.970	1.8	185	89.7
	1.113	2.1	136	75.7
	1.436	2.7	83	60.3
	1.999	3.8	56	56.0
	2.436	4.6	40	49.9
	2.941	5.6	34	50.0

* Values are mean of 3 runs.

about the influence of input carbon dioxide concentration on scrubbing ability. Generally, gas flow rate reduces absorbent efficiency by shortening the contact time of gas with absorbent (1). In closed and semiclosed circuit rebreathing apparatus, gas flows through absorbent only during the expiration. During inspiration, gas remains stagnant in the canister. High peak of expiratory flow can lead to dumping of gas beyond absorbent (2, 3). The residence time of gas in the canister can be prolonged by increasing the free volume of the canister, which should at least be equal to tidal volume (4).

It is obvious from Figs. 2-5 that with the same carbon dioxide injection at greater ventilatory rates, the scrubber broke through earlier. At the carbon dioxide isoconcentration points the difference is greater. At carbon dioxide injections lower than 0.75 liter/min and higher than 3 liter/min, differences dependent on ventilation rate diminish. Matching scrubber duration and capability at isoconcentration points of carbon dioxide in physiologic ranges, it is obvious that by doubling ventilation, the capability is halved, explaining the fourfold decrease of scrubber duration.

Favorable effects of lower concentration of carbon dioxide on scrubber

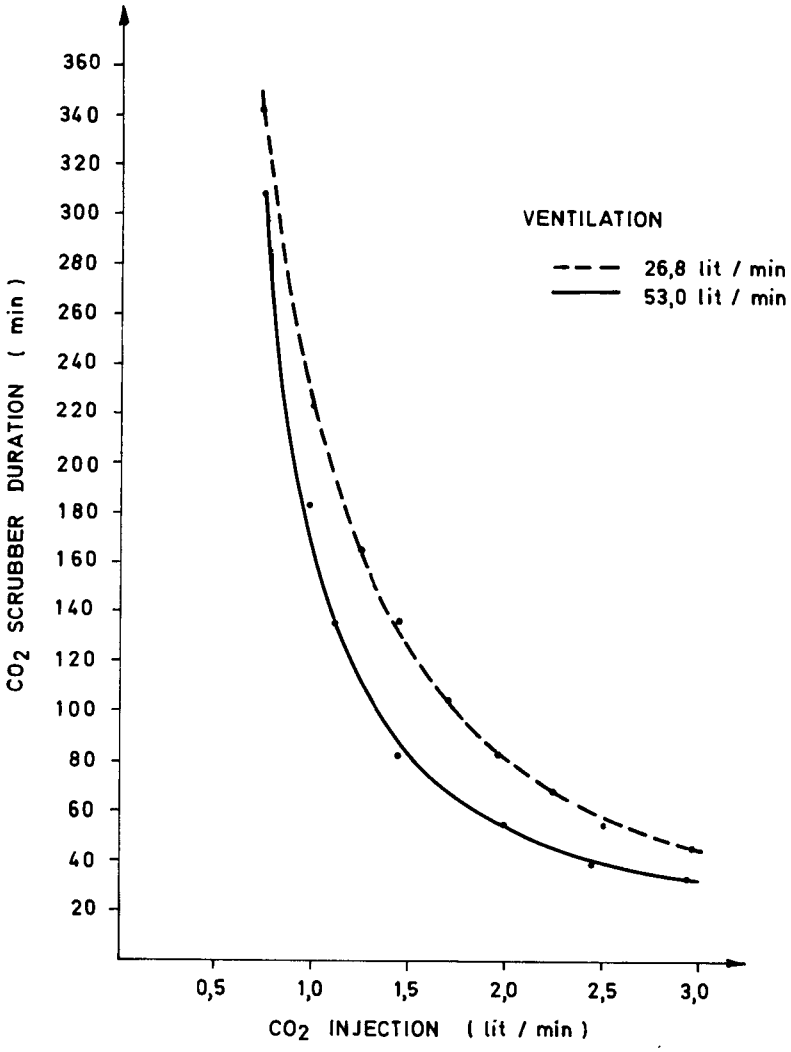

Fig. 2. Effects of ventilation and CO_2 injection on CO_2 absorbent duration.

efficiency were established in all conditions except in a range of hyperventilation where adverse influence of high gas flow predominates. From the physiologic standpoint this is rather favorable because elevated inspiratory carbon dioxide counteracts hypocapnia. In exercise-induced hyperpnea the effect of high gas flow on scrubber efficiency potentiates the hazard of carbon dioxide intoxication.

As can be seen in Table 2 and Fig. 6, for constant carbon dioxide input of 1.5 liter/min, the optimal absorbtion is reached at a ventilation rate of 30 liter/min, i.e., at 5% carbon dioxide in input gas. Generally, increasing tidal volume at constant ventilation rates decreases scrubber efficiency. This becomes clearer at greater ventilatory rates, i.e., in the hyperventilation range.

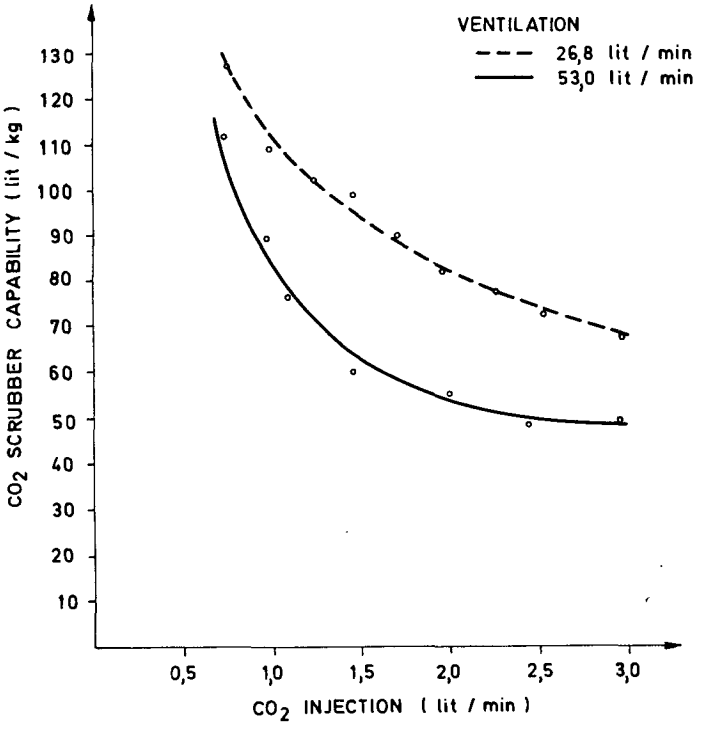

Fig. 3. Effects of ventilation and CO_2 injection on CO_2 absorbent capability.

Based on reported evidence, requirements for the carbon dioxide scrubber can be modified based on scrubber use. In closed circuit scuba, designed for use in moderate work at shallow depth, the need to minimize scrubber dimensions can be met by improving the scrubber design, regardless of free volume. However, in semiclosed circuit apparatus which is used at greater depth for even harder work, free volume in the canister should be equal to or greater than expected tidal volume.

CONCLUSION

The ventilatory pattern spontaneously adopted by divers in underwater swimming coincides with optimal carbon dioxide scrubbing conditions in our experiments. In exercise-induced hyperventilation, a diver could be endangered by the excess carbon dioxide in the inspiratory gas because of diminished scrubber efficiency. To maintain scrubber efficiency, in spite of increased ventilation or carbon dioxide input, the canister should be designed to take into account not only the duration but also the type and intensity of work in diving.

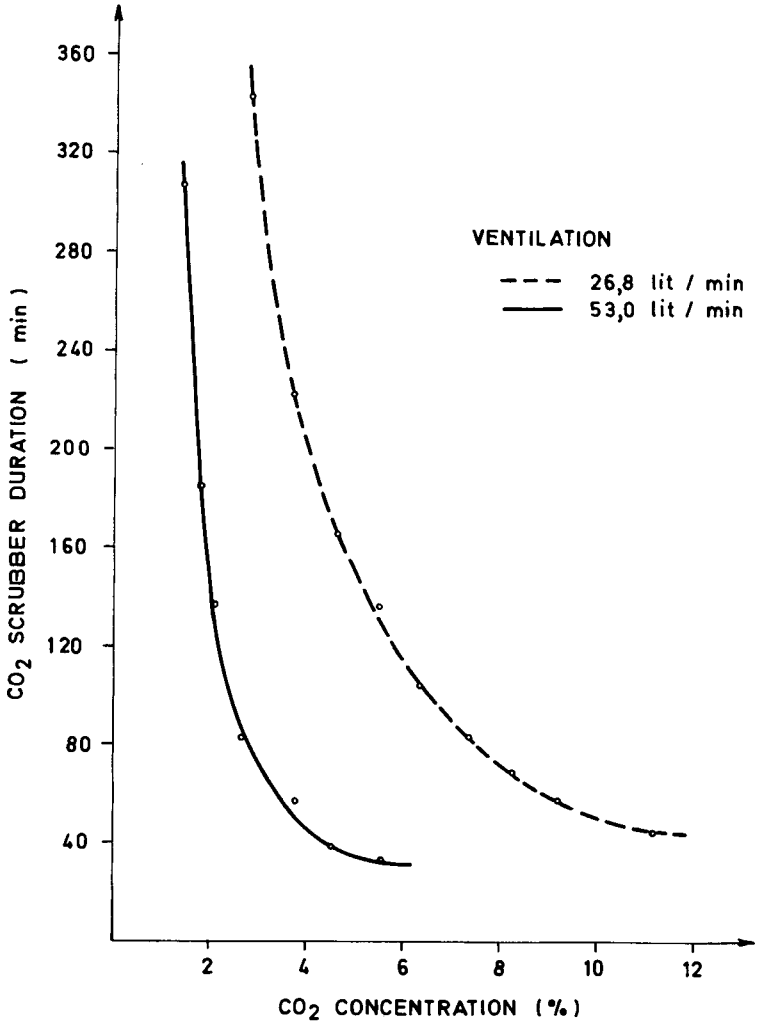

Fig. 4. Dependence of absorbent duration to CO_2 input concentration for ventilation rates of 26.8 and 53.0 liter/min.

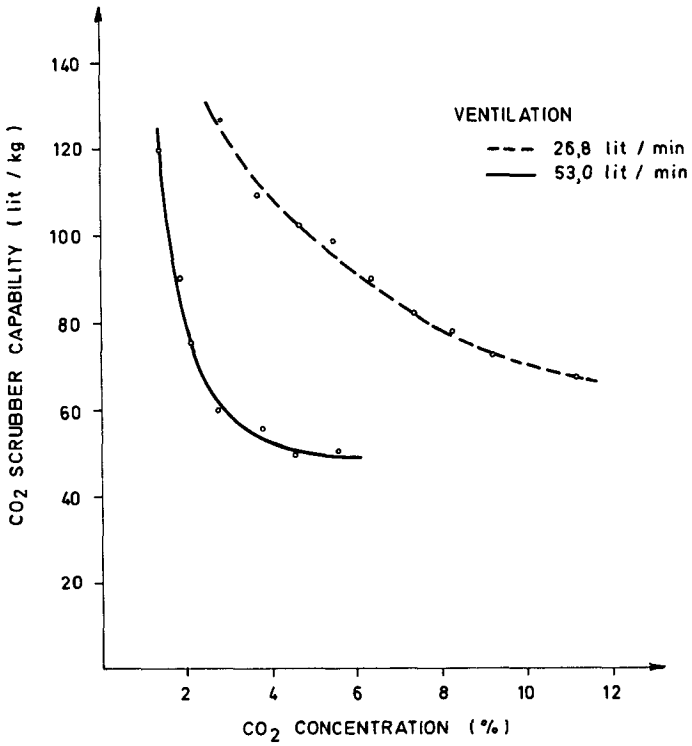

Fig. 5. Dependence of absorbent capability to CO_2 input concentration for ventilation rates of 26.8 and 53.0 liter/min.

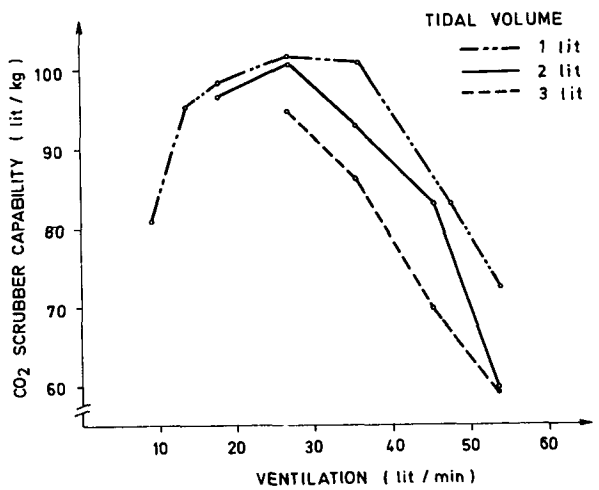

Fig. 6. Absorbent capability as a function of tidal volume at constant CO_2 injection of 1.5 liter/min.

Table 2
CO_2 Absorbent Efficiency in Relation to Tidal Volume

Tidal volume, $liter_{BTPS}$	Ventilation, liter/min	CO_2 Input,* %	CO_2 Absorbent Efficiency	
			Duration, min	Capability, liter/kg
1	9.07	16.2	112	81.9
	13.73	10.7	130	95.5
	18.03	8.2	134	98.5
	26.75	5.5	138	101.5
	35.93	4.1	137	100.8
	47.38	3.1	113	83.0
	53.69	2.7	99	72.5
2	17.76	8.3	132	96.7
	26.71	5.5	137	100.6
	35.32	4.1	128	92.9
	45.38	3.2	113	83.0
	53.44	2.7	81	59.5
3	26.72	5.5	129	94.8
	35.31	4.2	118	86.8
	45.25	3.2	95	69.7
	53.30	2.8	80	58.8

* Mean CO_2 injection is 1.4676 ± 0.0047 liter/min.

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CARBOXYHEMOGLOBIN IN RATS EXPOSED TO CARBON MONOXIDE AT 41 ATA

G. Bolstad, A. G. Lindrup, A. O. Brubakk, and S. Martini

Carbon monoxide (CO) is a chemical asphyxiant gas that is produced during welding, in particular during manual metal arc (MMA) welding. CO is also produced endogenously by human metabolism (1), which might be of importance for people living in closed environments over some time, such as in submarines, in spacecraft, or during saturation diving. A considerable amount of information exists regarding uptake and binding of CO to hemoglobin (Hb) at atmospheric pressure. However, for hyperbaric conditions very little information exists on uptake, elimination, or physiologic effects of this gas.

Rose et al. (2) have measured the carboxyhemoglobin (COHb) levels in rats, mice, and guinea pigs after CO exposure in a helium atmosphere up to approximately 8 ATA. Röckert (3) measured COHb in rats exposed to CO in air up to 6 ATA. CO exposures during more extreme helium pressures have so far not been investigated.

As diving and hyperbaric welding proceeds toward greater depths, the need for such information increases. The following investigation was therefore conducted to evaluate whether a helium pressure of 41 ATA has any effects on CO uptake, and whether the competitive binding of oxygen (O_2) and CO to Hb in vivo is affected by such pressures.

MATERIALS AND METHODS

Forty-three rats (250-300 g) which before exposure were maintained on food and water ad libitum were used in the investigation.

Experimental Procedure

Three rats were placed together in a hyperbaric chamber (approximately

17 liter). The chamber was flushed for 2 min with a certified, premixed gas consisting of 1000 ± 50 μbar (ppm) CO, 0.209 ± 0.002 (group 1A) or 0.418 ± 0.004 (group 2A) bar oxygen, the rest being helium (after this referred to as pressure groups). Pressure was then applied with pure helium to a total pressure of 41 ATA. Compression was linear at a rate of 2 bar/min.

Partial pressure of oxygen (Po_2) was controlled by a Servomex oxygen analyzer. The Po_2 was kept approximately constant by injecting O_2 approximately every 15 min, giving variations in O_2 pressure of ± 0.015 bar. Loss of CO in the chamber atmosphere due to uptake by the rats and sampling of gas for O_2 monitoring was not replenished during the exposure. Carbon dioxide was removed using a layer of soda lime inside the chamber.

After 2 h, including compression time, the animals were killed by rapid decompression to surface. Within 5 min after reaching surface the rats were decapitated and blood was collected in Na-heparinized tubes. The blood samples were either analyzed for COHb the same day or frozen immediately and analyzed for COHb within 1 wk. Lactate was analyzed the same day. Oxygen uptake was measured during the last 30 min of the exposure period.

Corresponding groups of rats were exposed to the same premixed gases containing 0.21 (group 1B) and 0.42 (group 2B) bar O_2, respectively (after this referred to as surface groups), as the experimental groups for the same amount of time. One half atmosphere of helium was added on top of this to obtain a total pressure of 1.5 ATA which was necessary to obtain gas samples for the O_2 analyzer. After 2 h exposure the control rats were killed by flushing the chamber with pure helium. Thereafter the rats were decapitated and blood was collected and analyzed as for the experimental groups. Blood from nonexposed, control rats was also analyzed for lactate and COHb.

Chamber temperature during exposure was 30.2 ± 2.0°C at 41 ATA and 24.4 ± 1.4°C at 1.5 ATA.

Analysis

Carboxyhemoglobin was determined in duplicate blood samples either by a gas chromatographic method (4) or by a spectrophotometric method (5).

Hemoglobin was determined by the hemoglobin-cyanide method (6).

Lactate was measured by fluorimetry in perchloric acid extracts of blood, according to an enzymatic method described by Lowry et al. (7).

Oxygen uptake was estimated on the basis of decrease in O_2 content in the chamber, monitored by a paramagnetic O_2-analyzer (Servomex). A more detailed description of the method will be published later by Martini et al.

Statistics

Mean values and their standard deviations (mean ± SD) were calculated

for each group. The significance of the differences between the mean values was calculated by Student's t test for independent groups.

RESULTS

The rats would usually lie down shortly after exposure started and they rested quietly during most of the exposure time. Toward the end of the 2 h a few of the rats may even have been unconscious, but no record was kept characterizing the rat's general behavior.

A few of the rats that were decompressed from 41 ATA were difficult to bleed because coagulation started very quickly. Due to technical problems, blood from 3 of the rats was discarded. The COHb values are presented in Table 1.

The COHb values in the surface groups decreased by 24% when Po_2 was increased from 0.21 to 0.42 bar. The corresponding value for the pressure groups was 46%. There was a significantly higher COHb value in the pressure group compared to the surface group when Po_2 was 0.21 bar. No such difference was seen between the groups exposed to 0.42 bar oxygen.

Blood lactate values were very high in all the rats investigated (Table 1), but there were no significant differences between any of the groups.

Oxygen uptake was measured in groups of 3 rats only during the last experiment in each group. Table 1 presents the average value for the last 30 min of the experiments, i.e., after 90 min of CO exposure. At 1.5 ATA the CO-exposed animals showed no change in oxygen uptake either with a low or with a high Po_2. At 41 ATA, on the other hand, a marked reduction in O_2 uptake was observed in the CO-exposed animal compared to nonexposed at low as well as at high Po_2.

In Table 2, measured COHb values in rats are presented together with calculated values according to Haldane's first law (8):

$$\frac{HbCO}{HbO_2} = M \cdot \frac{Pco}{Po_2} \text{ (M is the affinity constant).}$$

The results show that the measured values at surface as well as at pressure are not significantly different from the calculated ones when Po_2 is 0.42 bar. When Po_2 is reduced to 0.21 bar, however, the animals in the pressure group have higher values than were expected from the calculations.

DISCUSSION

In contrast to what has been shown by Rose et al. (2) and Röckert (3), who have been unable to show any effect of pressure on COHb values in rats, this investigation has shown that rats exposed to 41 ATA end up with a significantly higher COHb value than surface-exposed rats, when Po_2 is 0.21 bar and CO partial pressure is 1000 μbar. When Po_2 is doubled, however,

Table 1
Carboxyhemoglobin O_2 Uptake and Blood Lactate in Rats Exposed to CO at Different Environmental Pressures and Partial Pressures of O_2

Group	P_{tot} ATA	PO_2 bar	PCO 1 lbar	% COHb (mean ± SD)	O_2 Uptake $mlJg^{-1}Jh^{-1}$	Blood Lactate, mmol/liter (mean ± SD)
1A	41	0.21	1000	65 ± 19* (n = 9)	1.2 (n = 1)**	28.4 ± 4.6 (n = 3)
1B	1.5	0.21	1000	49 ± 5 (n = 11)	0.9 (n = 1)†	27.9 ± 4.8 (n = 3)
2A	41	0.42	1000	35 ± 5 (n = 8)	1.2 (n = 1)†	25.8 ± 0 (n = 2)
2B	1.5	0.42	1000	37 ± 8 (n = 8)	1.0 (n = 1)†	28.7 ± 6.9 (n = 3)
Controls	1	0.21	0	0.3 ± 0.2 (n = 7)	—	2.8 ± 1.7 (n = 5)
Controls	1.5	0.21	0	—	1.1 ± 0.3** (n = 27)	—
Controls	41	0.42	0	—	1.9 ± 0.5** (n = 10)	—

* $P < 0.05$ statistical significance of difference between surface and pressure groups.
** Unpublished result (Martini et al.). † O_2 uptake values given represent mean of 3 animals.

COHb levels were unchanged as compared to surface rats. The reason for this effect is not clear. It has been shown that not only high pressure, but also the nature of the inert gas used as pressurizing agent influence the affinity of red cells for O_2 by inducing a left shift of the O_2-dissociation curve, the magnitude of which depends on gas partial pressure. This left shift is reversible for pressure up to at least 100 bar (9). Such an effect of pressure and inert gas might also be expected on the affinity of red cells for CO, which combines with hemoglobin in competition with O_2, but with an affinity that is in the order of 220 times that of O_2 (10). If this ratio, i.e., the relative affinity of O_2 and CO, is changed as an effect of increased environmental pressure or a different breathing gas composition, a change in COHb value compared to a corresponding surface situation might be the result. It is, however, difficult to see how the increase in COHb at pressure observed in

Table 2
Calculated and Measured Values of COHb in Rats Exposed to CO at Different Partial Pressures of Oxygen and Total Pressures

P_{O_2} bar	P_{CO}, μbar	M Value	% COHb Measured	% COHb Calculated*
0.21	1000	220	65 ± 19 (41 ATA)	51
			49 ± 5 (1.5 ATA)	
0.42	1000	220	35 ± 5 (41 ATA)	34
			37 ± 8 (1.5 ATA)	

* Calculated according to Haldane's equation (see text).

this investigation can be caused by a change in relative affinity between CO and O_2, since it happens only at low P_{O_2}. The reason for the increased COHb values should therefore be sought elsewhere.

Salzano et al. (11) have shown that physiologic dead space was increased in man at 47 ATA. An increase in dead space may possibly lead to a decrease in the relative partial pressures of O_2 to CO in the alveoli. This effect will become more pronounced the lower the P_{O_2}. A change in relative alveolar P_{O_2} and CO might possibly explain the increase in the COHb at low P_{O_2}. The large variation of observed COHb values in the low P_{O_2} pressure group in this investigation may thus reflect a variation in the rat's ability to compensate for the hypoxia by increased ventilation.

Since O_2 uptake in our investigation was measured in groups of 3 animals at a time, individual differences in O_2 uptake have not been recorded. However, both CO-exposed groups showed a reduction in O_2 uptake compared to controls at 41 ATA, whereas no such change was seen at 1.5 ATA. Rats that are exposed to heliox at 41 ATA increase their O_2 uptake significantly as compared to 1.5 ATA. The reason this did not happen during severe CO exposure probably was because the animals were seriously disabled due to the intoxication.

Moon et al. (12) have shown that arterial lactic acid concentrations were increased by 70% at rest and 200% after exercise in subjects exposed to a helium-nitrogen atmosphere of 66 ATA. This happened despite an increased inspired P_{O_2} of 0.5 bar. During this dive, endogenously produced CO in the order of 20 μbar was later shown to be present (13). This indicates that O_2 availability to the tissues might be impaired both by pressure and small increases in CO. It is known that the O_2 dissociation curve is moved to the left at pressure (9, 13), reducing O_2 availability to the tissues even more. In our investigation extremely high levels of blood lactate were measured, but no difference between surface and pressure rats was observed. The lactate levels in all our groups were probably very close to what may be a maximum value in

a living mammal. A possible increase in lactate production due to high pressure might thus have been masked. Lactate values of 11 ± 2.2 mmol/liter have been reported by others during CO intoxications in man with COHb values of 31 ± 14% (14), and values of more than 20 mmol/liter have been produced as a result of very hard, brief exercise in man (15).

The physiologic implications of severe hypoxia during CO intoxication may be several. It has been shown (16) that hypoxia in the tissues leads to a redistribution of CO from Hb into muscle and possibly other tissues, and thus increases the effect on vital heme-containing enzymes involved in metabolism, such as cytochrome C and others. Another factor which might increase the severity of a CO intoxication at depth is the carbon dioxide retention that has been shown in divers (17). CO is shown to be more toxic for rats when carbon dioxide in the atmosphere is increased simultaneously (18).

In conclusion, this study has shown that CO uptake is higher at pressure than at surface. This fact, together with the observation that pressure can induce tissue hypoxia both at rest and during exercise (12), indicates that CO intoxication may be more serious at high pressure than at surface.

To evaluate this further, more information will have to be collected on CO uptake, binding, and elimination in vitro as well as in the intact body. Furthermore, the mechanisms of CO toxicity, which so far are only partly known, will have to be evaluated together with the various effects of pressure and inert gases on the body.

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SESSION 9: PERFORMANCE, NARCOSIS AND HPNS

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NEUROPSYCHOLOGIC AND NEUROPHYSIOLOGIC REACTIONS DURING A HELIOX DIVE TO 450 MSW

R. J. Værnes, H. Kløve, and H. Ursin

Heliox compression deeper than 16 ATA can lead to EEG changes associated with confusion and somnolence (1). Animal studies have shown similar changes as a prelude to the development of generalized seizures (2). This syndrome, with symptoms and signs such as EEG changes, tremor, and somnolence, has been termed the high pressure nervous syndrome (HPNS) (3). In man the syndrome includes tremor in the hands and arms, increased slow electrical brain wave activity (2-7 Hz) and a depression of alpha waves (8-13 Hz) in the EEG, memory problems, dizziness, nausea, and vomiting. With increasing depths and during fast rates of compression the symptoms and signs of HPNS become more severe, and at depths greater than 300 meters of seawater (msw) lapses of consciousness may occur (4, 5). Although human studies in several deep dives, animal experiments, and pharmacologic studies have been conducted in an attempt to elucidate the nature of this syndrome, there is still no agreement on its causes and mechanisms. Both the great individual susceptibility and the difference in individual response pattern show that we are dealing with a multifactorial CNS reaction.

Through extremely slow compressions one can more or less avoid HPNS in the divers. However, for operational diving it is crucial to reach working depth within a reasonable time. Some previous compression studies at NUTEC have shown that intermediate stops combined with progressively slower compression rates can minimize the HPNS problems down to 350 msw, but still be operationally acceptable concerning total compression time (6).

In the present study a compression profile extrapolated from these previous studies at our and other laboratories was tested going 100 msw deeper. Six divers participated. During the compression, the bottom phase and the decompression, neuropsychologic and neurophysiologic tests were per-

formed in addition to a logging of subjective status. During the bottom phase several wet lock-outs were conducted.

METHODS

Experimental Design

Six subjects breathed heliox during the compression to 450 msw. The planned compression profile (Table 1) was to be followed, providing that no severe symptoms occurred.

The Compression Phase. During compression the subjects rotated between the different test setups. The test sessions were at 100 msw, on reaching and before leaving 200 msw, on reaching and before leaving 300 msw, at 350 msw, on reaching and leaving 400 msw, and on reaching 450 msw.

The Bottom Phase. The same procedure was repeated the 2nd day at 450 msw, on dive Day 8 and dive D 12. Every day a morning and evening symptom questionnaire was administered.

The Decompression Phase. Morning and evening symptom questionnaires were administered every day, and the testing described below was performed at 400, 350, 200, and 100 msw.

Table 1
Compression Table

Depth, msw	Rate, msw/min	Event Time, min	Elapsed Time, min	Elapsed Time h:min
1-10	1.0	10	—	—
10	hold	(20)	00	00
10-100	1.0	90	90	1:30
100	hold	120	210	3:30
100-200	1.0	100	310	5:10
200	hold	300	610	10:10
200-300	1.0	100	710	11:50
300	hold	720	1430	23:50
300-350	0.5	100	1530	25:30
350	hold	120	1650	27:30
350-400	0.5	100	1750	29:10
400	hold	300	2050	34:10
400-450	0.5	100	2150	35:50
450	hold	720	2870	47:50

Instrument Description

The compression battery. The following 6 neuropsychologic and neurophysiologic tests were used: a) Electroencephalography with bipolar recording from both hemispheres (P3-F3 and P4-FA); data were Fast Fourier Transform (FFT) computer analyzed in 2-s epochs. b) The Static Steadiness Test for recording postural tremor. c) The Nihon Koden accelerometer for recording microtremor. d) The Finger Oscillation Test for Recording finger arthralgia. e) The Dynamometer Test for recording hand grip strength. f) The Trail B test for recording visuomotor speed and coordination. For further description of the tests, *see* Værnes et al. (5).

The Performance Tests. A battery of motor, visuomotor, and cognitive tests and a symptom questionnaire were administered, together with the compression battery. For further description of the tests *see* Værnes et al. (5).

Test Procedure

The test procedure was the same for all 6 divers. Predive testing, including all tests, was done repeatedly during the predive weeks. This was done to familiarize the subjects with the test procedures to be used in the pressure chamber and to reduce the effects of learning in-dive. The average control values were calculated from the five last samples for all variables in the predive period.

Both predive and during the dive, each test session started with the administration of the cognitive group tests. Thereafter, the compression battery was performed by 2 divers, while the 3rd filled out his symptom questionnaire. The motor tests were administered on the Performance Measurement System (PMS) individually, partly in parallel with the FFT EEG and the microtremor test.

RESULTS

The Compression Battery

FFT EEG. During compression, 2 divers had more or less normal FFT EEGs. The other 4 had a more traditional HPNS-EEG pattern with slow wave increase and alpha band inhibition. Two examples are given in Fig. 1. None of the 4 divers normalized in their FFT EEG during the saturation period, and it was not until they decompressed out of the HPNS zone and arrived at the surface that the FFT EEGs were similar to predive results. Only 1 diver had a sustained slow wave increase at surface postdive, but the center frequency had returned in the alpha band area around 10 Hz.

Postural Tremor. There were minor tremor increases during this dive compared with previous deep dives at NUTEC. Interestingly, it was the 2 divers with normal FFT EEGs who had the most marked increase in tremor.

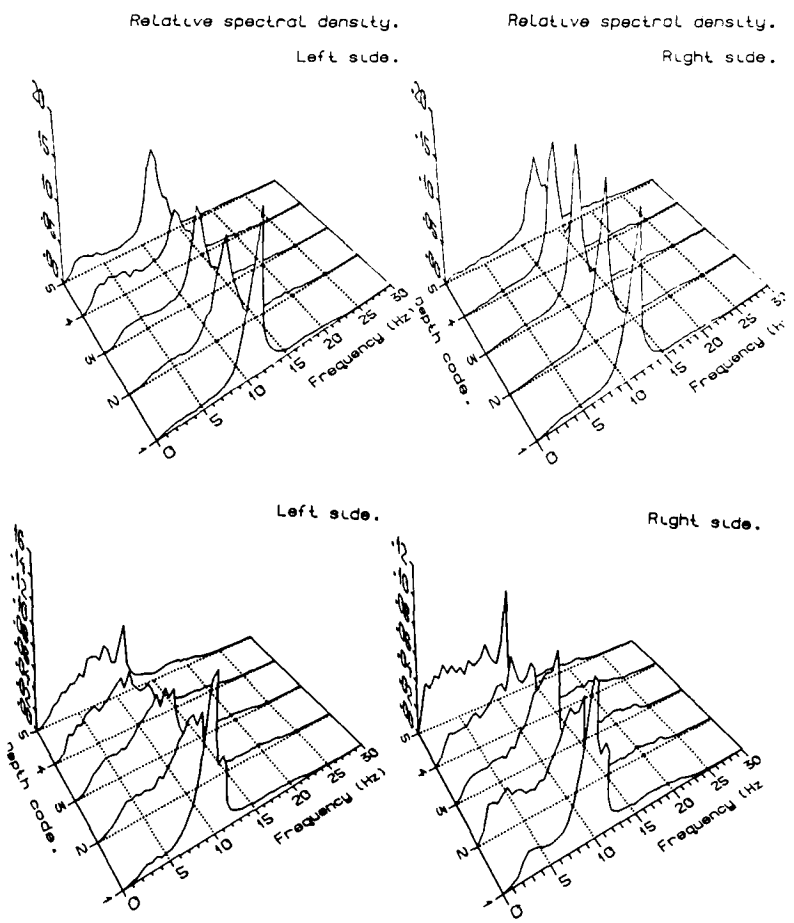

Fig. 1. Fast Fournier Transform EEG for 2 divers (left and right hemisphere) during the compression to 450 msw (1:predive, 2:300 msw, 3:350 msw, 4:400 msw, 5:450 msw).

There were no marked decreases in tremor for these 2 divers until reaching 350 msw during decompression (Fig. 2).

Microtremor. Although postural tremor was minimal in this group, all 6 divers had an increased amplitude in finer microtremor. But in contrast to the stability in postural tremor, microtremor seemed to vary to a greater extent and to be partly related to other mild CNS reactions that occurred periodically, such as nausea and dizziness. However, all divers had returned to predive levels of microtremor at 100 msw during decompression.

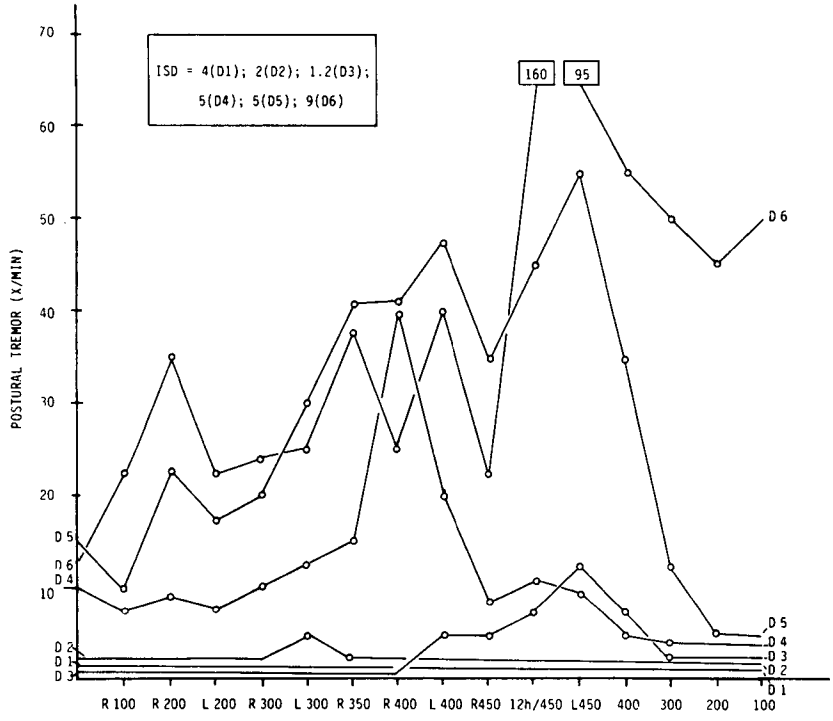

Fig. 2. Postural tremor (x/min) for all 6 divers during the compression to 450 msw.

Hand-Grip Strength. Except for 1 diver with wrist pain, all divers functioned within the normal range in hand-grip strength on arrival at 450 msw. During the bottom phase and decompression, there were no abnormal results on this test.

Finger Oscillation Speed. The results for this test were normal, except for 1 diver with impairment at 300 msw.

Visuomotor Speed. Two divers had impaired visuomotor performance on reaching 450 msw. Especially for 1 diver, this corresponded with experienced nausea and tremors. There were no abnormal results on this test during the bottom phase or in the decompression phase.

The Performance Tests

Motor Tests. On reaching 200 msw, 2 divers had a marked (35%) impairment in finger dexterity which continued at 300 and 350 msw. However, on reaching 450 msw, all 6 divers functioned within the normal range.

Except for an impaired manual performance for 1 diver at 300 msw, all functioned within the normal variation range throughout the whole compression on the manual dexterity test.

Three divers had stable impairment of about 2 SD below predive average on arm wrist speed during compression.

Cognitive Tests. Although the majority of the divers had impaired long-term memory during phases of compression, only 1 diver scored below normal on reaching 450 msw (Fig. 3). All divers functioned within the normal variation range on this test during the bottom and decompression phases.

As for memory performance, reasoning was affected by compression, but again the divers performed at predive levels on arrival at 450 msw. The same pattern was also found for the arithmetic test and perceptual speed, for which only 1 diver showed an impairment periodically during compression.

Two reaction-vigilance tests on the PMS, operational test and visual reaction time, confirmed that simple perceptual functions were generally only mildly impaired.

Symptom Questionnaire

The great majority of symptoms were only rated as "some impairment with no operational impact" (2). During compression the most frequent symptoms were dizziness, poor appetite, tremors, and upset stomach. As in previous deep dives at NUTEC, several symptoms occurred at 200 msw (Table 2). During the bottom phase and decompression, 2 divers frequently reported mild problems with memory, tiredness, and tremors. These symptoms were partly related to depth and partly to poor sleep.

Table 2
Total Number of Symptoms Scored During Compression
(R = Reaching, L = Leaving)

Depth	100	R200	L200	R300	L300	350	R400	L400	R450	12h/450
Symptoms	8	42	19	43	15	45	61	40	40	14

DISCUSSION

The HPNS testing during this dive revealed only very mild effects of compression and the stay at depth. Most of the findings during compression were isolated and did not approach levels of significant abnormality. Compared to previously monitored HPNS effects during compressions to deep depths, the symptoms and signs observed in this dive were mild (5, 7, 8). Two of the divers had normal FFT EEGs and 4 of the 6 divers had normal cognitive test results. The long-term memory test showed impaired function during compression, but normalized on reaching 450 msw. This confirmed previous results, where the same memory impairment was observed, even though only a mild HPNS was found.

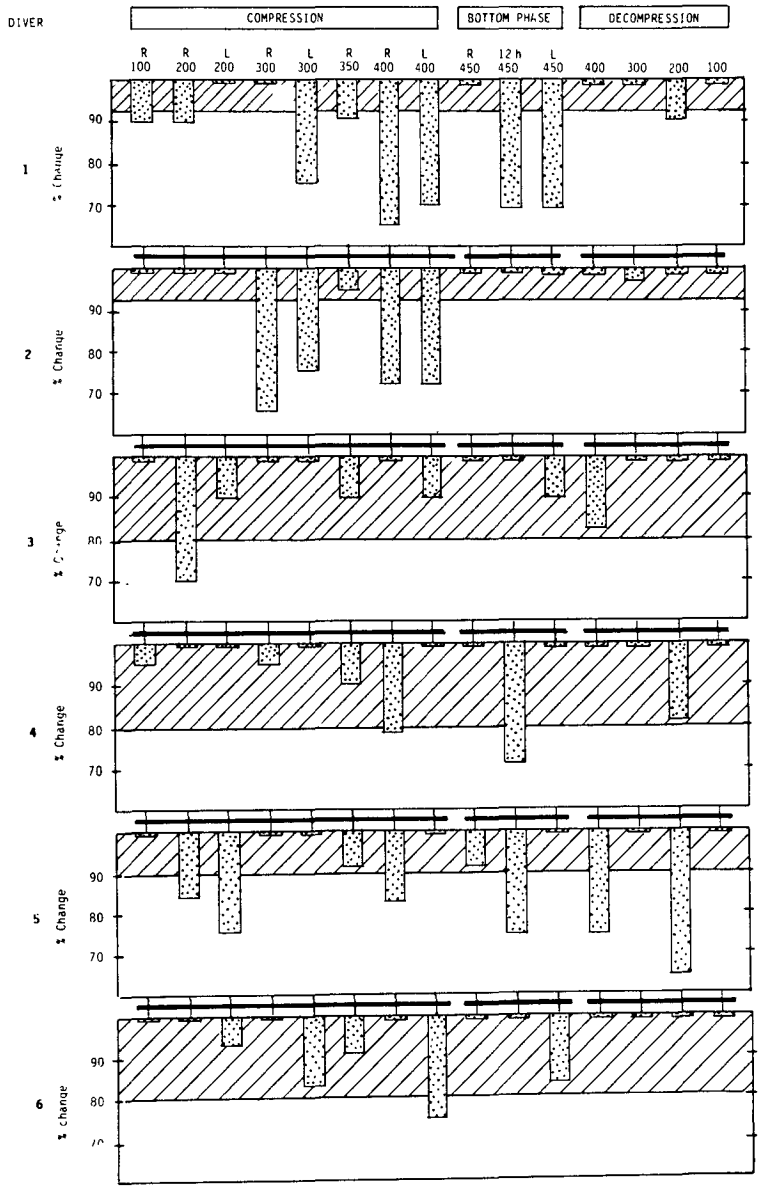

Fig. 3. Long-term memory for all 6 divers during the compression to 450 msw. One hundred percent is defined as the predive average. (Crosshatched area is 1 SD of predive control (R = Reach, L = Leave).

Individual variation was a striking feature. The divers often showed very different patterns of abnormal test results. This lack of a clear pattern in

symptoms and signs of HPNS is a strong argument against the hypothesis that the discussed pressure-related symptoms are to be considered as a single syndrome. The symptoms are more likely a result of many different neurophysiologic changes. These changes could then be further modified by the individual variability usually seen in neuropsychologic and neurophysiologic testing.

This variability in symptoms and signs presentation has been noted previously (5-8), and is the basis for using multiple tests for HPNS and performance to evaluate the total effect of the compression profile. Even with more severe symptoms of HPNS, a marked difference in the pattern of symptoms and signs between divers has been noted.

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EFFECT OF THE SHIFT FROM HYDROGEN-HELIUM-OXYGEN MIXTURE TO HELIUM-OXYGEN MIXTURE DURING A 450 MSW DIVE

J. C. Rostain, C. Lemaire, M. C. Gardette-Chauffour, and R. Naquet

The use of hydrogen in the breathing mixture for deep diving is interesting because of the low density of the mixture and because its narcotic potency may reduce some symptoms of high pressure neurologic syndrome (HPNS), according to the antagonism of narcotic substances-pressure described by several authors (1-4).

A previous study (Hydra IV) has shown that a hydrogen-oxygen mixture breathed during 30 min induced narcoticlike effects at 240 and 300 msw (5). Consequently, the narcotic potency of hydrogen was too high to enable us to use hydrogen alone in the mixture.

The present study (Hydra V) was performed with a hydrogen-helium-oxygen mixture. A group of 3 divers breathed a hydrogen-helium-oxygen mixture (56% H_2) and then were switched to a helium-oxygen mixture (0% H_2). We considered that the switch might induce problems (6, 7) so we analyzed the HPNS in every situation.

METHODS

The first group of 3 professional divers (COMEX and French Navy) was compressed to 450 msw in 38 h, according to the compression curve described elsewhere (8). The compression was performed in a helium-oxygen mixture to 200 msw and afterward with hydrogen. At 450 msw the divers stayed 64 h in a hydrogen-helium-oxygen mixture with 54 to 56% of hydrogen (25 to 26 bar).

Expecting some previously described troubles with these gas shifts, the schedule was changed so that the first slight change occurred from 56 to 30% H_2, then followed 8 h later by the shift from 30 to 0%. The subjects stayed in helium-oxygen mixture for 128 h. The decompression lasted 15 d.

The humidity was around 50% and the partial oxygen pressure was maintained at 0.4 bar during compression and the stay, at 0.5 bar during the decompression.

Our data for EEG and tremor were obtained continuously from 57% hydrogen to 0%. References were based on the days preceding the switch and values of recovery are given for days after the switch. EEG activities were recorded (twin bipolar leads) on an EEG and magnetic tape for subsequent computer power spectral analysis from electrodes made of platinum wires fixed on the skull for the duration of the experiment. They were implanted on frontopolar, central, midtemporal, and occipital positions of the right hemisphere. The tremor was measured with an accelerometer placed on the middle finger of the dominant hand.

For performance, we considered results obtained 14 h after arrival at depth as a reference, then results obtained after the first shift and, due to the schedule, 3 d after the shift of 0% H_2.

The tests used were the same as currently performed by our divers during all the deep dives as evaluation of performance at depth (manual dexterity, visual choice reaction time, number ordination) (8, 9) with an additional test called "psychomotor and mental promptness," more adapted to test narcosis.

RESULTS

EEG Analysis

Compression with the hydrogen-helium-oxygen mixture induced a decrease in alpha and beta activities in all the leads. This decrease persisted during the stay at 450 msw in H_2-He-O_2 or He-O_2 mixtures. The increase of slow waves (theta frequency band) was recorded during compression from 400 msw (200% increase) in 1 subject (A3). This 100% increase persisted during the stay in H_2-He-O_2.

A second subject (A1) had an increase of theta activity (100%) during the stay in H_2-He-O_2. A 3rd diver (A2) did not present significant changes in slow waves.

The switch of the mixture produced an increase of slow waves, especially theta in frontocentral and centrotemporal region in all the subjects. This increase occurred 1 h after the switch to 30% H_2 and the maximum value (500 to 600% increase) was recorded during the test performed 3 h later.

The second switch to 0% H_2 induced a new increase of theta waves in 2 subjects. Subject A2 seemed to have a depression of his EEG activities in every frequency band analyzed. During the days of the stay at 450 msw, after the switch to He-O_2 mixture, we recorded a decrease of the peak of theta activity. The EEG returned to control values during decompression after 300 msw.

Hyperbaric Tremor Analysis

The compression to 450 msw and the stay at the bottom in hydrogen-

helium-oxygen mixture did not induce tremor in all the subjects. The switch to the helium-oxygen mixture induced an increase of tremor in all of the subjects (around 50 to 100%) which persisted during the stay at 450 msw and during decompression until 200 msw.

Performance

Manual Dexterity: Previously altered by the compression to 450 msw, the manual dexterity decreased 6% during the stay at 30% H_2, and 7% in helium-oxygen mixture 3 d later.

Visual Choice Reaction Time: Recovery obtained before the switch was abolished by the shift (-9% at 30% H_2). Three days later, in helium-oxygen mixture, no more decrement was noted (a slight recovery to -6%)

Number Ordination and Mental Promptness: These tests were not performed after the first switch to 30% H_2. No change was noted 3 d later in the helium-oxygen mixture.

COMMENTS

EEG Changes

The results of EEG studies indicate that the H_2-He-O_2 mixture with 56% of hydrogen induces similar changes to those recorded with He-N_2-O_2 or He-O_2 at 450 msw with the same compression curve (10, 11).

During the change of the mixtures, the increase in theta activities was accentuated. This increase appeared rapidly and was intense in the 3 subjects. The decrease, which appeared during the test performed 7 h after the first switch, suggests that this manifestation is related to the rapid change of the mixture and the sudden increase of helium partial pressure; it might be equivalent to a fast compression effect.

The decrease of theta waves after the change of the mixture, which was never seen before in He-O_2 mixture, could be related to a secondary effect of the switch of the mixture.

Hyperbaric Tremor

The suppression of tremor and other clinical symptoms of HPNS (dysmetria, fasciculation, drowsiness) indicate that the hydrogen-helium-oxygen mixture with 56% of H_2 prevents the appearance of these symptoms. This effect is probably related to the "narcotic potency" of hydrogen, which antagonizes the pressure according to the observation or hypotheses of several authors (1, 2, 12, 13).

The tremor appears rapidly after the mixture is switched. This phenomenon might result from the disappearance of hydrogen, which was necessary to suppress the tremor; it might also be the consequence of the sudden increase of helium, in which case it would be equivalent to a rapid compression.

Performance

For the psychometric test, the decrements observed after the shift to the helium-oxygen mixture are equivalent to those noted just after the compressions with helium-nitrogen-oxygen mixtures (ENTEX series) (7, 10, 11). The effects of the switch to heliox at 450 msw are the same as the effects of compression to 450 msw in $He-N_2-O_2$ or $He-O_2$ mixtures. Consequently, the decrements noted for the deep dives can be attributed to the increase of the partial pressure of helium.

In conclusion, the switch to a helium-oxygen mixture produces troubles that were not present with the hydrogen-helium-oxygen mixture (increase in EEG changes, appearance of tremor, decrease in psychometric performance). Consequently, this procedure must be avoided to prevent neurophysiologic and psychologic impairments.

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HYDROGEN NARCOSIS, NITROGEN NARCOSIS, AND HPNS: A PERFORMANCE STUDY

C. Lemaire

Behavior changes and performance decrements induced by hyperbaric exposures are now well documented. Many factors influence these modifications, the most important being: depth, rate of compression (related to depth), and breathing mixture (depending on depth). The two first points have been well studied during the breathing of oxygen-helium or oxygen-nitrogen-helium mixtures, and their effects on performance are well established (1-4). Addition of hydrogen at great depths had not been studied in man before the HYDRA IV dive (5). The use of hydrogen in breathing mixtures at great depths is supposed to reduce the high pressure nervous syndrome (HPNS), but this gas is known to have narcotic effects when breathed under high partial pressures. Divers' efficiency can be altered, and at great depths it can only be used in addition to oxygen-helium mixtures. From another point of view, nitrogen also induces narcosis, but at shallower depths. This narcosis includes: behavior alteration, decrease of manual dexterity and vigilance, and decrease of intellectual performance (6, 7).

By adding a small amount of nitrogen to the oxygen-helium mixtures it has been possible to reach very great depths (up to 686 msw) with a minimum of perturbations (8, 9), but HPNS is still present enough to prevent much greater depths being reached. Another question concerns the relative role of partial pressure and total pressure. More precisely, does 5 ATA nitrogen have the same effect while breathing air at 40 msw or when added to oxygen-helium at 450 msw? Are the effects the same while breathing 25.6 ATA hydrogen with oxygen at 250 msw or while breathing 25.6 ATA hydrogen with oxygen-helium at 450 msw? To compare the perturbations induced by hydrogen at 450 msw and nitrogen narcosis, we evaluated the performance with the same psychomotor tests under two different situations: breathing air at shallow depth and

breathing H_2-He-O_2 at 450 msw.

METHODS

For many years we have had the opportunity of testing divers during all of the French deep dives, and it was possible during this time to use the same performance tests, i.e., pegboard tests to evaluate manual dexterity, a red light-green light reaction time to measure vigilance, and a paper-pencil test of number ordination or letter recognition for mental ability.

Among other dives, the tests have been performed by 8 subjects breathing air at 42, 60, and 75 msw, then at 75, 60, and 42 msw (to evaluate habituation) from a 15-msw saturation lever (NEREIDE 1) (10), and by 6 subjects breathing a H_2-He-O_2 mixture to 450 msw (56% H_2, 43% He, 1% O_2) (HYDRA V) (11).

RESULTS

Manual dexterity (MD)

For each mixture, decrement in performance was a function of depth. Breathing air, the decrease reached 5% at 42 msw, 6% at 60 msw, and 12% at 75 msw. Breathing H_2-He-O_2 mixture, the performance decreased by 2% at 250 msw and by 5% at 450 msw. These values measured upon arrival at maximum depth tested were not modified during the stage under pressure.

Visual Choice Reaction Time (VCRT)

The same effect of depth was noted: with air, the decrease of performance ranged from 1% at 42 msw to 8% at 60 msw, and 12% at 75 msw. With H_2-He-O_2, the values measured were -2% at 250 msw and -7% at 450 msw. Recovery was evident 24 h later (Table 1).

Table 1
Psychomotor Performance

	Air			H_2-He-O_2	
Depth msw	42	60	75	250	450
MD	- 5%	- 6	-12	- 2	- 5
VCRT	- 1%	- 8	-12	- 2	- 7

Intellectual Tests

Breathing air, the decrease of performance was less important at 60 msw (6%) than at 75 msw (16%). No marked variation was noted at 450 msw for the number ordination test (-3%), but the test of mental and psychomotor prompt-

ness was more affected (-20%).

DISCUSSION

The two situations altered the performance, and the amplitude of the modifications is approximately the same while breathing air at 60 msw or breathing H_2-He-O_2 at 450 msw. The performance decrement being the same, it may be presumed that the same narcosis is present in both cases.

First, psychometric tests must not be considered outside the context of the dive. Divers are in a particular environment (chamber ambient temperature, noise, heavy breathing mixture, etc.) and their behavior is partly dependent on this environment and partly on the effects of pressure (or on the breathing mixture under pressure). Performance tests are only indicators, and for these 2 dives we had subjects whose behavior led us to think that test results would be bad. Especially during HYDRA V the divers were perfectly able to concentrate during the tests and to maintain their performance level. The slight euphoria manifested could be well controlled for the duration of the tests. For the intellectual tests, which had been chosen as sensitive to narcosis, we noted more perturbations; this is the translation of a certain level of difficulty in assimilation of orders and in speed of executing orders.

NEREIDE results are similar to the ones described in previous experiments. A slight habituation is present as a function of the duration of stage at saturation level or as a function of the number of dives. Saturation at 15 msw does not improve the performance of the divers.

The HYDRA V dive showed better results with psychomotor tests than the ones noted previously with He-O_2 or He-N_2-O_2 mixtures for the same compression curve. Compression is better tolerated and the results showed an improvement 24 h after arrival at depth. H_2-He-O_2 seems now to be a good mixture to ensure good performance of working divers in this depth range.

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INTERACTIONS BETWEEN ETHANOL, AMPHETAMINE, AND INERT GAS NARCOSIS ON THE PERFORMANCE OF A MEMORY SCANNING TASK

B. Fowler, H. Hamilton, and G. Porlier

It is well known that inert gas narcosis disrupts human performance on a variety of tasks. Lately, for both practical and theoretical reasons (1), there has been a good deal of interest in how drugs may influence this impairment. Practical concern is generated by the increase in drug usage and the consequences for the operational safety of divers. Theoretically, there exists a distinct possibility that patterns of drug effects may provide insight into the mechanisms underlying narcosis. In this regard, it has been demonstrated that both nitrous oxide (2) and hyperbaric air (3) increase the intercept but not the slope of the Hick-Hyman choice reaction time function. This function represents a stage of processing involving the choice of a response to a stimulus (4). It has also been found that ethanol, a depressant, exacerbates narcosis while amphetamine, an excitant, ameliorates it (2, 3). These experiments used an identical serial choice reaction time task. This raises the question whether this pattern of results can be generalized to other reaction time tasks representing different stages of information processing. The purpose of this experiment was to investigate this issue by determining how nitrous oxide, ethanol, and amphetamine influence the slope and intercept of the linear short-term memory scanning function generated by Sternberg's item-recognition paradigm (5, 6).

MATERIALS AND METHODS

Subjects

Three male and 3 female, paid volunteers, 20 to 25 yr old were used in this experiment. Each subject was medically screened and signed informed

consent documents. All were social drinkers and had experienced the effects of breathing nitrous oxide. As part of the screening procedure, they were also exposed to a 5-mg dose of dextroamphetamine. The experimental protocol was approved the Human Ethics Committees at both York University and DCIEM.

Apparatus

The stimuli were digits displayed on a computer screen (Commodore model SP9000). The subject was seated in a reclining chair at a distance from the screen such that the maximum visual angle subtended by the stimuli was approximately 2°. Responses were made by pressing one of two finger-operated pushbuttons (Microswitch model PK 85022) mounted on each armrest of the chair. Reaction time (RT) was defined as the latency from stimulus onset to depression of a pushbutton. A PDP 11/04 computer controlled presentation of the stimuli and recorded RT as well as the number of errors (pressing the incorrect pushbutton).

Narcosis was induced with a 20% N_2O: 80% O_2 mixture and air was used as a control. Both mixtures were breathed via a nonrebreathing, oronasal mask. Ethyl alcohol (40% by volume) was administered orally, using a dose of 1.5 ml/kg of body weight, mixed with orange juice to a total volume of 350 ml. It was determined with an alcoholmeter (Thomas model AE-D1) that this dose produced a peak blood ethanol level of 0.06 to 0.09 g/ml during the testing session. The control consisted of an equivalent volume of juice with 1 ml of ethanol layered on top. A fixed dose of 15 mg of dextroamphetamine sulfate (Dexadrine) was administered orally in two capsules. Dextrose in identical capsules was used as a control.

Design and Procedure

A repeated measures, single-blind design was used with 6 conditions tested in separate sessions: placebo, ethanol, dextroamphetamine, nitrous oxide, ethanol combined with nitrous oxide, and dextroamphetamine combined with nitrous oxide. The order of presentation of conditions was partially counterbalanced across subjects.

The varied set procedure of Sternberg's item-recognition paradigm with equi-probable responses was used (6). Random digits in sets of 1, 3, and 5 were presented for 2000 ms, followed 300 ms later by a test digit presented for 1395 ms. The intertrial interval was 600 ms. The subject responded to each test digit by pressing one of the pushbuttons to indicate whether or not the digit had been included or excluded from the preceding set. In every condition, each set size was administered in a block of 30 trials with 15 included and 15 excluded test digits presented in a random order. Presentation of the 3 set sizes and the assignment of left and right pushbuttons to included and excluded test digits were counterbalanced across subjects.

Before the experiment the subjects were practiced on the task while breathing air, nitrous oxide, and after 5 mg of dextroamphetamine until their performance was stable with an error rate of 1 to 2%. On the days of the

experiment, subjects were restricted to a bowl of soup for lunch and then tested individually in the late afternoon. At least 1 d elapsed between each session. The capsules were ingested 2 h and the liquid 35 min before each session. Breathing via the mask commenced 10 min before the session. When the error rate exceeded 1 to 2% during a block of trials, the data were discarded and the block repeated immediately.

RESULTS

For each subject in each condition, mean correct RT was plotted against digit set size and fitted with a linear function by the method of least squares. The placebo and nitrous oxide conditions are illustrated in Fig. 1.

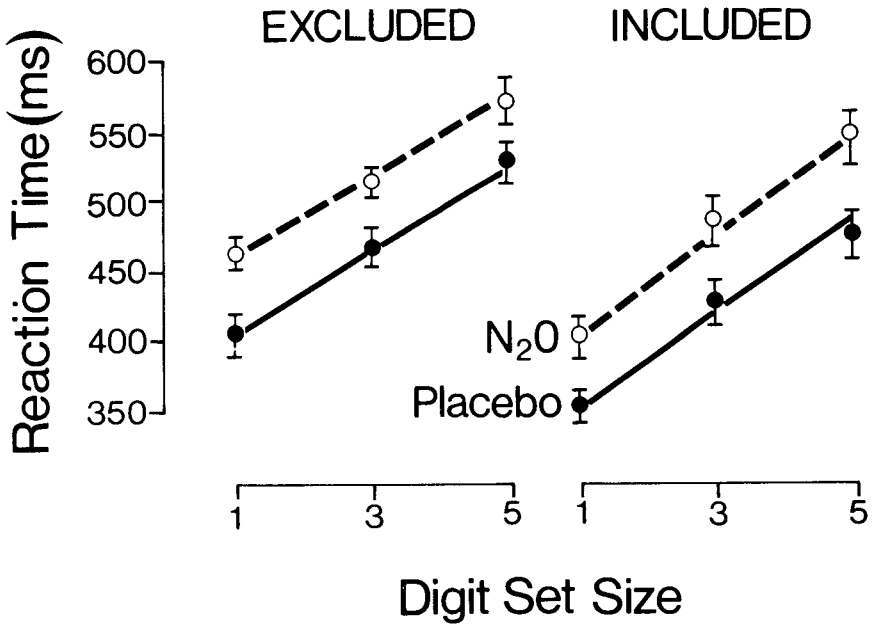

Fig. 1. The relationship between reaction time and digit set size in the memory scanning task for both excluded and included digits. The placebo and nitrous oxide (N_2O) conditions are used as examples. Bars represent SEM.

Repeated measures analyses of variance, comprising 2 test digits (included-excluded) × 6 conditions, were conducted separately on the slopes and intercepts of the individual memory-scanning functions. For the slopes, no effects were significant. Thus, the various drug conditions did not alter the slopes. The mean slope, pooled across all conditions, was 35 ms/digit. This falls within the normal range of experimental results (6). For the intercepts, test digits ($F1, 5 = 60.26$, $P < 0.001$) and conditions ($F5, 25 = 12.5$, $P < 0.001$) were significant but not the interaction. These results show that RT was faster to

included than excluded digits (353 vs. 406 ms), a well-established finding for equi-probable test stimuli (6). The intercepts of the various conditions differed from one another and these results, pooled across included and excluded digits, are illustrated in Fig. 2. Duncan's post hoc test revealed that, compared to the placebo, ethanol and nitrous oxide increased the intercept ($P < 0.05$ in both cases) while dextroamphetamine had no significant effect. With respect to nitrous oxide alone, ethanol combined with nitrous oxide increased the intercept ($P = 0.05$) whereas dextroamphetamine combined with nitrous oxide decreased it ($P = 0.01$). In the latter case, this decrease was sufficient to make the intercept virtually identical to the placebo condition. The size of the effect predicted, if ethanol and nitrous oxide were additive, is also illustrated in Fig. 2. It is evident that the data fit this prediction reasonably well (actual vs. predicted, $t\ 5 = 1.44$, n.s.).

DISCUSSION

The results of this experiment are consistent with a wide body of evidence that has been uncovered in recent years. This evidence is believed important for the development of an adequate theory of the behavioral effects of inert gas narcosis.

Nitrous oxide increased the intercept of the memory scanning function while leaving the slope unaffected. This outcome is consistent with the results of a previous item-recognition experiment which used enflurane to induce narcosis (7). The link between nitrous oxide and enflurane provides further support for the qualitative equivalency hypothesis, which proposes that all inert gases exert identical effects on behavior (1).

When combined with nitrous oxide, ethanol increased the intercept of the memory-scanning function additively while dextroamphetamine decreased it. In the latter case, it was not possible to establish whether the decrease was additive or synergistic because amphetamine alone did not affect the intercept significantly. This paradoxical effect has been observed previously (2) and explained by assuming that this drug cannot improve performance beyond some optimum, but can ameliorate deterioration from this level. The pattern of effects for ethanol and dextroamphetamine is identical to the pattern obtained with the Hick-Hyman function (2, 3). This congruency is consistent with the hypothesis that ethanol, dextroamphetamine, and narcosis have a common effect on different information processing stages.

What insights can be gained from the results of this experiment about the behavioral mechanisms that are disrupted by narcosis? It has been theorized that the linear function obtained with the item-recognition paradigm reflects the processing rate of an exhaustive serial-scanning stage (6). In this view, the test digit is compared successively to the set of digits held in short-term memory. The slope of the function represents the scanning rate of this stage while the zero intercept represents the sum of times of all other stages involved in the task. Thus, the finding that enflurane and nitrous oxide

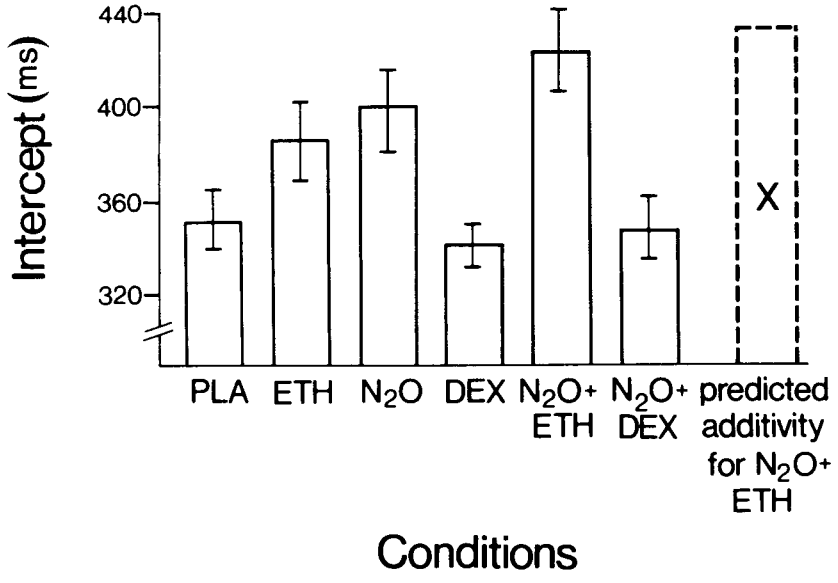

Fig. 2. Mean intercept for the regression functions obtained from the memory scanning task for each experimental condition. Bars represent SEM. PLA = placebo, ETH = ethanol, N_2O = nitrous oxide, DEX = dextroamphetamine.

influence the intercept but not the slope implies that the efficiency of the scanning mechanism is unaffected and that some other stage is slowed. However, it has not been possible to identify a stage that is slowed by the inert gases (1). Moreover, narcosis is exacerbated by ethanol and ameliorated by dextroamphetamine. Therefore, it has been proposed that slowing occurs before stage processing and that narcosis represents a decreased state of arousal (2). This assumption is an important element of the slowed processing model of inert gas narcosis (1).

These drug experiments have implications for diving operations. The weight of present evidence clearly favors the view that ethanol exacerbates rather than ameliorates narcosis (8). Consequently, ethanol should not be ingested before diving. Furthermore, a combination of ethanol and narcosis could conceivably have more far-reaching effects on performance than the slowing demonstrated in the present experiment. For instance, it has been suggested that ethanol disrupts the effector system in addition to decreasing arousal (1). If this is the case, a good deal of caution is required in extrapolating to underwater operations. Even more care is required in the case of dextroamphetamine. In our laboratory, it has been observed that the subjects have difficulty in maintaining a low error rate after ingesting dextroam-

phetamine, either alone or in combination with an inert gas. An increased error rate in diving operations could have catastrophic consequences which far exceed any benefits gained from decreasing the slowing induced by narcosis. On the other hand, it may be possible to overcome problems of this kind if appropriate diver training and indoctrination procedures are used (9). This question awaits further research.

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9th International Symposium on Underwater and Hyperbaric Physiology
Undersea and Hyperbaric Medical Society, Bethesda, MD, 1987

NEUROPHYSIOLOGICAL AND BEHAVIORAL CORRELATES OF COLD WATER IMMERSION

D. L. Reeves, M. M. Winsborough, and A. J. Bachrach

Changes associated with immersion in cold water have long been a source of concern for divers and for diving researchers interested in assessing effects of cold water on operational effectiveness and safety. In recent years the technology of electrophysiologic evaluation of central nervous system activity has advanced significantly, particularly in the development of measures of evoked potentials (1). The main purpose of the present series of studies was to apply the measurement of visual and auditory event-related potential latencies to an assessment of the effects of cold water immersion on neurophysiologic processes such as nerve conduction. Related performance measures such as tapping the grip strength were of interest to determine behavioral factors in nerve conduction velocity. Mood check lists were also administered to assess possible emotional alterations associated with cold water immersion.

MATERIALS AND METHODS

Subjects

Seven male volunteers were recruited under informed consent from the research staff of the Naval Medical Research Institute in Bethesda, MD. Requirements for participation were normal vision or vision corrected to 20/20; normal audition; robust health (as determined by our staff medical officer); and claimed nonuse of medicinal drugs or alcohol for at least 24 h before time of testing. Means of age, height, body mass, and percent body fat were 25.9 yr, 1.77 m, 75.4 kg, and 10.46%.

Experimental Design

A repeated measures design was used in which pre- and posttest data

were recorded during 3 independent experimental sessions designated as cold water immersion, thermoneutral water immersion, and thermoneutral dry.

Independent and Dependent Measures

Colonic and hand temperature were monitored continuously by means of flexible thermocouple leads connected to a Bailey digital electronic thermometer. Temperature recordings were taken at the beginning and end of each testing session and then again immediately after collection of data on each dependent measure.

Colonic temperature was indexed by use of a rectal thermocouple and hand temperature was measured by a disk-type skin thermocouple that was taped to the center and back of the dominant hand.

The dependent variables were: the latency of the P100 component of the pattern-reversal evoked potential (PREP); the event-related P300 potential; grip strength; finger-tapping rates; and test scores on the anxiety, hostility, and depression scales of the Multiple Affect Adjective Check List (MAACL).

Apparatus: Nicolet Compact-4

Electrophysiologic data were recorded and averaged by a portable Nicolet Compact-4 evoked potential analyzer. Settings for recording the pattern-reversal component P100 were as follows: upper and lower bandpasses, 100 and 1 Hz; automatic artifact rejection, ± 100 μV; span of time for response analysis was 250 ms.

Electrode placement was achieved by means of an electrode cap with scalp electrodes coupled to the signal averager by a flexible set of conductive leads. Two-channel PREPs were recorded from electrodes at occipital locations (01 and 02) with a forehead (Fpz) reference; the ground electrode was attached to the right earlobe.

The visual stimulus consisted of a black-and white checkerboard generated by a Nicolet NIC-1015 video monitor which measured 16 x 18 cm and presented a pattern-reversal rate of one reversal every 500 ms. The video screen was positioned at eye level and 1 m from the subject's nasion. Each check subtended approximately 0.5° of visual angle and the whole screen subtended approximately 15° of visual angle. Approximately 110 repetitions of the stimulus were presented to each subject to permit averaging of 100 artifact-free potentials.

Averager settings for the event-related P300 were: 30 and 0.5 Hz for upper and lower bandpasses; artifact rejection, ± 100 μV; and span of time for response analysis was 750 ms.

Electrode placement for single-channel recording was at the vertex (Cz) with a right earlobe reference and a left earlobe ground.

Stimuli for eliciting a P300 consisted of 250 binaural tone bursts (50 ms duration 10 ms rise/fall time, 65 dB SPL sound intensity, and 0.7/s recurrence rate). These were delivered to the subject through Amplivox shielded earphones. Ninety percent of the tone bursts (designated as "frequent tones")

occurred at a frequency of 1500 Hz. The sequence of frequent and rare tones was random.

The subject's task was to attend only to the "rare" tones and keep a mental count, which was reported after the recording.

Apparatus: Tapping Board and Hand Dynamometer

Grip strength of the dominant hand was recorded in kilograms by use of a hand dynamometer. The test procedure consisted of holding the dynamometer with the hand hanging loosely at the subject's side while standing, and then simply squeezing his hand as hard as he could. Scores consisted of the average of 2 consecutive trials.

Maximal tapping speed of the index finger of the dominant hand was measured by use of the Reitan adult tapping board (Fig. 1). The test procedure called for 5 consecutive 10-s trials that were within a 5-point range from fastest to slowest. Intertrial intervals were 30 s and a maximum of 10 trials was recorded. The score was the mean for 5 consecutive trials.

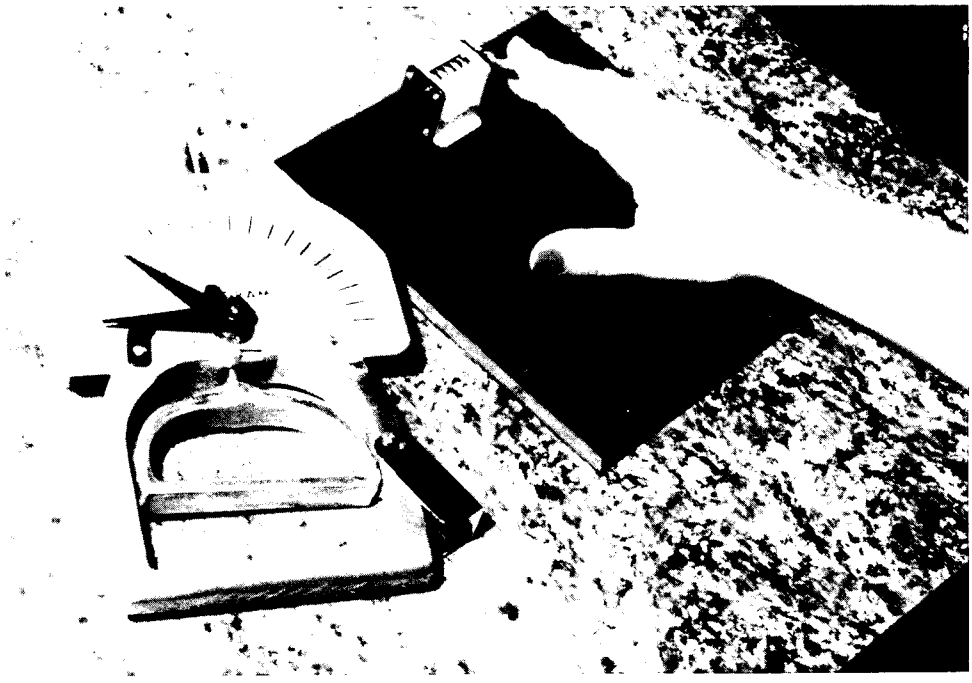

Fig. 1. The Lafayette hand dynamometer and the Reitan tapping board.

Test Facility and Cold Tubs

The experiment was conducted in the Environmental Stress immersion tank facility located at the Naval Medical Research Institute, Bethesda, MD. The room was well lit except during PREP recordings, at which time lighting

was reduced to low illuminance originating from a shielded 25-W lamp. Ambient room temperature was maintained between 24 and 26°C as indexed by a WGBT monitor. Immersion tanks were constructed from redwood, and water temperature was thermostatically controlled to ± 1.0°C. The cold-water immersion tank measured 1.2 m in diameter and 1.1 m deep (measured at water level). The immersion tank used for thermoneutral runs measured 1.9 m in diameter and 1.1 m deep.

PROCEDURE

Upon arrival at the laboratory, subjects underwent a standardized orientation procedure, physical examination, donned a pair of swimming trunks, and were fitted with the electrode cap and hand and rectal thermocouples. Then they were taken to the experimental facility, seated in a reclining chair, and tested on the battery of dependent measures.

Following baseline measurements they either remained in the chair (for the thermoneutral dry condition) or entered and sat in the thermoneutral water tank (the thermoneutral wet condition) for 60 min, followed by administration of the posttest battery.

During cold-water runs, subjects entered and sat (immersed up to their chins) (Fig. 2) in the cold-water tank for a period of 60 to 90 min or a reduction of core temperature 1.5° below their baseline temperature (whichever came first). Water temperature, which ranged between 10 and 14°C was predetermined and adjusted for each individual's physical composition using a procedure presented earlier by Winsborough et al. (2).

As the core temperature neared the criterion (about 1.2° below baseline) a second set of PREPs was recorded. At the criterion temperature the tapping

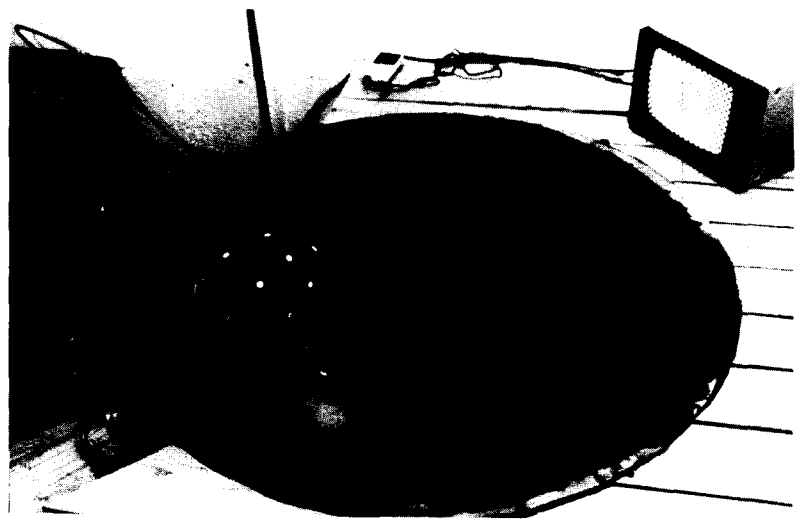

Fig. 2. A subject in the cold immersion tank ready for visual evoked response testing.

test and MAACL were administered while the subject remained in the water. Then the subject exited the tank, and grip strength was immediately recorded, the subject was reseated in the reclining chair, and a final set of PREPs was recorded.

A slow rewarming procedure followed which consisted of drinking warm water or decaffeinated coffee while sitting wrapped in a cotton blanket in the experimental facility. Upon recovery to normal temperature, subjects underwent a final physical examination by the medical officer and were released to return home.

RESULTS

Comparison of data from the thermoneutral-dry and thermoneutral-wet runs yielded *no* significant differences. Hence, only comparisons of results from the thermoneutral wet and cold-water exposure will be discussed.

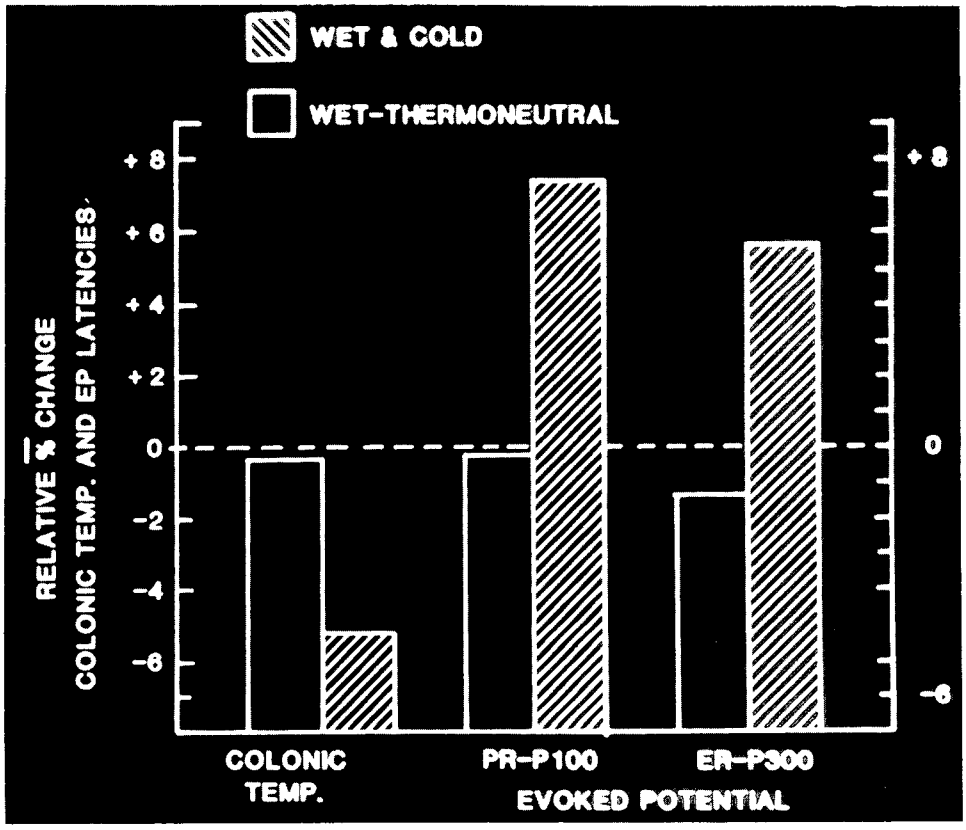

Fig. 3. Bar graphs relating the percent change in colonic temperature and evoked potential latencies.

As illustrated in Fig. 3, no significant changes occurred as a result of sitting in thermoneutral water. However, considerable changes did result when the subjects were both cold and wet.

First, our cooling procedure effectively induced reductions of colonic temperature to an average 35.5°C (with a SE of ± 0.30). This is illustrated in the figure as a 5.1 relative mean-percent reduction below baseline temperature.

An associated average 7.7 ms (9 ± 2.0 SE) *increase* of latency to the pattern-reversal P100 component was observed. This is illustrated as a 7.28 relative mean-percent increase above baseline, a difference found to be reliable at the $P < 0.05$ level.

A corresponding mean increase of 18.89 ms (± 8.5 SE) of latency to the event-related P300 relative mean-percent increase above baseline and was found to be a reliable difference at the $P < 0.05$ level.

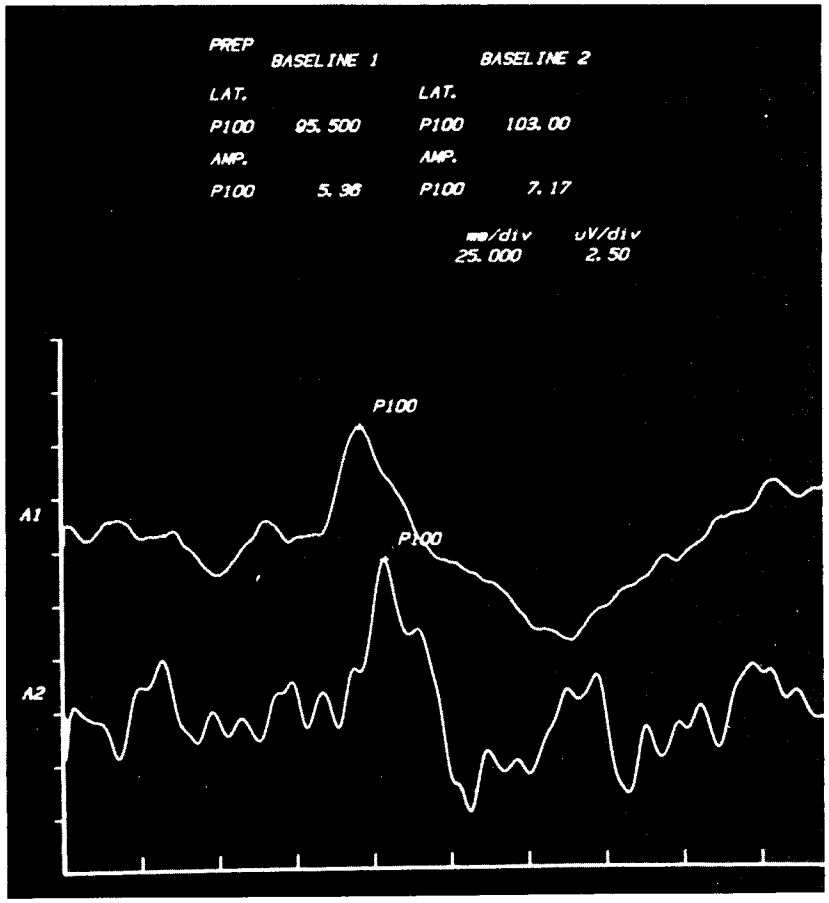

Fig. 4. Display relates changes in microvolts at O_1 and $_2$ as a function of time in milliseconds. Record A1 is the thermoneutral response; record A2 is the wet cold response; A2 shows a prolonged latency compared to A1.

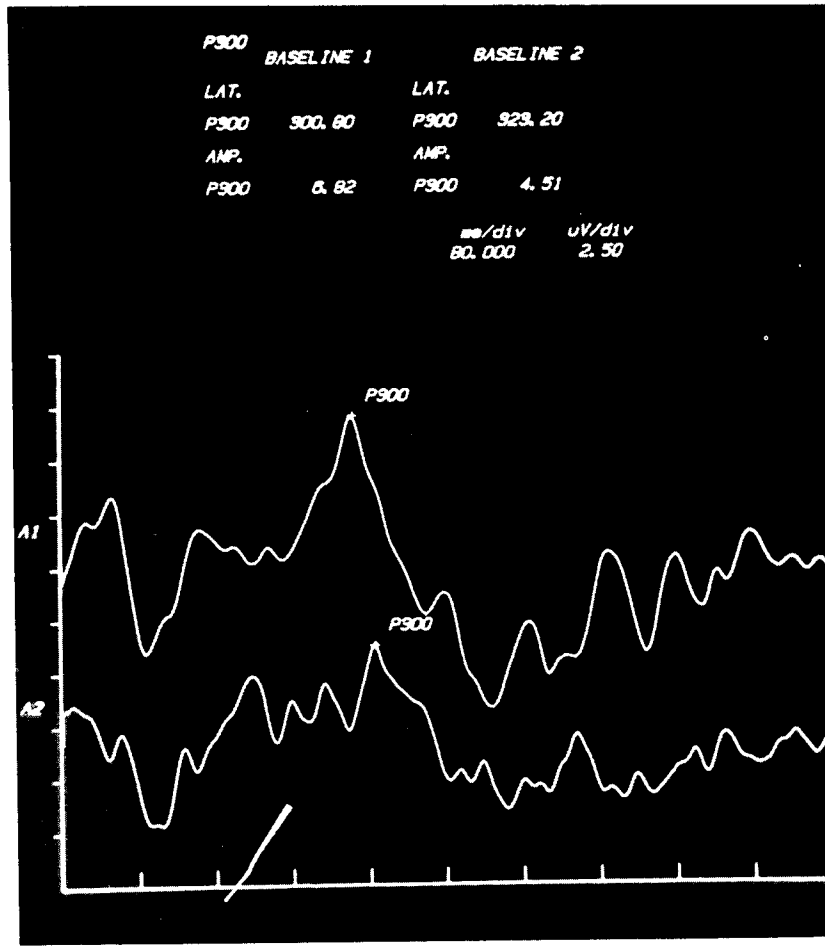

Fig. 5. Display relates change in microvolts at C2 as a function of time in milliseconds. Record A1 is the thermoneutral response; record A2 is the wet cold response; A2 shows a prolonged latency in response compared to A1.

These results are further illustrated in Fig. 4 showing a representative P100 baseline latency (*top waveform*) of 95.5 ms and a wet and cold delayed latency of 103.0 ms (*bottom waveform*).

In Fig. 5, the baseline P300 (*top*) latency is 300.8 ms which was slowed to 323.20 ms (*bottom*) following a reduction of colonic temperature of 1.6°C.

Although the subject's forearm and hands remained dry below the elbow throughout all experimental runs, hand temperature was reliably reduced by an average of -6.43°C below baseline (SE = 0.8), and is shown in the figure as a 22.9 relative mean-percent reduction.

As a result, grip strength and tapping rates were reliably affected by the cold. Grip strength was reduced by 15.17 relative mean-percent, and tapping

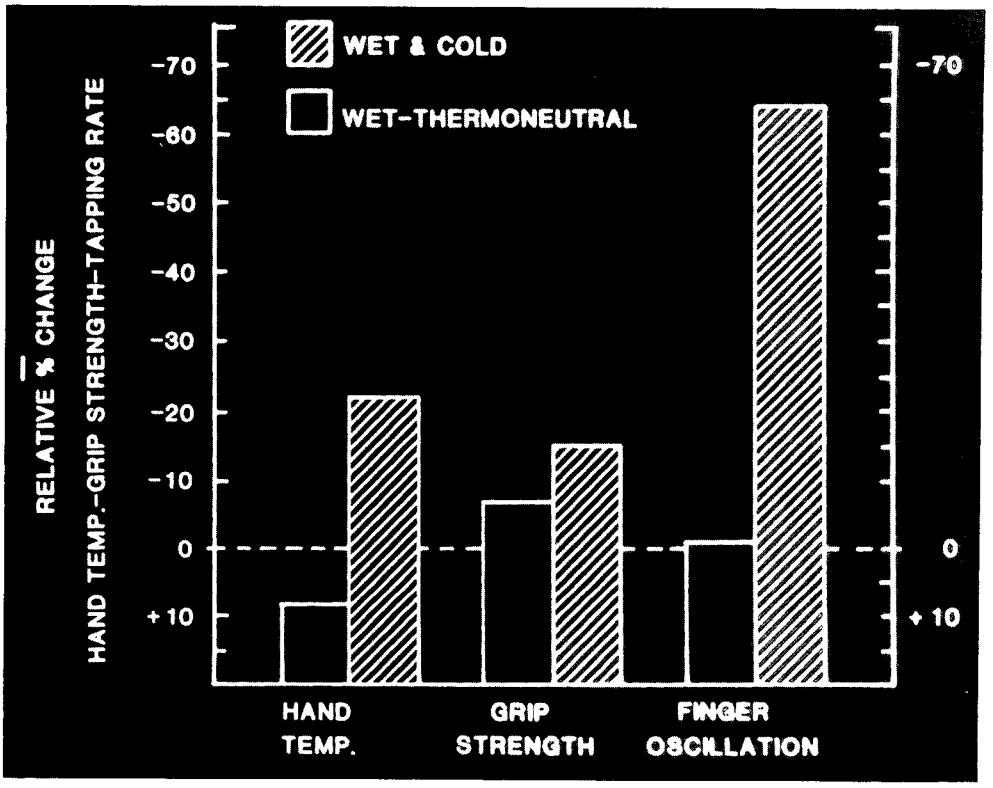

Fig. 6. Bar graphs relating the percent change in hand temperature and grip strength and tapping rate.

rate, which was most severely affected, was reduced by 64.27 relative mean-percent below baseline (Fig. 6). A marked correlation was found between reduction in conduction velocity and tapping rate whereas no correlation seemed to exist between conduction and grip strength at these temperatures (3). A possible explanation for this finding is the difference between the two performance measures; tapping involves an intermittent neuromuscular activity whereas grip strength is a static and steady neuromuscular effort less susceptible to marked interference from nerve conduction alterations (4-8). There may also be muscle fiber type effect (9) and an E-C coupling effect, which is itself affected by muscle ChE (10).

Results from the MAACL yielded reliable test-retest stability for the thermoneutral test sessions. However, they tended to be highly variable, yielding interesting individual differences with respect to the subjects emotional response to cold-water induced hypothermia. Results ranged from no pre- and posttest differences to dramatic elevations of anxiety and hostility scores; in contrast, 1 subject yielded scores that indicated a moderate eleva-

tion positive affect. Unfortunately, our *n* of 7 is too small to deduce much more than that individuals apparently respond individually to sitting for an hour in very cold water.

SUMMARY

The overall conclusion is that multineuronal conduction velocity in both the central and peripheral nervous system can be reliably slowed as a result of reducing the core temperature by an average of 1.5°C.

The visually evoked pattern-reversal P100 potential provided a robust index of a purely physiologic response to changes of core temperature, whereas the event-related P300 provided an electrophysiologic index of a cognitive event. Results indicate that the reduction of temperature accounted for the increased latency of the P100. However, a follow-up study needs to be conducted which includes a "sham" cold-water control (i.e., an immersion condition with water cold enough to be noxious but not cold enough to result in reduction of core temperature) before we will know if the increased latency of the P300 is associated with cold or discomfort-induced distraction or both (11).

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SESSION 10: CLINICAL OXYGEN TOXICITY

9th International Symposium on Underwater and Hyperbaric Physiology
Undersea and Hyperbaric Medical Society, Bethesda, MD, 1987

HYPEROXIA INCREASES HYDROGEN PEROXIDE GENERATION IN RAT BRAIN IN VIVO

T. Yusa, B. A. Freeman, and J. D. Crapo

An increased rate of generation of toxic, partially reduced oxygen species such as superoxide, hydrogen peroxide, and hydroxyl radical has been postulated to be responsible for tissue damage during hyperoxia.

In the rat brain, we demonstrated that augmentation of brain superoxide dismutase and catalase-specific activities after intravenous injection of liposome-entrapped antioxidant defenses inhibited the CNS oxygen toxicity in rats exposed to 6 ATA of oxygen (1). This finding implies that superoxide and hydrogen peroxide are important mediators of oxygen toxicity to the CNS.

To confirm the concept that overproduction of partially reduced oxygen species in the brain is an important factor in CNS oxygen toxicity, we studied the effect of clinically useful normobaric and hyperbaric oxygen pressures on the rate of hydrogen peroxide generation in rat brain in vivo.

MATERIAL AND METHODS

The method employed to detect hydrogen peroxide in vivo was based on the irreversible inhibition of endogenous catalase by 3-amino-1,2,4-triazole (aminotriazole). Aminotriazole irreversibly inhibits catalase by reaction with an intermediate of the enzyme-substrate complex, compound-I (2-4). Therefore, inhibition of catalase by aminotriazole implies the metabolism of H_2O_2. In the presence of high tissue concentrations of aminotriazole and endogenous tissue catalase activity, an increased rate of catalase inactivation implies increased rates of tissue H_2O_2 generation. This technique, however, reflects relative rather than quantitative rates of tissue H_2O_2 generation. Using this approach, H_2O_2 generation in vivo has been detected in rat brain during normobaric metabolism (5).

Male Sprague-Dawley rats weighing 250 to 350 g were used. Rats were injected i.p. with 1 g/kg 0.15 *M* aminotriazole in saline, then divided into 4 groups depending on exposed oxygen pressures: 0.2 ATA O_2 (breathing room air), 0.6 ATA O_2 (exposed to 3 ATA hyperbaric air), 1.0 ATA O_2 (exposed to normobaric 100% oxygen), and 3.0 ATA O_2 (exposed to 3 ATA hyperbaric 100% oxygen). Rats were exposed to each oxygen pressure either immediately or 30 min after aminotriazole injection.

Rats were placed singly in a cage enclosed within a polyethylene bag, thus separated from the environmental atmosphere. For 100% oxygen exposure at normobaric or hyperbaric condition, oxygen concentration (>95%), CO_2 concentration (< 0.1%) and temperature (22-23°C) were maintained in the cage during exposure, using humidified and heated 100% oxygen. The hyperbaric chamber was compressed or decompressed with air at the rate of 1 ATA/min.

Rats were killed by decapitation at indicated times up to 120 min after aminotriazole injection. The brains were immediately removed and processed for biochemical analyses.

Aminotriazole content of the brain homogenates was measured by the colorimetric method of Green and Feinstein (6), as modified by Sinet et al. (5), using chromotropic acid. Catalase activity in brain homogenates was assayed according to Bergmeyer (7).

Statistical analyses for significance of data were evaluated by the unpaired Student's *t* test and Duncan's multiple range test with significance defined as $P < 0.05$.

RESULTS

Tissue Level of Aminotriazole

The aminotriazole content of rat whole brain after i.p. injection (1 g/kg body weight) increased up to 120 min after injection, confirming the appearance and persistence of aminotriazole in the brain over the time period of this study. Brain homogenates that had less than 10 μg aminotriazole/g tissue were excluded from further study because the rate of H_2O_2-induced inactivation of catalase depends in part on aminotriazole concentrations (Table 1).

Time Course of Inhibition of Endogenous Catalase in Rat Brain After Aminotriazole Injection

Catalase activity of brain tissue obtained from rats breathing air (0.2 ATA O_2) was 74.95, 60.90, and 40.26% of control at 30, 60, and 120 min after aminotriazole injection, respectively. Significant inhibition of catalase activity was obtained after 30 min and directly related with time over 60 min. This represents normal metabolic hydrogen peroxide generation rate in the rat brain in vivo (Fig. 1).

Table 1
Aminotriazole Content in Brains of Treated Rats*

Time After Aminotriazole Injection, min	Aminotriazole, μg/g tissue
30	60.18 ± 4.30 (n = 18)
60	94.18 ± 7.80 (n = 15)
120	119.68 ± 11.88 (n = 7)

* Values represent mean ± SE with n in parentheses. Rats were injected i.p. with 1 g/kg of aminotriazole. All values are corrected for endogenous chromogenic material in rat brain homogenates.

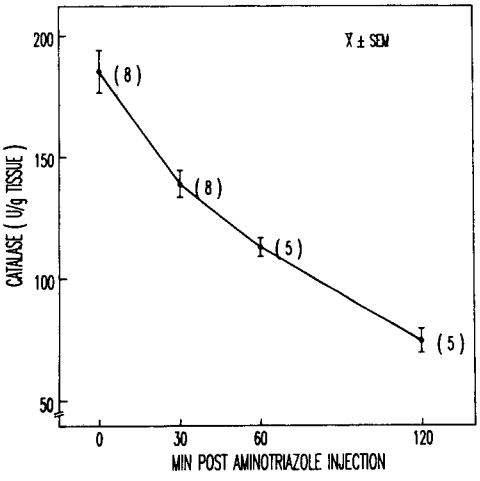

Fig. 1. Time course of inhibition of catalase in rat brain by aminotriazole (0.2 ATA O_2 group). Each data point is the mean ± SE for the indicated number of rats. Each rat brain catalase activity is the mean of at least five catalase measurements. Catalase activity at 0, 30, 60, and 120 min after aminotriazole injection are significantly different from each other.

The Effect of Hyperoxia on the Inhibition of Endogenous Catalase in Rat Brain In Vivo After Aminotriazole Injection

Brain catalase activity decreased more rapidly at higher oxygen pressures, whether aminotriazole was injected immediately or 30 min before oxygen exposure.

Longer periods after aminotriazole injection, especially when hydrogen peroxide generation was augmented by hyperoxic exposure, would not permit detection of changes in catalase activity due to the presence of insufficient levels of aminotriazole. Moreover endogenous hydrogen peroxide generation results in the virtually complete inhibition of endogenous catalase activity at

extended times after aminotriazole injection. Experimentally, when hyperoxic exposure time was extended to 60 min, we could not differentiate hydrogen peroxide generation rate differences between hyperoxic exposure groups.

When the rats were exposed to hyperoxia for 30 min immediately after aminotriazole injection, the extent of catalase inhibition increased as a function of oxygen pressure, although the 0.6 ATA O_2 group was not significantly different from either 0.2 ATA O_2 or 1.0 ATA O_2 groups (Fig. 2). When the rats were exposed to hyperoxia for 30 min beginning 30 min after aminotriazole injection, the extent of catalase inhibition increased as a function of oxygen pressure. The effect of oxygen pressure on brain catalase inactivation was more pronounced than that shown in Fig. 2 (Fig. 3).

At the point of 30 min exposure to hyperoxia, the calculated relative K (min^{-1}) of the hydrogen peroxide generation by rat brain increased as a function of oxygen pressure (Fig. 4).

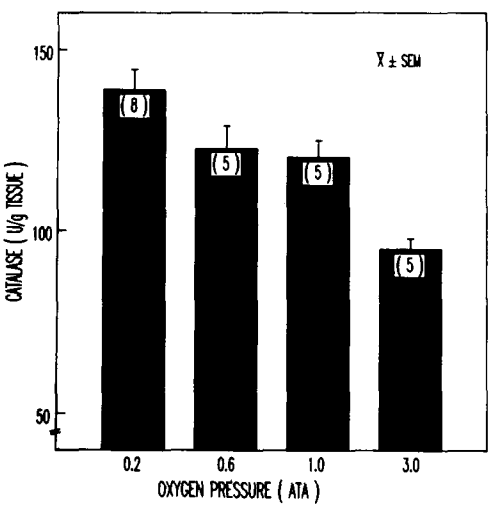

Fig. 2. The effect of hyperoxia on the inhibition of rat brain catalase 30 min after aminotriazole injection. Hyperoxic exposures were for 30 min. Values are the mean ± SE, with (*n*) in bars. Except 0.6 ATA O_2 group, the other three exposure groups were significantly different from each other.

DISCUSSION

The CNS and lungs are highly sensitive to the toxic effect of hyperoxic exposure, which has been postulated to be due to increased generation of partially reduced oxygen species (8).

Hydrogen peroxide is generated both by enzymatic and spontaneous dismutation of superoxide and by direct divalent reduction of oxygen as a normal metabolic by-product of aerobic metabolism (9, 10). In the brain, this is confirmed by the group of rats breathing air in this study and by Sinet et al. (5). Hydrogen peroxide can be further reduced by transition metals to generate the highly reactive hydroxyl radical or to initiate lipid peroxidation (11).

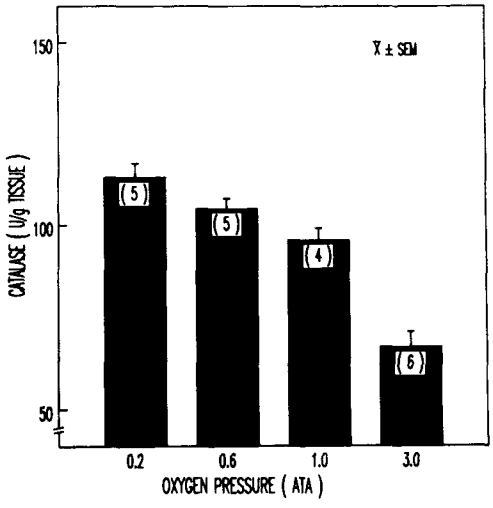

Fig. 3. The effect of hyperoxia on the inhibition of rat brain catalase 60 min after aminotriazole injection. Hyperoxic exposures were for 30 min. Values are the mean ± SE with (n) in bars. As in Fig. 2, with the exception of 0.6 ATA O_2 group, the catalase activity of the other 3 exposure groups were significantly different from each other.

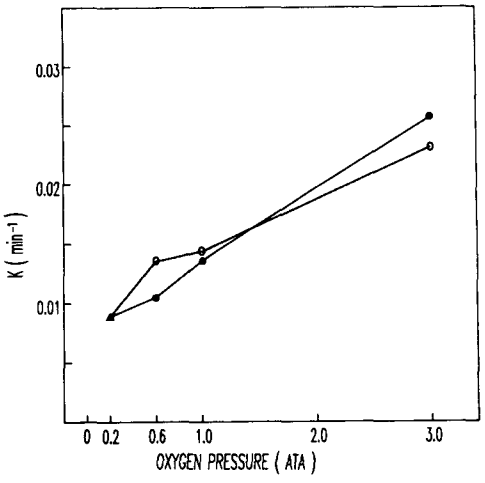

Fig. 4. The effect of hyperoxia on the relative K (min^{-1}) of hydrogen peroxide generation by rat brain. (▲) is the mean of K (min^{-1}) of 0.2 ATA O_2 at 30 min after aminotriazole injection. (○) and (●) indicate K (min^{-1}) for 30 min hyperoxic exposures calculated at 30 and 60 min after aminotriazole injection, respectively.

In the brain, increased rates of partially reduced oxygen species generation have been indirectly demonstrated during hyperbaric oxygen exposure by measuring increased lipid peroxidation and increased oxidation of brain glutathione (12-14).

In the present study, hydrogen peroxide generation in the rat brain in vivo increased as a function of oxygen pressure. This was shown by an increased rate of H_2O_2-dependent inactivation of endogenous catalase in the presence of aminotriazole. Previous studies have demonstrated that liposome-mediated augmentation of brain superoxide dismutase and catalase activities protected rats from the CNS effects of 6 ATA of oxygen (1). These findings show that hyperoxia results in excess generation of partially reduced oxygen species in the brain.

The brain contains relatively low specific activities of hydrogen peroxide scavenging enzymes, including glutathione peroxidase, catalase, and other hemperoxidases (5). Thus, brain tissue may be especially sensitive to increased rate of superoxide and hydrogen peroxide generation by hyperoxia or other pathologic processes including trauma, acute inflammation, and ischemia-reperfusion phenomena.

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AXIAL LENGTH IN HYPEROXIC MYOPIA

B. Anderson, Jr. and D. L. Shelton

Therapeutic use of oxygen breathing at elevated atmospheric pressure has often been observed to cause myopia (1-4). In a previous study (1) we followed the development of myopia in a series of patients being treated with hyperoxia for osteoradionecrosis and similar necrotic lesions. Baseline determinations of refraction, corneal curvature, and intraocular tension were followed by similar measurements in the middle and end of the treatment periods. Posttreatment observations were also made at intervals in some patients. We demonstrated an increasing magnitude of refractive change with increasing hyperoxic treatment days and a complete regression of the myopia over time in most, but not all, patients after cessation of the hyperoxic exposure. We also documented that this myopic change was not associated with any change in corneal curvature or intraocular tension.

If corneal dioptric power does not change then the observed myopic change must be the result of either changes in lenticular power or position, or changes in the axial length of the globe, or both. In this study we investigate the axial length of eyes in which hyperoxic myopia developed.

Although the axial length of the human eye in situ has been determined radiologically, the advent of ultrasonic measurement techniques have made its determination clinically feasible. The time required for a sound pulse to travel from the corneal surface and to echo back from the retina to the corneal surface again is measured. Echoes of low amplitude indicating off-axis reflections are ignored, and with the appropriate constant for sound velocity in the eye, a distance from corneal surface to retinal surface may be calculated. Although we have expressed our measurements in distance units, we could also have used transit times as each eye was its own control.

METHODS

All patients accepted for hyperoxic treatment were screened for entry into the study. Aphakic and pseudophakic patients were excluded. The majority of the remaining patients had osteoradionecrosis of the head and neck area following radiation of cancers in this area and were treated as previously described (5). The initial measurements were made before or within several days of the first hyperoxic exposure. The patients breathed 98% oxygen delivered by head tent for 2 h each day while at 2 atmospheres absolute pressure. The usual regimen consisted of 40 consecutive d of treatment excepting Sundays and lasted slightly less than 2 mo.

Refraction was determined using a fogging technique without the use of mydriatics. The highest plus lens power accepted for sharpest vision was the endpoint used. The observer was masked as to the results of previous refractions, and patients were retinoscoped at the start of the procedure. The spherical equivalent of astigmatic refractions was used in the calculations and in plots of the data.

Corneal curvature was measured with the Baush & Lomb keratometer and the result expressed in diopters. The measurement was made by an observer who was unaware of previous measurement values, if any. The keratometer measured the anterior radius of curvature of the central 2 mm of the cornea.

Axial length determinations were made using the Sonometrics 400 Ocuscan machine. A speed of sound propagation within the eye of 1550 m/s was assumed. The eyes were anesthetized with proparacaine hydrochloride 0.5% drops, and the patient asked to look at the fixation light in the transducer which was gently brought into contact with the cornea. At least three measurements of axial length were made of each eye, at each sitting, alternating between eyes. The echo spike was observed directly on the display screen in making the measurements. No measurements were made in the hyperbaric chamber. All subjects were breathing air at ambient pressure and most had decompressed several hours before coming to the eye clinic. In this study the time between baseline and second measurements was variable but all were made after at least 20 hyperoxic exposures. No measurements were made on Sunday, and in some cases the second measurement was made a few days after the last hyperoxic exposure.

During the treatment period the patients received many medications but these did not consistently differ from those given before or after the hyperoxic exposures. Some patients underwent surgical debridement of necrotic tissue during the treatment period. All patients were accompanied during their hyperoxic exposures by attendants who did not breathe oxygen and did not become myopic.

Many more patients were entered into the study than completed it. Patients were often unwilling or unable to complete the full 2 mo. of treatment, or their surgeons discharged them before their final measurements could be made. It is reasonable to assume that patients experiencing a change in

vision would be somewhat more likely to return for second refractions than those without such changes in refraction.

RESULTS

Twenty-six eyes of 13 patients developed significant myopic changes. Theses changes ranged from -0.13 to -2.74 diopters. The mean refractive change (all eyes) in this study was -1.37 diopters. The axial lengths of the eyes did not correlate with the change in refraction. The mean change in axial length (all eyes) was -0.014 mm. In other words, no significant systematic change was found, but the mean difference that did occur was in the opposite direction from that expected. On the average, eyes measured very slightly shorter rather than longer. The range of differences in axial lengths was from -0.19 to +0.37 mm. No significant change in keratometer measurements occurred. The mean pretreatment keratometer reading was 43.28 diopters whereas the mean posttreatment measurement was 43.17 diopters. A scatter plot (Fig. 1) of refractive change vs. axial length change demonstrates the lack of correlation between these two variables. Calculation of a correlation coefficient results in a value of 0.066, indicating no significant correlation.

DISCUSSION

The laws of optics dictate that the myopic change that occurs in older patients undergoing prolonged hyperoxic treatment regimens must be the result of a change in one or more of three variables: the power or position of the lens of the eye, the dioptric power of the cornea, or the length of the eye. Since neither the power of the cornea nor the length of the eye can be demonstrated to change significantly in these patients, the observed change in refraction must be lenticular. This is not surprising, because transient myopia lasting for hours or a few days can be produced by osmotically active drugs such as diuretics and sulfonamides (6). Diabetics may also become transiently myopic if their blood glucose varies markedly.

The lenticular change that we observed is probably not osmotic. Both the development and the regression of hyperoxic myopia require weeks rather than hours or days, unlike ordinary osmotic refractive changes. There is no evidence that blood osmolarity is affected by hyperoxic exposures, nor do these patients develop aqueous flare as might be seen if there were a marked increase in aqueous protein concentration.

Edematous swelling of the ciliary body has been postulated as a cause of transient myopia. Such swelling would produce relaxation of the zonule with thickening of the lens and possible associated forward movement of the lens (6). It seems unlikely that ciliary body edema could progressively increase over the course of months and resolve just as slowly, but this possibility has not been excluded.

Some evidence exists that the changes we observed are intrinsic to the

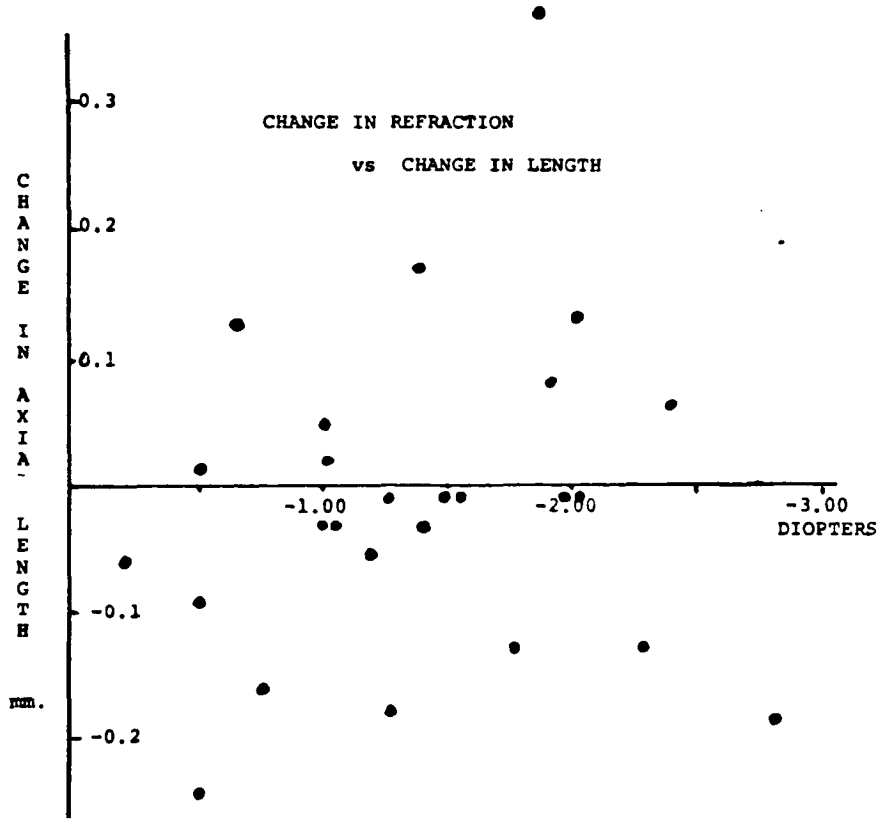

Fig. 1. Change in refraction vs. axial length of the eye.

lens. Myopia is a common concomitant of the nuclear sclerotic cataract of aging. Indeed the "second sight" of folklore is the ability to read again without glasses as a result of this myopic change. Metabolic changes of hyperoxic myopia may therefore be similar in some respects to those occurring in nuclear sclerotic cataract. This has been hypothesized before (1) and the observation that hyperoxia may produce nuclear cataract has been made (4). Since normally cataractous lens changes progress and are difficult to quantitate, careful prospective matched controls with masked observers would seem essential before attributing causation or progression of cataract to hyperoxic exposure.

Such a hypothesis is unreasonable. Nichols et al. (7) have demonstrated damage to lens epithelium in guinea pigs exposed to elevated oxygen tensions, and Shocket et al. (8) have produced such cataracts in mice with oxygen. The lens may be peculiarly susceptible to oxygen toxicity as it is normally relatively hypoxic. The lens has no circulation of its own and is separated from the nearest capillary bed by distances that are biologically very large. In patients given oxygen by head tent where both blood oxygen tension and oxygen tension at the corneal surface are markedly elevated, the magnitude of the

change in aqueous and lens tissue Po_2 must be quite large. Stefansson at our institution has found a change in aqueous Po_2 from 37 to 192 mmHg in cats given 100% oxygen at ambient pressure and with air at the corneal surface. Our patients were given oxygen by head tent at 2 atmospheres absolute pressure with the corneas exposed to the oxygen atmosphere. Aqueous and lens Po_2 would therefore be correspondingly much higher.

Hyperoxic myopia rarely occurs under age 50 unless the patient is diabetic. Its development requires weeks of daily oxygen exposure at 2 atmospheres absolute or above and takes just as long to resolve as to develop. In some patients refraction never returns to baseline. Since we have shown the myopia to be lenticular in origin, it is reasonable to assume that a metabolic change intrinsic to the lens is responsible for the myopia. Such a change may well be similar to that occurring in the myopia that commonly parallels the development of nuclear cataract. Ultrasonic measurement of lens position and thickness in nuclear cataract and in hyperoxic myopia may demonstrate further similarities.

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ROLE OF OXYGEN IN DYSBARIC OSTEONECROSIS

D. N. Walder and I. Holloway

An analysis of the data held at the Newcastle upon Tyne Decompression Sickness Central Registry (1) shows that very little bone damage (a prevalence of 0.6%) was found in divers who had only performed short, shallow dives (20 m for < 4 h) but that a lot of bone damage occurred in those involved in long, deep dives; for example, the prevalence in men who had been to depths greater than 200 m and carried out saturation diving was 15.8%. A similar situation has been observed in compressed air workers where those who had carried out long shifts (> 4 h) at high pressures (> 30 psig) were at greatest risk of developing bone necrosis showing a prevalence of 15.8% (2) (Fig. 1).

It has been noted that, in the human, some areas of the skeleton are more vulnerable to bone necrosis than others (1) (Fig. 2). This is also true for the minipig in which it has been shown that the midshaft of the femur is vulnerable (3).

It transpires that all the affected sites correspond to regions where fatty marrow is located in the bones. It is thus suggested that the presence of fat in the bone marrow cavity might be an important factor in determining where bone necrosis occurs.

Because marrow fat cells are confined in the marrow cavity of bones together with the blood vessels that are supplying both them and the surrounding bone, any increase in marrow fat cell size would inevitably leave less space for the interposed blood vessels, which would thus be narrowed. This would not only lead to a reduction in blood flow to the marrow and bone but also to the trapping of any circulating emboli and total blockage of the blood flow. If this persisted for several hours it would result in the death of both bone cells and fat cells.

It has been shown that animal fat cells do enlarge as a result of exposure to increased partial pressures of oxygen not only in vitro (4), but

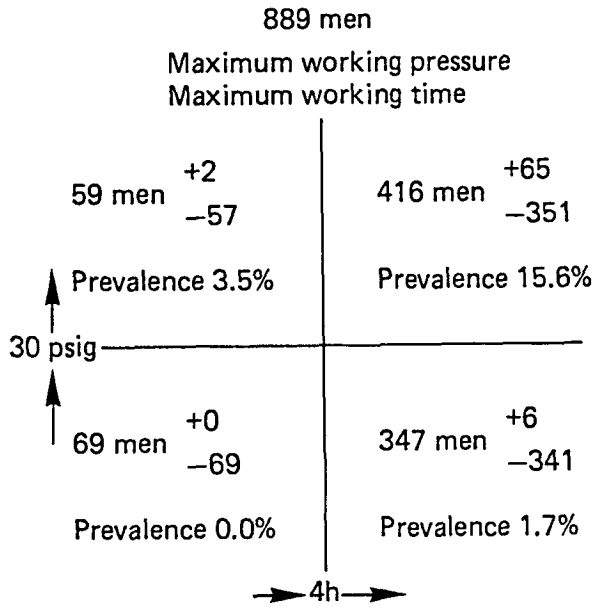

Fig. 1. Aseptic bone necrosis and the Blackpool tables.

also in vivo (5). The explanation for the swelling is possibly that a raised oxygen partial pressure leads to interference with the "sodium pump" mechanism of the cell. This is normally responsible for maintaining the osmotic equilibrium of the cell. When the pump malfunctions the sodium content of the cell rises and water enters to reestablish the osmotic equilibrium. Unfortunately this inevitably results in an increase in the volume of the cell.

HYPOTHESIS

Our hypothesis is that breathing oxygen at a greater than normal partial pressure leads to an increase in the size of fat cells. This would not cause a problem with subcutaneous fat because there is no hindrance to its expansion, but in the case of marrow fat cells, which are confined in the rigid cavity of a bone, any increase in size can only be accommodated by a corresponding reduction in the space available for blood vessels. Consequent compression of these would impair the blood flow to the marrow and to the surrounding bone. Even if this in itself were insufficient to cause death of the cells, the narrowed vessels in the marrow cavity would act as a trap for any circulating bubbles or other emboli and could thus lead to complete obstruction of the circulation. The compromised blood flow would also mean that the rate at which gas could be cleared from the tissues after an exposure to pressure

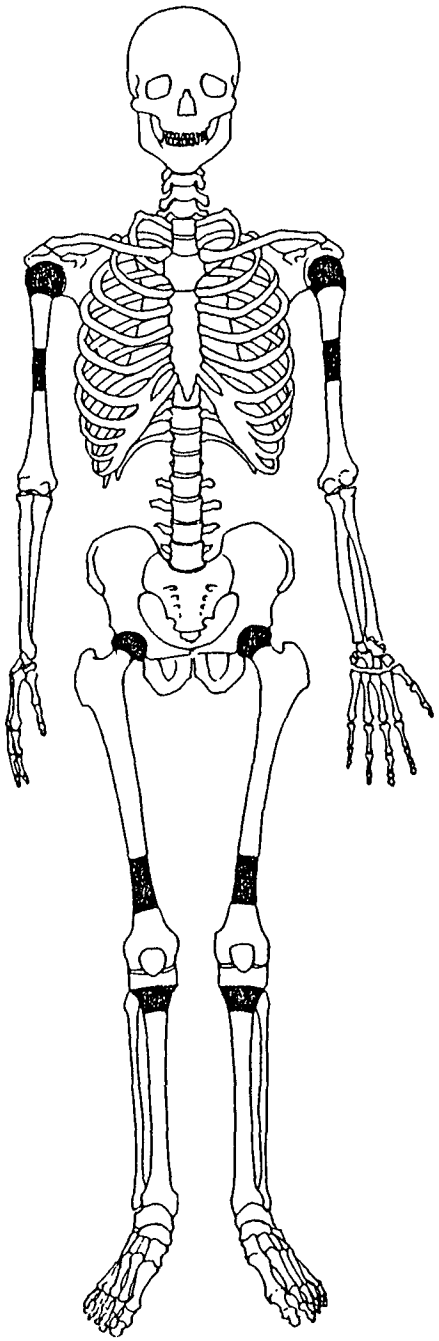

Fig. 2. Common sites of lesions in divers and compressed air workers.

would be reduced so that the fatty marrow would remain supersaturated with gas much longer than expected, and hence any bubble emboli arriving in the marrow circulation during or after decompression would undergo a rapid growth and further obstruct the circulation, thus setting up a vicious circle. Such a hypothesis explains why only some parts of the skeleton are potentially at risk of developing bone necrosis during inadequate decompressions and thus emphasizes the existence of an additional (but avoidable) factor: that is, breathing a gas mixture with a partial pressure of oxygen greater than normal for some critical length of time.

OBJECTIVE

The object of our experiments was to determine in man whether breathing a gas mixture with a raised partial pressure of oxygen (0.4 or 0.6 bar) for some critical length of time (4 h) reduces the blood flow (as determined by measuring the clearance half time of radioactive xenon gas) of bone and fatty marrow at a site known to be commonly affected by bone necrosis in divers and compressed air worker, namely the lower end of the femur.

Rationale of Experimental Design

There is reason to believe from experiments on minipigs that bone and fatty marrow have about the same rate of blood flow and hence the same $t_{\frac{1}{2}}$ (6) and that this is similar to that of subcutaneous fat (7).

By comparing the effects of breathing oxygen-rich mixtures on the half time for the clearance of Xe from all the tissues of the lower thigh (including subcutaneous fat, bone, and marrow fat) with that on the subcutaneous fat alone it should be possible to determine if there is a specific effect on the bone and marrow fat.

If a dose of radioactive Xe-133 gas dissolved in normal saline is given directly into the subcutaneous fat of the lower thigh it will be cleared by the lungs. It is estimated that more than 95% of Xe diffuses from the blood stream into the alveoli at each passage through the lungs so that there is virtually no recirculation (8). When a breathing circuit is appropriately arranged, the exhaled gas can be exhausted to the open air and cannot be rebreathed by the subject. Radioactive Xe can be detected by externally placed counters. The clearance rate ($t_{\frac{1}{2}}$) as determined by a scintillation counter placed over the site of the injected Xe will be proportional to the blood flow through the subcutaneous fat.

If a dose of radioactive Xe gas dissolved in normal saline is given i.v. to a subject while he is breathing from a closed circuit, it will be distributed throughout the tissues of the body. Since Xe is cleared via the lungs, equilibrium between the tissues and the gas in the closed-circuit breathing apparatus will be reached in 1 or 2 min. Once this has been achieved the breathing circuit is changed so that the exhaled gas exhausts to the open air and cannot therefore be rebreathed by the subject. Each tissue of the thigh

will then be cleared of its Xe at a rate proportional to the blood flow through it, and the clearance can be monitored by suitably placed scintillation counters.

Experimental Detail

All experiments were carried out at 1 ATA on human volunteers at room temperature. The subjects sat on a chair with the thigh under investigation comfortably supported so that a constant counting geometry could be ensured. To aid the correct positioning and repositioning of the sodium iodide scintillation detectors, all subjects wore trunks, and indelible ink marks were made on the skin surface so that the detectors could be readily checked and realigned when necessary, using a flexible probe placed between the detector and the ink marks.

The scintillation detectors consisted of 3 cm diameter sodium iodide crystals coupled to photomultipliers. The detectors were housed in lead collimators. The necessary associated electronics were to Nuclear Instrument Modular System (NIMS) standards set to detect 80 keV radiation from the Xe-133.

The subject breathed from a circuit which could be used in two modes; either as a closed circuit in which carbon dioxide was absorbed and oxygen added as it was used up, or as a circuit exhausting to the open air.

For the experiments in which the radioisotope was injected directly into the subcutaneous fat at the lower end of the thigh, a single detector was used as shown in Fig. 3. Background corrected count rates were recorded. In these cases the observed counting rates were determined solely by the radioactivity in the subcutaneous fat.

For the experiments in which the radioactive Xe was administered i.v., two scintillation detectors were positioned at right angles to each other and directed toward the center of the lower end of one thigh as shown in Fig. 4. The count rates were recorded from each detector then summed after correction for the contribution from background radiation. In these circumstances the observed counting rates represented a combination of all the radioactivity from Xe-133 in the thigh including that from the subcutaneous fat, bone, and marrow fat.

A total of 18 subjects took part in these experiments. Each subject acted as his own control and attended on two occasions separated by an interval of several weeks. The same site of the same thigh in each individual was studied on both occasions.

On one of the two visits the subject was given air to breath (0.2 bar oxygen) and on the other visit a gas mixture containing either 0.4 bar oxygen in nitrogen or 0.6 bar oxygen in nitrogen.

In the 1st group of experiments 6 volunteers were involved. On one of his two visits the subject breathed air (0.2 bar oxygen) and on the other of his visits 0.6 bar oxygen in nitrogen. On both visits the subject was given a dose of radioactive Xe-133 (135 μCi in approximately 0.1 ml normal saline) into the subcutaneous fat of the lower thigh while breathing from a

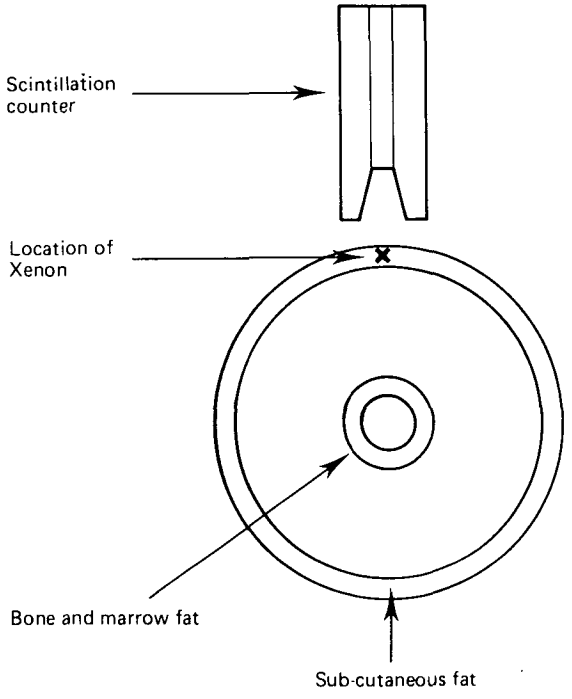

Fig. 3. Local injection of Xe. Cross section of the lower end of the thigh showing location of fat and position of scintillation counter.

circuit arranged to exhaust to the open air. The scintillation counter measured the counting rate determined solely by the clearance of Xe from the subcutaneous fat. Counting for periods of 10 s started immediately after the injection and was continued for 4 h at 5-min intervals.

In the 2nd group of experiments, 12 volunteers were involved. Each subject breathed air (0.2 bar oxygen) on one of his two visits, 6 breathed a gas mixture containing 0.6 bar oxygen, and 5 subjects (a technical failure occurred during one visit) breathed a gas mixture containing 0.4 bar oxygen on the other visit.

On both visits each subject was given a dose of radioactive Xe-133 (1.90 mCi in approximately 0.1 ml normal saline) into an antecubital vein (i.e., i.v. while breathing air from a closed circuit. Once the counting rate, measured by the two scintillation counters placed over the thigh, reached a plateau, the selected breathing gas for the experiment was connected into the breathing circuit. Simultaneously the breathing circuit was arranged to exhaust to the open air. Counting was then carried out for periods of 100 s every 5 min for 4 h.

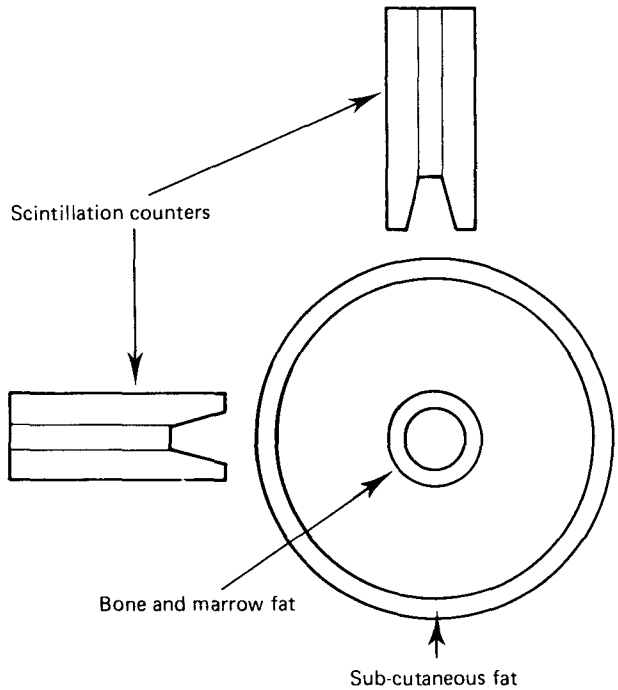

Fig. 4. Intravenous injection of Xe. Cross section of the lower end of the thigh showing location of fat and position of scintillation counters.

The total radiation dose given to each volunteer was within Category II as defined in a report of a WHO/IAEA Consultation on The Use of Ionizing Radiation on Human Beings for Medical Research and Training, Including the Use of Radio-Active Materials (1972). It is the dosage category permitted by the ICRP to individual members of the public who form only a small part of the population, and is of the same order of magnitude as that received annually from natural sources. Ethical approval was obtained from the District Health Authority and from the University of Newcastle upon Tyne.

RESULTS

It was found that Xe injected into the subcutaneous fat of the thigh cleared in a monoexponential manner (Fig. 5). It can be seen from Table 1 that the half time for the clearance was not statistically different whether air or a gas mixture containing 60% (0.6 bar) oxygen in nitrogen was breathed. ($P > 0.5$).

When the Xe was introduced i.v. into the circulation of the subject it cleared in a multiexponential manner which could be most readily resolved into two components. (Fig. 6*A*, *B*). There was a slow component and a fast compo-

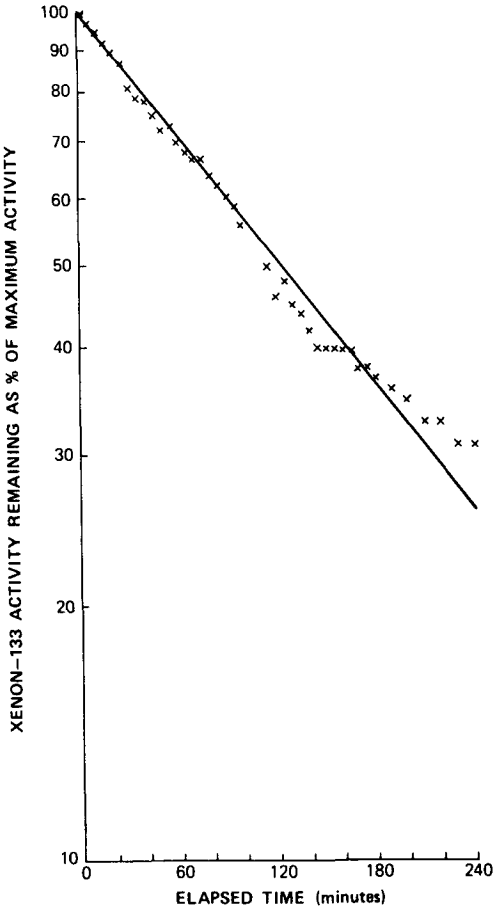

Fig. 5. Retention washout of Xe-133 after subcutaneous injection. Subject 6: half time: 124 min; breathing gas: air.

nent. The faster phase was assumed to represent clearance from the muscle and skin and the slower phase was assumed to represent clearance from the fatty tissues of the thigh (i.e., subcutaneous fat and marrow fat and bone). The clearance rate of the faster phase did not show any statistically significant difference if the subjects were switched from air to a gas mixture of either 40% (0.4 bar) oxygen in nitrogen ($P > 0.1$) or to a gas mixture of 60% (0.6 bar) oxygen in nitrogen ($P > 0.5$) (Table 2).

However, when the slower phase representing the Xe clearance from the subcutaneous fat, bone, and marrow fat was examined it became apparent that both when breathing 40% (0.4 bar) oxygen in nitrogen, and when breathing 60% (0.6 bar) oxygen in nitrogen as opposed to air the clearance half time increased to a statistically significant extent in both cases, $P < 0.05$ and $P < 0.001$, respectively (Table 3). The effect with 0.6 bar oxygen was greater than the effect with 0.4 bar oxygen (Fig. 7).

Table 1
Clearance Rate of Locally Injected Xe into Subcutaneous Fat of Lower Thigh; Comparing Air with 60% oxygen: 40% Nitrogen as the Breathing Gas

Subject	Clearance Rate When Breathing Air ($t_{\frac{1}{2}}$), min	Clearance Rate When Breathing 60% Oxygen ($t_{\frac{1}{2}}$), min
1	255	320
2	440	640
3	460	250
4	110	70
5	560	270
6	124	150
Mean	324.8	283.3
SD	188.8	196.5
SEM	77.1	80.3

$t = 0.36$ ($P > 0.5$) not significant.

DISCUSSION AND CONCLUSIONS

Since the clearance rate of Xe from the subcutaneous fat is not altered by increasing the partial pressure of oxygen breathed, whereas the clearance rate of Xe from the subcutaneous fat, bone, and marrow fat combined is significantly slowed, it must be concluded that the observed change in clearance rate must be due to alterations in the contribution made by the bone and marrow fat to the clearance curve; that is, breathing an above-normal partial pressure of oxygen results in a slowing of the clearance rate and hence blood flow to bone and marrow fat at the lower end of the femur.

If this is so, it might be argued that when breathing a raised partial pressure of oxygen, since the clearance of locally introduced Xe from subcutaneous fat remained unchanged, an additional phase should have been seen in the exponential clearance curve of the Xe introduced i.v. to represent the even slower rate of clearance from bone and marrow fat. Unfortunately the limitations of experimental accuracy were such that this could not be expected.

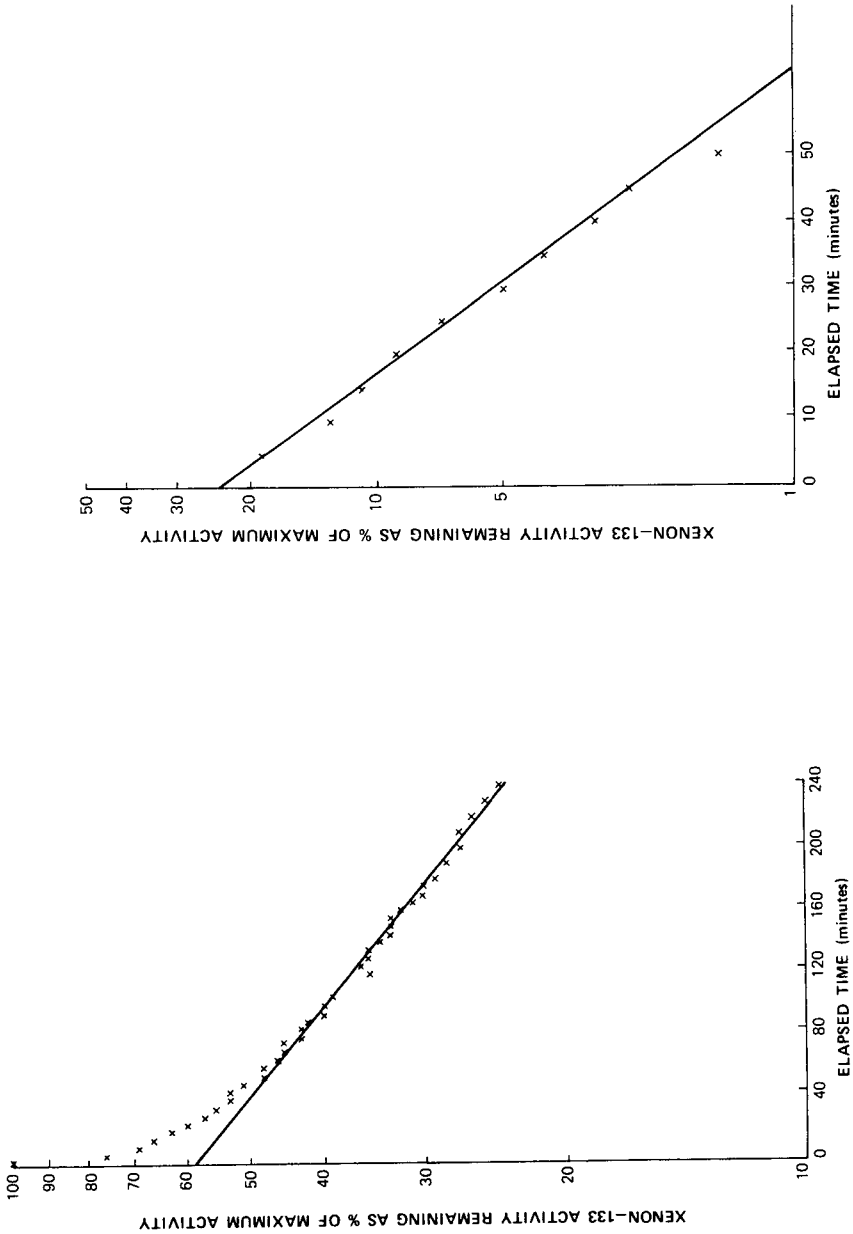

Fig. 6. A. Retention/washout of xenon-133 after intravenous injection. Subject: 14; half times: 14 min, 186 min; breathing gas: air. B. Retention/washout of xenon-133 after intravenous injection. Subject: 14; half times: 14 min, 186 min; breathing gas: air.

Table 2
Clearance Rate of i.v. Injected Xe from the Lower Thigh;
Comparing Air with 40% Oxygen: 60% Nitrogen
and 60% Oxygen: 40% Nitrogen as the Breathing Gas

	Faster Phase		
	A	B	C
Subject	Clearance Rate When Breathing Air $t_{\frac{1}{2}}$, min	Clearance Rate When Breathing 40% Oxygen $t_{\frac{1}{2}}$, min	Clearance Rate When Breathing 60% Oxygen $t_{\frac{1}{2}}$, min
7	13	21	-
8	23	22	-
9	15	26	-
10	10	16	-
11	10	19	-
12	34	14	-
13	9	-	20
14	14	-	28
15	3	-	30
16	21	-	12
17	14	-	30
Mean	15.1	19.7	24.0
SD	8.3	4.3	7.9
SEM	2.5	1.8	3.5

B is not significantly different from A; $t = 1.24$ $(0.5 > P > 0.1)$.
C is not significantly different from A; $t = 2.01$ $(0.1 > P > 0.05)$.

SUMMARY

In summary, our suspicions that breathing a raised partial pressure of oxygen adversely influences bone and marrow circulation are entirely confirmed, and this will almost certainly have an important role in the cause of dysbaric osteonecrosis.

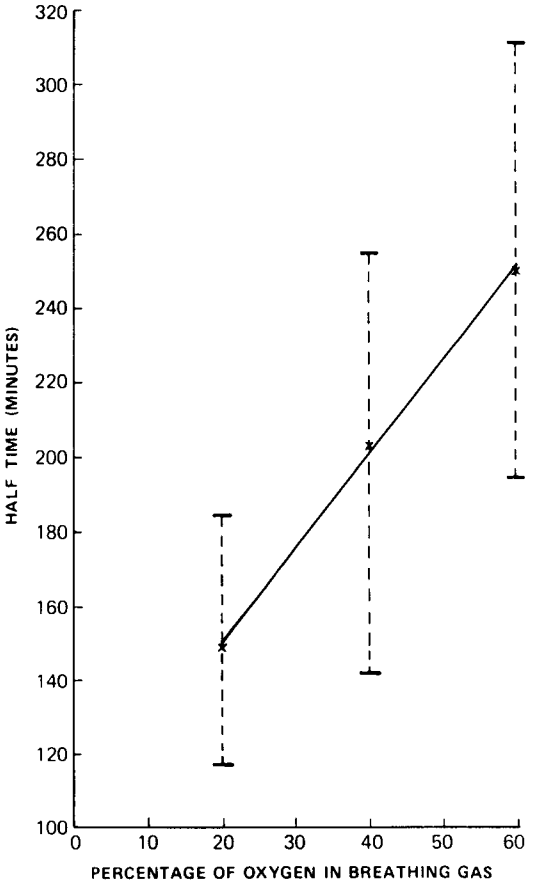

Fig. 7. Change in mean half time with increasing oxygen concentration. Long half time component ± 1 SD.

Table 3
Clearance Rate of i.v. Injected Xe from the Lower Thigh, Comparing Air with 40% Oxygen: 60% Nitrogen and with 60% Oxygen: 40% Nitrogen as the Breathing Gas

	Slower Phase		
	A	B	C
Subject	Clearance Rate When Breathing Air $t_{\frac{1}{2}}$, min	Clearance Rate When Breathing 40% Oxygen $t_{\frac{1}{2}}$, min	Clearance Rate When Breathing 60% Oxygen $t_{\frac{1}{2}}$, min
7	135	180	-
8	133	250	-
9	180	210	-
10	95	175	-
11	125	265	-
12	156	110	-
13	190	-	276
14	186	-	262
15	148	-	260
16	200	-	310
17	116	-	155
Mean	151.3	198.3	252.6
SD	34.1	56.3	58.1
SEM	10.3	23.1	26.0

B is significantly different from A; $t = 2.16$ $(0.05 > P > 0.02)$.
C is highly significant from A; $t = 4.43$ $(0.001 > P)$

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IDENTIFICATION OF THE SITES OF FLUID LEAKAGE INTO THE AIR SPACES DURING EARLY STAGES OF HYPERBARIC OXYGEN EXPOSURE

R. R. Mercer, P. B. Bennett, and K. E. Pinkerton

Trauma to the CNS, convulsions, or short-term exposure to O_2 at pressures in excess of 1 atm may result in the development of pulmonary edema. The purpose of our study was to investigate the mechanisms of hyperbarically induced, noncardiac pulmonary edema by identifying the earliest sites of fluid leakage into the air spaces of the lungs. To do this, we made use of the fixation properties of osmium tetroxide vapor in conjunction with the ability of vascular perfusion fixation to preserve the surface lining layers as well as the microarchitecture of the lungs at various time points during hyperbaric oxygen exposure in 6 atm 100% O_2. By this combination of avenues of attack, i.e., vapor fixation of the airways via osmium and perfusion fixation of the lungs via the vasculature, it was possible to preserve the mucous lining layers in the airways as well as the surface lining layers of the alveolar structure in normal control animals (Fig. 1). Because edema fluid in noncardiac pulmonary edema has a composition similar to the mucous lining layers of the airways it was expected that the fixation procedure would also preserve the early sites of fluid leakage into the air spaces.

MATERIALS AND METHODS

Male, Sprague-Dawley rats (250-300 g body weight) were used in these experiments. Rats for the exposure were placed in a sealed Plexiglas box which was flushed with oxygen. After 1 h of exposure to normobaric oxygen the box was transferred to the exposure chamber and the chamber compressed to 6 ATA at a rate of 30 ft/min. Temperature was maintained at 28°C using a heat

exchanger in the oxygen supply line with a CO_2 absorber in series with the heat exchanger. During the course of the exposure, the animal was monitored constantly and the time to seizure was recorded independently by two observers. The chamber was then decompressed after the conditions for the exposure had been reached. The conditions for exposure of each group were:

1. Normal air controls.
2. 6 ATA 100% O_2 preseizure — Animals exposed to hyperbaric oxygen for 15 min, which was 3 to 5 min before the onset of seizure activity.
3. 6 ATA 100% O_2 seizure — Animals exposed to hyperbaric oxygen until the onset of seizures.
4. 6 ATA 100% O_2 5 min — Animals exposed to hyperbaric oxygen postseizure for 5 min after the onset of seizures.

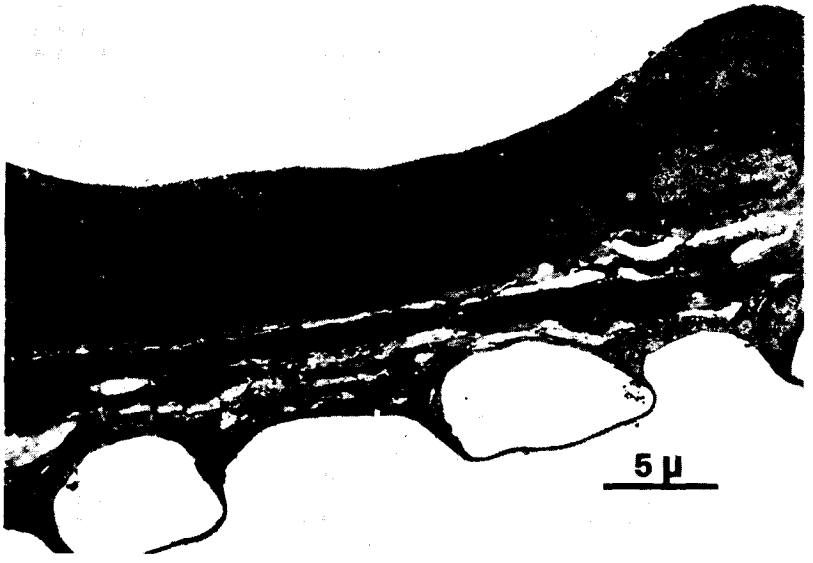

Fig. 1. Mucous lining layer preservation in the bronchiole of a normal rat. ×4800.

The animal's lungs were fixed by vascular perfusion and osmium vapor. To carry this out the animal was anesthetized by intraperitoneal injection of pentobarbital, and a tracheostomy was performed by insertion of a Luez stub adaptor into the trachea. A laparotomy was then made, and the inferior vena cava and the abdominal aorta were exposed. The inferior vena cava was then cannulated, and blood within the lungs cleared by perfusion with 0.9% NaCl, 0.3% sodium nitrate, and 100 U/ml of heparin at a perfusion pressure of 20 cmH_2O, with the femoral arteries being severed for drainage of the perfusate.

During the perfusion of the clearing solution the tracheal cannula was attached to a pressure reservoir and inflation of the lungs was maintained at 5

cmH_2O. A midline incision of the thorax was then made. The perfusate was switched to a fixative solution containing 2% glutaraldehyde and 1% formaldehyde in cacodylate buffer adjusted to pH 7.4. By means of a small valve attached to the tracheal cannula, the pressure source maintaining the 5-cmH_2O inflation pressure was switched to a bubbled solution of osmium tetroxide connected to a pressure source. Due to its proximity to the tracheal cannula, this source maximized the delivery of osmium tetroxide-laden gas into the trachea for vapor fixation of the lungs. After 2 min of coagulative and cross-linking fixative perfusion, the fixative solution was switched to 2% osmium tetroxide in cacodylate buffer to obtain better preservation of lipid structures. At this time sufficient fixation of the lungs had occurred for small leaks to be made in the pleural surface without any noticeable deflation of the lungs. Enough such small leaks were made until the flow rate of osmium vapor-laden gas into the trachea was approximately equal to the minute ventilation of the animal (for the rat this is approximately 80 ml/min). After approximately 30 min of vapor fixation the lungs were dissected free.

Measurements consisted of examination of bronchiole-pulmonary artery profiles, morphometric evaluations of the distal alveolar region, as well as serial section reconstructions. For examination of the bronchiole and adjacent pulmonary artery profiles, isolation procedures similar to those described for terminal bronchioles (1) were used. Basically this consists of cutting the right upper and lower lobes into small slabs for embedding in Epon. After embedding the tissue samples, the tissue cubes were softened by heat and cut with a razor blade. Bronchioles cut in perfect cross section on the face of these slices can be identified with reflected light. The region surrounding the bronchiole and pulmonary artery was then cut out and mounted.

These oriented samples were ultrathin sectioned for electron microscopy. The sections were picked up on 1 × 2-mm slotted-hole grids, coated with 0.5% Formvar, and stained with uranyl acetate and lead citrate for viewing. Sections were then photographed at ×1200 and enlarged to ×4700 on 11 by 14 in. photographic paper.

The measurements of the distal alveolar region used standard morphometric procedures such as those described in the study of normobaric oxygen toxicity (2). The basic modification in the present case was to include determination of air-space fluid as another category in the point and intercept counting methods.

Briefly, tissue samples (2 × 2 mm) containing no major airways or blood vessels were selected at random and embedded as described in the perivascular cuff isolation procedure. Using a diamond knife, thin sections large enough to cover 30- to 40-grid spaces on a 200-mesh, uncoated grid were cut. At ×2000 the upper-left corner of 8 grid squares of the tissue were photographed. These were then enlarged to ×8000 on 11 by 14 in. photographic paper. Point and intercept counting were then used to determine the thickness of the various parenchymal compartments (epithelium, interstitium, and endothelium) as well as the volume of air-space fluid.

Serial section reconstructions were utilized to identify the relationship between edema pockets and tissue elements. The blocks for serial sectioning were obtained following perfusion fixation and embedding similar to that described for morphometric analysis. Each section of the series was then photographed and printed. Each print of the series was examined and the structures for reconstruction were identified on the print for subsequent tracing. The structures for reconstruction included capillaries, areas of edema fluid in the air spaces, areas of interstitial edema, alveolar epithelial surfaces, and appropriate macroscopic structures such as the pulmonary artery endothelium or alveolar septum.

For each series the X-Y-Z coordinates representing the profiles of each structure were entered into an IBM PC by tracing the profile on a digitizer tablet. Using point-to-point spacing of 0.01 in. on the digitizer surface a typical reconstruction required storage of approximately 50,000 X-Y-Z coordinates. After the structures were entered into the computer, use was made of standard computer graphic techniques to allow interactive viewing of the structures in the appropriate rotation with hidden lines removed (3).

RESULTS

Initial evaluations were made by examining sections of the lung for the presence of edema fluid, perivascular cuffing, and overall quality of fixation. As is shown in Fig. 1, the mucous lining layer(s) in the bronchioles of normal animals was preserved, with the depth and appearance being similar to that reported by others (4, 5). In the preseizure group the lymphatics adjacent to the small airways occasionally seemed to be abnormally inflated. For animals exposed to the point of seizure activity, interstitial edema in the perivascular cuff and electron lucent alveolar epithelium were noted. Occasional areas of edema fluid in the air spaces adjacent to the periarterial cuff were also found in this group (Fig. 2). In the animals exposed for 5 min past the onset of seizures, large quantities of edema fluid were found with consistent fluid accumulation in the perivascular cuffs (Fig. 3). Although edema fluid was consistently found in the air spaces of animals exposed for 5 min past the onset of seizure, the mucous lining layer(s) of adjacent bronchioles was not increased. For example, Fig. 4 shows a bronchiole epithelium with a normal mucous lining layer(s) adjacent to a pocket of edema fluid.

The results of the morphometric determination of the thickness of the epithelial, interstitial, and endothelial compartments of the distal alveolar region are given in Fig. 5. Although the changes between the control, preseizure, and onset of seizure groups follow an obvious trend, the results were not statistically significant. The parenchymal air-space fluid of the animals exposed to the onset of seizure (15 ± 5 μl/lung) was not statistically different from the control group (16 ± 6 μl/lung, mean ± SE).

Reconstructions were carried out on the liquid lining the layer pools of the alveolar epithelium adjacent to the pulmonary artery-periarterial cuff.

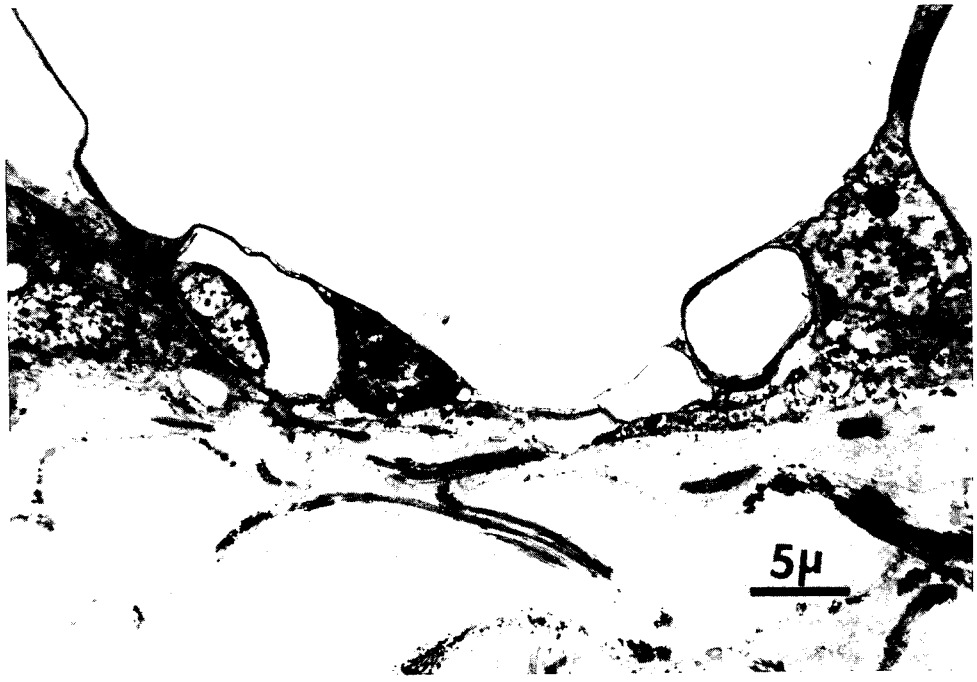

Fig. 2. Focal area of fluid accumulation in the air spaces of the alveolar epithelium adjacent to the periarterial cuff of an animal exposed to the onset of seizure activity. ×625.

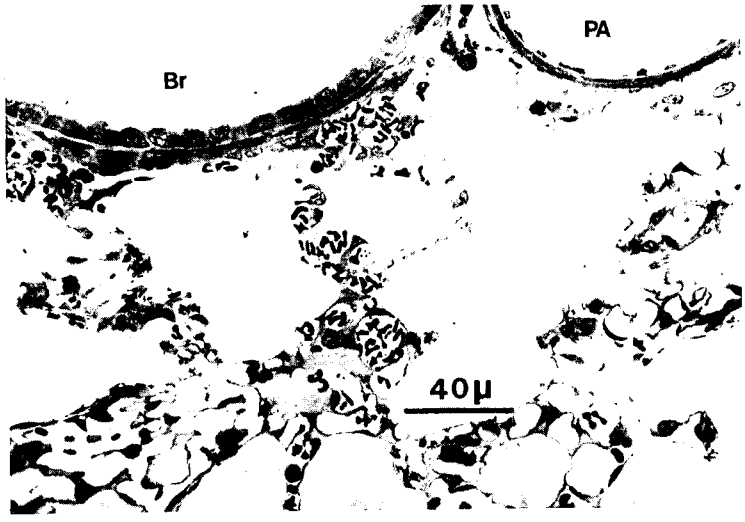

Fig. 3. This figure demonstrates the considerable degree of edema fluid found in the air spaces of animals exposed for 5 min past the onset of seizure activity. Bronchiole (Br), pulmonary artery (PA). ×2600.

Fig. 4. Airway epithelium of an animal exposed for 5 min past the onset of seizure activity. Although the air space adjacent to the airway's epithelium had edema fluid, the mucous lining layer appears normal. ×3100.

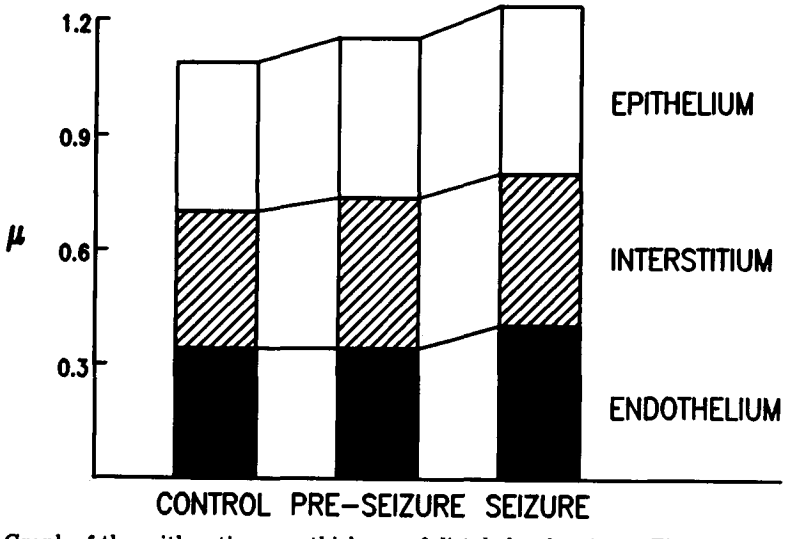

Fig. 5. Graph of the arithmetic mean thickness of distal alveolar tissue. The apparent increases in the thickness of the various alveolar tissue compartments of animals exposed to the onset of seizures were not statistically significant.

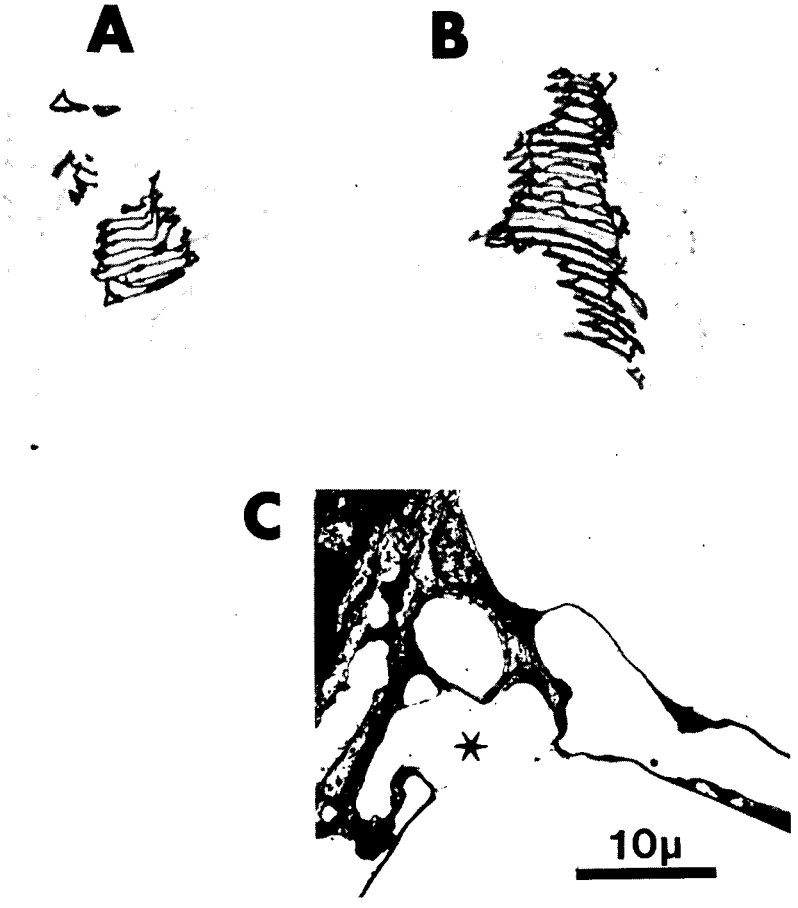

Fig. 6. 3-D Reconstruction of the fluid pool and capillaries adjacent to the periarterial cuff of an animal exposed to the onset of seizure activity. In A of this figure, the reconstruction shows the pool viewed from the interstitium. In B the pool is viewed from the air space. One of the sections used in this reconstruction is shown in C with the fluid pool indicated by the asterisk. Alveolar capillaries (light lines) fluid pool (dark lines).

The pool and adjacent capillaries of one such reconstruction are shown in Fig. 6. In one of the serial sections in this reconstruction the probable site of injury was found. This is shown in the electron micrograph (Fig. 7) where the intracellular junctions between two endothelial cells seem to have separated, exposing the basement membrane beneath the endothelial cells. This defect in the endothelial cell junctions was limited in extent and exposed less than 1 μm^2 of the basement membrane. Such abnormalities in the endothelial cell junctions of capillaries adjacent to the periarterial cuff were found associated with liquid lining pools in several series.

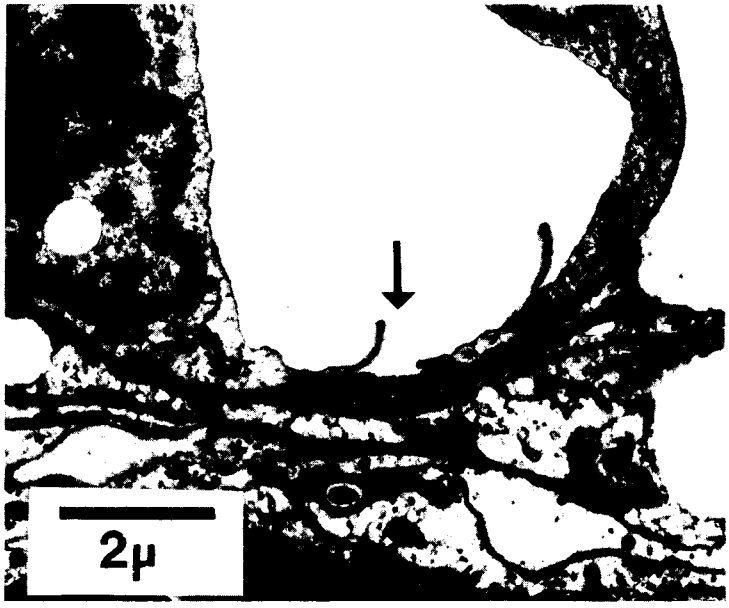

Fig. 7. Electron micrograph from a capillary in one section of the reconstruction given in Fig. 6. The potential injury site responsible for the formation of the fluid pool is indicated by the arrow. This arrow points to a region where the intercellular junctions between the endothelial cells have separated exposing the underlying basement membrane. ×10,000.

DISCUSSION

By identifying the site(s) of the earliest fluid leakage into air spaces, one can identify the anatomic regions damaged in different types of injury that lead to pulmonary edema. Electron microscopic examination of animals exposed to the onset of seizure activity demonstrated significant involvement of the periarterial region of the lungs. In particular, the alveolar epithelium adjacent to the periarterial cuff seems to be the earliest identifiable site where fluid could be found in the adjacent air spaces. Other possible pathways, such as leakage of fluid into the air spaces via the small airways (6) or alveolar epithelium of the distal alveolar region, do not appear to be involved at this stage.

In the animals exposed for 5 min past the onset of seizures, the fluid in the air spaces was widely distributed, with the individual pools of edema fluid in the air spaces being interconnected. Thus localization of the sites of fluid leakage was not possible, and it cannot be concluded from the present study whether the alveolar epithelium adjacent to the periarterial cuff is the sole or

predominant pathway during later stages of edema formation. For instance, in animals exposed for 5 min past the onset of seizures the air-space fluid (Fig. 3) could be due to either the formation of new sites of injury widely distributed throughout the distal alveolar region or to the redistribution of edema fluid formed from the periarterial sites.

With respect to these two possibilities it is interesting to note that red blood cells and fibrinlike masses were rarely seen in animals exposed to the onset of seizure activity, whereas numerous red blood cells and fibrinlike masses were seen in the air-space fluid of those exposed for 5 min past seizure onset. These differences may reflect either the development of new sites sufficiently large to allow extravasation of red blood cells or the formation of a plasma ultrafiltrate while the injury is limited in size, followed by an extravasation of larger molecules and cells as the injury site is widened by prolonged exposure to the high vascular pressures during seizures.

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THE PATHOBIOLOGY OF HYPERBARIC OXYGEN: EFFECTS OF TEMPERATURE ON SENSITIVITY

K. E. Pinkerton, R. R. Mercer, and P. B. Bennett

Little is known concerning the sequence and extent of structural changes that occur in the lungs following exposure to hyperbaric oxygen. Prolonged exposure to oxygen (60 h) under normobaric conditions is known to cause perivascular edema, capillary congestion, and airspace fluid accumulation. Exposure to oxygen at higher pressures (2.8 ATA and above) is primarily associated with disturbances of the central nervous system (1), although pulmonary changes secondary to neurologic injury have been described. Whether environmental temperatures alter sensitivity of oxygen under hyperbaric conditions is also not known. The goals of this study were a) to identify and quantify changes in lung morphology following hyperbaric oxygen exposure and b) to examine the effects of temperature on oxygen-induced toxicity.

MATERIALS AND METHODS

Male Sprague-Dawley rats (250-300 g body weight) were exposed to oxygen at 1, 3, or 6 ATA in a 1.33 m^3 Bethlehem chamber at 8, 24, or 37°C. Twelve animals were observed at each pressure and temperature. Compression and decompression rates for exposures at depth were 9.2 m/min. All animals were observed for the duration of the study for signs of altered behavior and/or neurologic change in the form of seizures. Skin and core body temperatures were recorded for a portion of the monitored animals at 15-min intervals. Chamber oxygen and carbon dioxide concentrations were measured before and during each exposure and found to be greater than 98.5% oxygen and less than 0.15% for carbon dioxide. Experiments done at 1 and 3 ATA were for 6 h, whereas experiments at 6 ATA were for approximately 40 min. Following decompression animals were anesthetized by an i.p. injection of pentobarbital,

and the lungs were inflation-fixed by intratracheal instillation or intravascular perfusion of 2% glutaraldehyde in sodium cacodylate buffer adjusted to pH 7.4, and with a total osmolality of 350 (2).

RESULTS

Animals exposed to 6 ATA of oxygen demonstrated the most severe degree of pulmonary change in the shortest period of time (bottom time at 6 ATA was 24 min). All changes in the lungs of affected animals were associated with various phases of edema formation. The onset of neurologic change (i.e., seizures) at 6 ATA alone was not sufficient to induce edema. However, persistence for an additional 5 to 8 min of those conditions which had originally induced seizures resulted in rapidly progressive changes in the lungs as noted by the general appearance of the lungs (Fig. 1) and the accumulation of blood and fluid in the airspaces and tissues surrounding the pulmonary vasculature (Fig. 2). This relationship of altered neurologic function to pulmonary vascular change was further confirmed by observation of other animals exposed to 6 ATA of oxygen. Lung edema was consistently more marked in animals having an earlier onset of seizures during hyperbaric exposure compared to animals seizing at a later time or during the period of decompression.

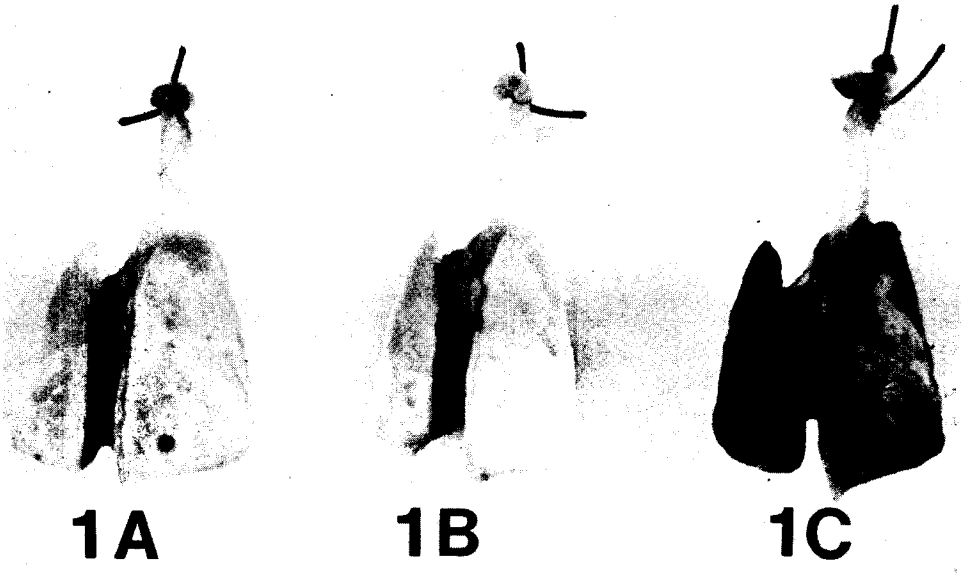

Fig. 1. Gross appearance of vascularly perfused lungs following exposure to hyperbaric oxygen. A, control lung; B, exposure to 6 ATA of oxygen to the point of the onset of seizures; C, exposure to 6 ATA of oxygen for 5 min beyond the onset of seizures.

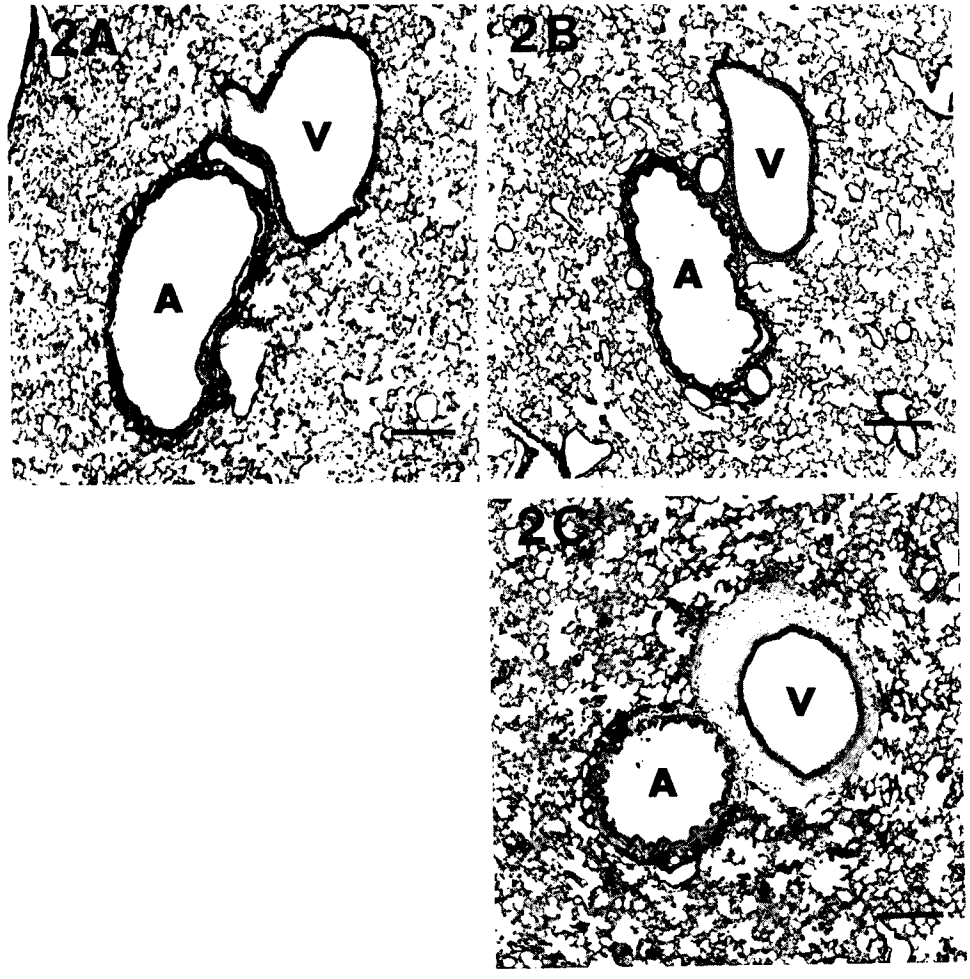

Fig. 2. Light micrographs of bronchovascular bundles and surrounding parenchymal tissues from the respective lungs shown in Fig. 1. A, control lung; B, 6 ATA oxygen to the initial onset of seizures; and C, 6 ATA oxygen 5 min beyond the onset of seizures. Cuffing is most prominent around the vessel (V) and minimal around the airway (A). The scale bar in each micrograph is equal to 250 μm.

The distribution of perivascular cuffing (fluid accumulation within the interstitial space surrounding vessels) in the pulmonary circulation following edema formation was determined by computer-aided, three-dimensional reconstruction of the arterial and venous systems of the right middle lobe (Fig. 3). It was found that perivascular cuffing was most prominent along the arterial and venous vessels accompanying the major airway entering the lobe. Vascular cuffing was also noted along the branches of these vessels. The extent of cuffing appeared to be greater toward the hila region of the lobe compared to the more peripheral regions. In addition, vascular cuffing around arterial vessels extended out to the level of the capillary bed, whereas cuffing around veins could only be noted beginning several vessel generations away from the capillary bed. An interesting finding for pulmonary veins was that the most prominent cuffing around these vessels occurred in those regions where the vein shared a common interstitial sheath with an arterial vessel.

To further examine the involvement of the pulmonary vasculature in edema formation, randomly selected circular-to-oval profiles of arteries and veins were photographed from step sections taken through the lung of an animal exposed to 6 ATA of oxygen. Since gradients of pulmonary involvement were noted for animals exposed to identical hyperbaric conditions, a single lung demonstrating edematous changes was used to determine the proportional degree of cuff formation for arteries and veins of various lumenal sizes in the pulmonary circulation. The thickness of the perivascular cuff surrounding each vessel was measured to determine an average cuff thickness. The results of these measurements are given in Table 1 and illustrate that a more prominent interstitial cuff surrounds arteries compared to veins of similar lumenal size. The ratio of cuff thickness to vessel lumen radius further demonstrates a more severe involvement of arteries compared to veins.

Table 1
Interstitial Cuff Size of Pulmonary Vessels Following Exposure to 6 ATA of Oxygen.

	Vessel Lumen Size (μm)					
	< 20		20 - 50		50 - 80	
Vessel Type	Artery	Vein	Artery	Vein	Artery	Vein
Cuff thickness* μm	38 ± 4	N.D.**	41 ± 3	18 ± 4	74 ± 5	32 ± 4
Ratio of cuff thickness to vessel lumen radius	5.11	—	2.59	1.00	2.35	0.99

* ± SEM. ** = not detectable.

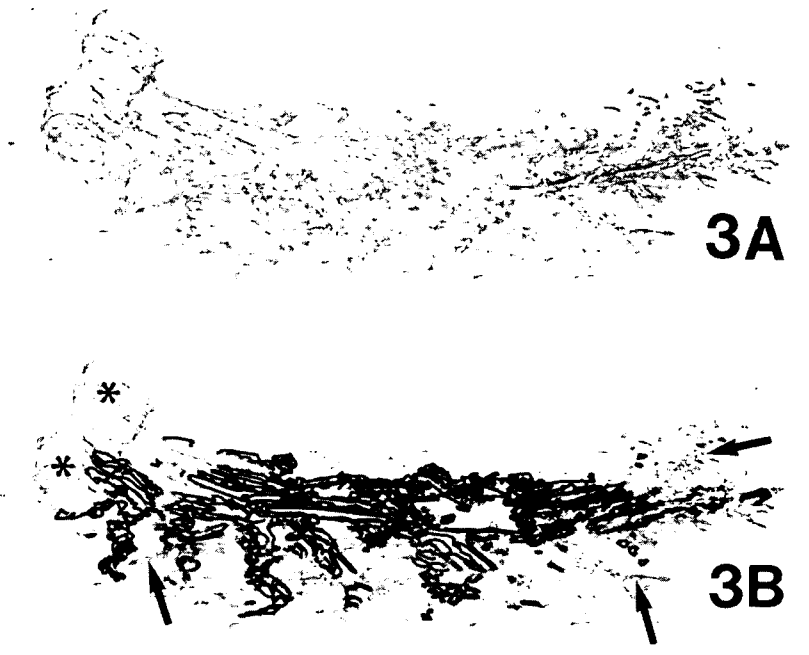

Fig. 3. The arterial and venous circulations of the right middle lobe (A) and distribution of perivascular cuffing (B) following hyperbaric exposure to 6 ATA of oxygen. The extrapulmonary vessels (*) demonstrate minimal cuffing, as do many of the most peripheral branches of pulmonary vessels which are almost exclusively veins.

The effect of temperature on oxygen sensitivity was most marked in animals exposed to 3 ATA for 6 h. Mortality rates, changes in skin and core body temperatures, and the relative degree of perivascular cuffing and airspace fluid accumulation are shown in Table 2. Measurements have been compared to those values in animals exposed to 1 and 6 ATA of oxygen.

DISCUSSION

These findings confirm those of previous studies. A number of investigators have speculated that pulmonary edema following exposure to hyperbaric oxygen is due to central nervous system disturbances, resulting in massive sympathetic discharge and central pooling of blood into the pulmonary circulation (3). We have found that persistent perturbations of the central nervous system induced by exposure to hyperbaric oxygen are associated with marked perivascular cuffing in the lungs. This cuffing is more pronounced for arterial vessels compared to venous vessels of the pulmonary circulation. Venous cuffing may be due in large measure to fluid seepage from the arterial side at those points where the arterial and venous vessels share a common

Table 2
Response to Hyperbaric Oxygen for Different Temperature Settings*

Temperature, °C	Mortality Rate, %		Skin Temperature Δ/6 h		Core Body Temperature Δ/6 h		Lung Pathology**		
	1 ATA	3 ATA	1 ATA	3 ATA	1 ATA	3 ATA	1 ATA	3 ATA	6 ATA
8	0	0	-3	-12	-2	-11	0	2+	3+
24	0	17	1	-1.5	-1	-2	0	2+	4+
37	8	83	—	+5†	—	+7†	±	1+	4+

* Based on 12 animals for each temperature and exposure setting. ** Only lungs from animals surviving the exposure were examined. A score of 0 represents no pathology and a 4+ score represents severe pulmonary edema with extensive perivascular cuffing and hemorrhage associated with fluid accumulation in the airspaces. † Temperature changes noted above were for the first hour of exposure only.

interstitial sheath. Brain dysfunction following exposure to hyperbaric oxygen is also manifested by the loss of body temperature regulation (4), in particular for cold and hot temperatures at 3 ATA of oxygen. Although pulmonary edema appears to be the final insult leading to death in hyperbaric oxygen exposure, this process can be accelerated or delayed by hot or cold temperatures, respectively.

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REEXPANSION PULMONARY EDEMA: A PROPOSED FREE RADICAL MECHANISM

R. M. Jackson, C. F. Veal, Jr., J. D. Fulmer, and C. B. Alexander

Since its initial description in the 19th century, reexpansion pulmonary edema (RPE) has been attributed to intrathoracic changes in mechanical forces resulting from rapid reexpansion of previously collapsed lungs. Riesman's (1) classic clinical report of several cases contained detailed analyses of the edema fluid, which unequivocally established that the fluid was rich in protein, suggesting an increase in alveolar-capillary permeability. Finally, however, he attributed the edema to "congestion by recoil," and did not propose a unilateral inflammatory response as a possible mechanism of RPE.

In addition to interruption of oxygen supply because of absent alveolar ventilation, collapsed lung tissue is hypoperfused. The pulmonary blood supply to collapsed lung in both open and closed chest preparations is significantly reduced, predominantly as a result of hypoxic pulmonary vasoconstriction. Glasser and his coworkers (2) found that left lung atelectasis in dogs caused reduction of the left lung blood flow from 43 to 12% of cardiac output throughout 4 h of observations. Similar findings have been reproduced in other models, but the degree of hypoxic pulmonary vasoconstriction depends on the presence or absence of systemic hypoxemia and respiratory alkalosis and on the type of experimental ventilation (3).

Since absent ventilation and a reduction in pulmonary artery blood flow occur together during atelectasis, collapsed alveolar units are relatively hypoxic (although lung cells retain aerobic metabolism even at very low Po_2), given a normal alveolar Po_2 of > 100 mmHg. The abrupt simultaneous resumption of ventilation and pulmonary artery perfusion during reexpansion of a collapsed lung would reintroduce excess molecular oxygen into a tissue that had approached Po_2 of mixed venous blood, 40 mmHg.

Parks and his coworkers (4) have developed a hypothesis that explains

increased postischemic vascular permeability (quantified as permeability of feline gut to protein) attributable to reoxygenation. In this model, xanthine oxidase is the major intracellular source of oxygen free radicals, and hydroxyl radicals ($OH^{\bullet}$) have been shown to be the proximate cause of cytotoxity. Importantly, tissue injury in this model can be minimized by either intracellular or extracellular free radical scavengers such as dimethylsulfoxide or superoxide dismutase (5).

We proposed a similar mechanism in lung, and specifically postulated that during reexpansion and reoxygenation of previously collapsed lungs, hydroxyl radicals were formed and caused increased alveolar capillary permeability. This study tested the hypothesis by administration of hydroxyl radical scavenger, dimethylthiourea (DMTU), alone or in combination with the antioxidant enzyme catalase (CAT) (from bovine liver, Sigma Chemical Co, St. Louis, MO), to rabbits immediately before reexpansion of their right lungs, which had been previously collapsed for 7 d. Our results confirm the importance of free radicals in RPE, and further suggest formation of a chemoattractant for neutrophils which enter the reexpanded lung within 2 h, resulting in marked, unilateral lung inflammation.

METHODS

Experimental Animals

New Zealand white male rabbits weighing 2 to 3 kg (Myrtle Farms, Thompson Station, TN) were used in these experiments. The protocol we used for lung collapse and reexpansion in these animals paralleled that already described (6).

Lung Collapse and Reexpansion

On the day of collapse ~75 to 100 ml of air was injected through a 23-gauge needle into the right pleural space after the rabbit had been sedated (methohexital, 3-5 mg i.v.). The initial procedure and subsequent daily injections of 15 to 40 ml of air were visualized by fluoroscopy to ensure that the right lung was completely collapsed and the mediastinum slightly shifted. We maintained total lung collapse for 7 d because that period results in a high incidence of reexpansion edema.

Immediately before reexpansion of the collapsed lung, the rabbits were sedated with ketamine (50 mg/kg i.m.) and promazine (1.5 mg/kg i.m.) with methohexital (3-5 mg i.v.) administered as needed. The rabbit lay supine throughout subsequent preparation. A tracheostomy was performed and a tube inserted and tied in the trachea, through which the animal spontaneously breathed 100% oxygen. A 10 French neonatal chest tube was then inserted through a right midintercostal space and secured so as to be leak-free. After respiratory fluctuations of a water seal confirmed the intrapleural position of the tube, the lung was reexpanded rapidly by applying -100 mmHg pressure. Complete lung reexpansion was confirmed by fluoroscopy in several animals.

After reexpansion of the previously collapsed lung the animals were allowed to breath spontaneously for 2 h before death by rapid i.v. injection of 100 mg pentobarbital. The abdominal aorta and vena cava were transected before removal of the lungs.

To evaluate the effects of prolonged lung collapse without reexpansion, 4 rabbits were prepared as above. After 7 d of lung collapse the animals were killed. The lungs were removed and processed exactly as described for the reexpanded lungs, so that the degree of edema and inflammation before reexpansion could be assessed.

Administration of Antioxidants

To assess the effects of oxygen radical scavengers on the formation of RPE, pairs of animals were prepared as described. In the first series (7 pairs) the experimental rabbits received an i.v. injection (500 mg/kg) of DMTU (1,3-dimethyl-2-thiourea; Aldrich Chemical Co, Milwaukee, WI), an efficient intracellular hydroxyl radical scavenger which protects lung tissue from several types of free radical-mediated injuries. We chose this compound to test whether an intracellular source of free radicals was responsible for edema formation because it is freely diffusible into lung interstitial fluid and Fox (7) has shown that DMTU prevents hyperoxia-induced lung edema, a form of tissue injury directly attributable to increased intracellular superoxide production. These rabbits are denoted as DMTU-pretreated. Before reexpansion the control rabbit in each pair received equivolume vehicle i.v. (normal saline, 5 ml/kg). In the second series (6 pairs) the experimental animals were pretreated with both the intracellular hydroxyl radical probe DMTU as above and CAT (150,000 U/kg, i.v.), an antioxidant enzyme capable of eliminating extracellular hydrogen peroxide (8). These rabbits are designated as DMTU+CAT-pretreated. The paired controls similarly received equivolume vehicle.

Processing of Lung Tissue

Two hours after the lung had been reexpanded the animals were killed and the lungs removed for assessment of edema formation. The lungs were inspected for evidence of acinar filling and edema fluid in the tracheal tube. The lungs were manually reinflated once and allowed to collapse to residual volume, so that the following histologic studies were all done at comparable lung volume. (The paired animals were matched for weight so that alveolar size would not differ.) The lungs were removed from the thorax and 2-mm thick sections were cut from the midportion of the right (reexpanded) and left (contralateral control) lungs in a vertical plane from ventral to dorsal surface. One section was placed in 10% buffered formalin for processing as 5-μm hematoxylin and eosin (H&E) sections for light microscopy, and the other section was weighed. That section was oven-dried for 72 to 96 h (80°C) to constant weight for determination of wet weight-to-dry weight (wet:dry weight) ratio. The remainder of the lung was frozen (-20°C) and later homogenized in (1:10 w/vol) in 50 m*M* phosphate buffered saline (PBS, pH 7.4)

for measurement of protein and DNA content.

Quantitation of Pulmonary Edema

The degree of edema formed during reexpansion was assessed by two independent means. Lung wet:dry weight ratios were used as an indicator of fluid content in the lung as in previous studies. Intraalveolar edema fluid and inflammatory cells were also estimated by light microscopy using the semi-quantitative technique describe by Frank (9). The 5-μm H+E sections were coded and examined by a blinded observer. Alveoli which contained visible edema fluid in each of 50 random ×450 microscope fields were counted. Since the lungs were sectioned at residual volume and alveolar size is comparable in rabbits of similar weight, this technique provided a separate and reasonably quantitative estimate of intraalveolar edema. Intraalveolar cells (either alveolar macrophages or neutrophils) were quantitated by counting those alveolar spaces in 50 random ×450 fields which contained two or more nucleated cells.

Protein and DNA Contents

Protein content of lung homogenates was measured using the Coomassie blue dye binding assay of Bradford (10). The DNA content was measured by reacting DNA extracted from the tissue with diphenylamine reagent (11).

Data Analysis

The data are expressed as arithmetic means ± 1 SEM. Within each group the data from reexpanded (right) lungs were compared to those from contralateral (left) lungs in the same animal using the paired t test. To further define the effects of reexpansion, the experimental interventions, and their interactions the groups were also compared using 2 × 2 factorial analysis of variance (ANOVA) with post-hoc analysis by a t test appropriately modified for orthogonal comparisons (12). The level $P < 0.05$ was accepted as significant.

Description of Lungs

Lungs from those 4 rabbits killed before reexpansion appeared liverlike on opening the thorax, but were completely free of edema, hemorrhage, or signs of infection when reinflated. Wet:dry weight ratios of the collapsed (right) lungs (4.91 ± 0.13) were identical to those of the left (4.92 ± 0.11, $P > 0.05$), confirming that no edema developed during collapse. Except for some residual evidence of collapse, the nonreexpanded lungs were histologically indistinguishable from the contralateral lungs. There was no visible perivascular or intraalveolar edema, and the alveolar spaces were free of inflammatory cells. Protein and DNA contents did not differ from the contralateral lungs (Table 1).

In the absence of free radical scavenger pretreatment, reexpanded lungs were evidently edematous and collapsed more readily than the contralateral lungs. Alveolar edema was obvious in many reexpanded lungs because acinar filling was visible on dependent pleural surfaces and edema froth flowed from

Table 1
Data From Nonreexpanded Lungs*

	Right ($n = 4$)	Left ($n = 4$)	P**
Wet:dry weight	4.91 ± 0.13	4.92 ± 0.11	> 0.05
DNA, mg/g dry wt	120.32 ± 0.84	106.63 ± 3.70	> 0.05
Protein, mg/g dry wt	466.98 ± 18.47	472.66 ± 13.40	> 0.05

* Data are means ± SEM from 4 rabbits killed after the right lung had been collapsed for 7 d.
** Right compared to left by paired t test.

the endotracheal tube after death. In all animals the contralateral left lung appeared normal. The reexpanded lungs of DMTU- or DMTU+CAT-pretreated rabbits closely resembled the undamaged contralateral lungs. Qualitative assessment of light microscope sections of reexpanded lungs from vehicle-treated rabbits revealed changes consistent with acute, diffuse alveolar injury. Many alveoli were filled with proteinaceous edema fluid, and hyalinelike membranes were present in some. The alveolar septae were hypercellular, and many neutrophils and macrophages were present in alveolar spaces. Perivascular edema was present and septal widening suggested interstitial edema. In most cases the left lungs appeared normal, although on occasion intraalveolar cells or edema were noted. These pathologic changes were largely absent in reexpanded lungs from animals which received DMTU or DMTU+CAT before reexpansion. The antioxidant or vehicle pretreatments had no detectable effects on the contralateral (left) lungs (Fig. 1).

Lung Fluid Content

In the first 7 pairs (DMTU compared to vehicle), significant pulmonary edema only developed in the reexpanded lungs of vehicle-pretreated animals. Mean wet:dry weight ratio in the reexpanded lungs was 6.53 ± 0.52, 33% greater ($P < 0.02$) than that of the contralateral lungs (4.91 ± 0.07) indicating an increase in lung fluid content. In contrast, the reexpanded lungs of rabbits pretreated with only DMTU, the hydroxyl radical scavenger, had a mean wet:dry weight ratio of 5.35 ± 0.34, only slightly higher than the contralateral controls (4.95 ± 0.10, $P > 0.20$) demonstrating significant protection from edema formation. However, ANOVA revealed that the wet:dry weight ratio of the right lungs from DMTU-pretreated rabbits (5.35 ± 0.34) was not significantly less than the ratio of the right lungs from vehicle-treated rabbits (6.53 ± 0.52, $P > 0.05$). No significant differences were detected between the left lungs' wet:dry weight ratios (Fig. 2).

Similar, but even more pronounced, protection from edema formation occurred after pretreatment with both DMTU+CAT. In vehicle-pretreated rabbits of that second series the mean lung wet:dry weight ratio was 7.30 ± 0.68 in reexpanded right lungs, and 5.08 ± 0.08 in contralateral left lungs (an

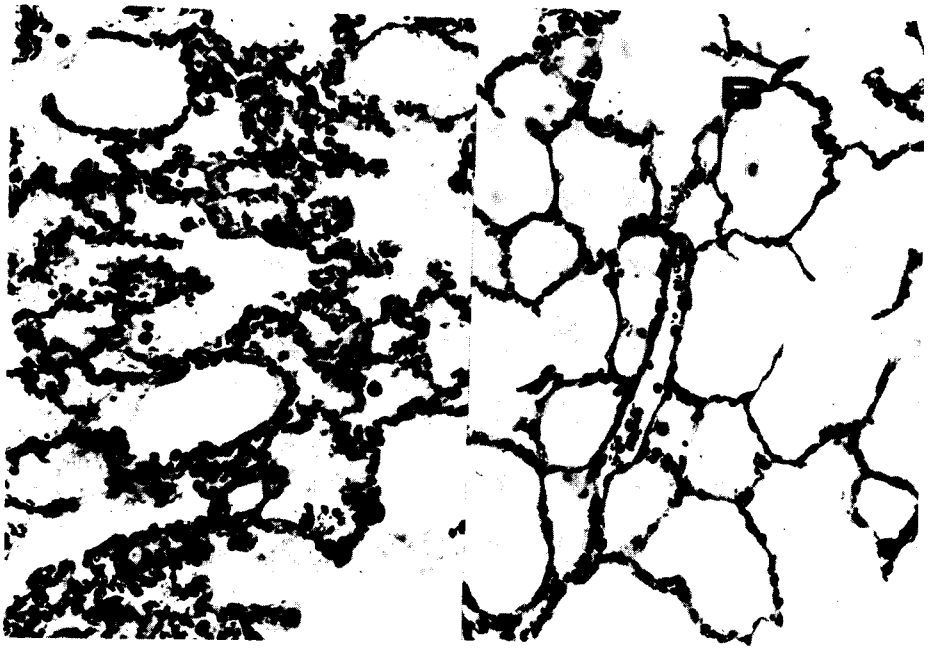

Fig. 1. Histologic changes in reexpanded right lungs from vehicle-pretreated (A) and DMTU+CAT-pretreated (B) rabbit lungs. The reexpanded lung from the vehicle-pretreated rabbit shows changes of acute lung injury with intraalveolar edema and inflammatory cells, and a cellular infiltrate in the interstitium. In contrast, the reexpanded lung from the DMTU+CAT-pretreated rabbit appears normal with no intraalveolar or perivascular edema; there are no inflammatory cells in alveolar spaces or interstitium (μm H&E sections, original magnification x200).

increase of + 44%, $P < 0.03$). This increase was completely prevented by DMTU+CAT, as indicated by wet:dry weight ratio of 4.95 ± 0.19 in reexpanded lungs, compared to 4.74 ± 0.17 in contralateral lungs ($P > 0.40$). The addition of the extracellular antioxidant enzyme CAT was associated with a significantly lower wet:dry weight ratio of reexpanded lungs from the DMTU+CAT-pretreated rabbits (4.95 ± 0.19) when compared with the ratio of reexpanded lungs from the vehicle-pretreated, paired controls (7.3 ± 0.68, $P < 0.05$) by ANOVA (Fig. 3).

Exactly corresponding results were obtained by semiquantitative assessment of light microscope sections. In the first series of experiments, reexpanded lungs in vehicle-pretreated rabbits contained significantly more alveoli (per ×450 field) with visible edema fluid ($P < 0.01$), whereas reexpanded lungs from the DMTU-pretreated rabbits did not have significantly more visible edema than the contralateral lung ($P > 0.30$). More alveoli in reexpanded lungs from vehicle-pretreated rabbits also contained two or more intraalveolar cells ($P < 0.02$), whereas no significant increase in intraalveolar cells occurred after DMTU pretreatment (Table 2).

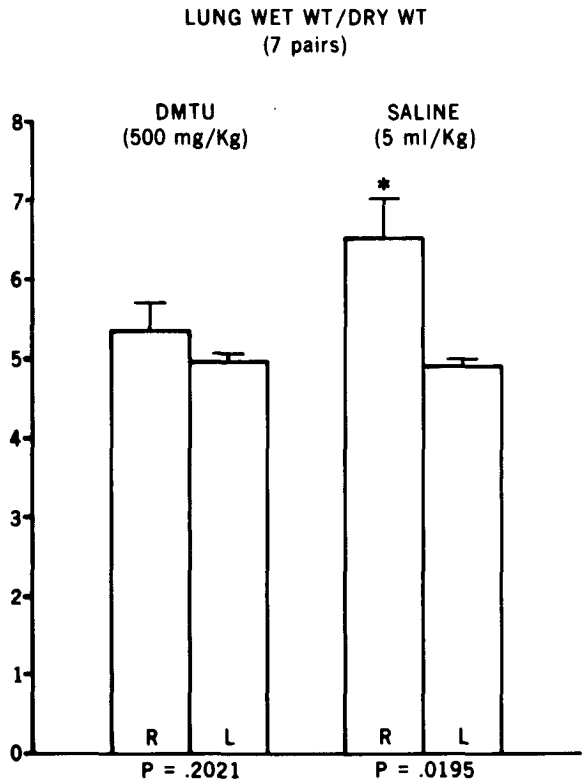

Fig. 2. Wet:dry weights in DMTU- and saline-pretreated rabbits' lungs. Bars represent the means with 1 SEM indicated. The P values for paired t test comparison of reexpanded vs. contralateral lungs in each treatment group are shown. However, the wet:dry weights of the DMTU-right did not differ significantly from the saline-right lungs ($P > 0.05$, 2 × 2 factorial ANOVA).

In the second series (pretreatment with DMTU+CAT), histologically evident edema was frequently present in reexpanded lungs from vehicle-treated rabbits, whereas the left lungs held significantly less intraalveolar fluid ($P < 0.03$). No detectable difference in numbers of edema-containing alveoli occurred in reexpanded lungs after DMTU+CAT-pretreatment $P > 0.68$), reconfirming the marked protective effect demonstrated by wet:dry weight ratios. Intraalveolar cells were also increased in vehicle-pretreated rabbits' reexpanded lungs ($P < 0.05$), whereas the increased cellularity was likewise prevented by pretreatment with DMTU+CAT ($P < 0.05$) (Table 3).

Lung protein content (expressed as milligram of protein per gram of dry lung tissue) tended to be higher in the reexpanded lungs of both series, but the only apparent increases that reached statistical significance occurred when the protein content of vehicle-pretreated right lungs (735.26 ± 146.99 mg/g) was compared to the protein content in the paired DMTU+CAT-pretreated

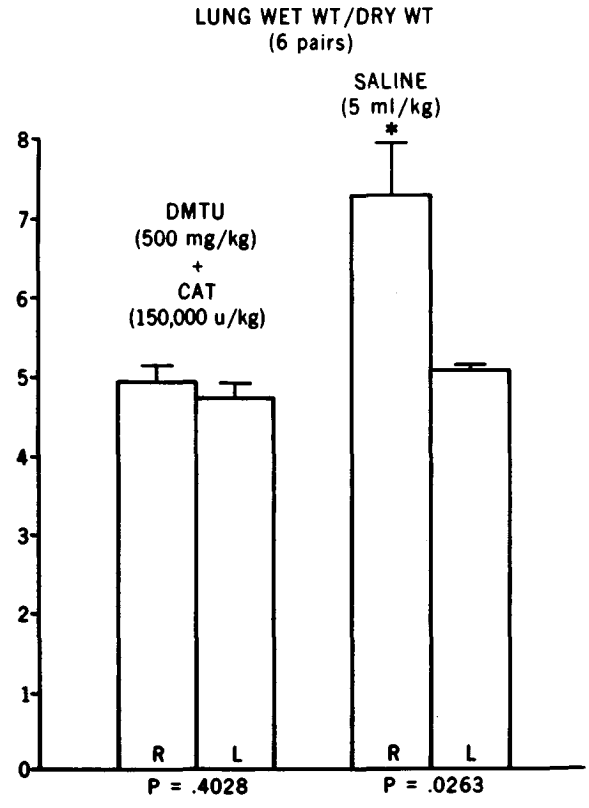

Fig. 3. Lung wet:dry weights in DMTU+CAT- and saline-pretreated rabbit lungs. The format is identical to Fig. 1. In addition, ANOVA revealed that the DMTU+CAT-right lungs were significantly less edematous than the saline-right lungs ($P < 0.05$), suggesting additive protective effect of extracellular CAT.

Table 2
Histologic Assessment of Inflammation and Edema*

	DMTU, 500 mg/kg, $n = 7$		Saline, 5 ml/kg $n = 7$	
	R	L	R	L
Alveolar with edema/HPF	0.93 ± 0.51	0.54 ± 0.25	3.65 ± 0.83**, †	0.27 ± 0.12
Alveolar with > 2 cells/HPF	1.48 ± 0.51	0.59 ± 0.20	3.52 ± 0.81**, †	0.59 ± 0.28

* Data are means ± SEM of 50 ×450 fields. ** $P > 0.05$ paired t (right compared to left lung in each treatment group). † $P < 0.05$, 2 × 2 factorial ANOVA (DMTU-right compared to saline-right lungs).

Table 3
Histologic Assessment of Inflammation and Edema*

	DMTU+CAT (150,000 U/kg) $n = 6$		Saline, 5 ml/kg, $n = 6$	
	R	L	R	L
Alveolar with Edema/HPF	0.26 ± 0.18	0.29 ± 0.15	2.69 ± 0.71**, †	0.22 ± 0.14
Alveolar with > 2 cells/HPF	0.54 ± 0.16	0.52 ± 0.14	3.58 ± 0.97**, †	0.59 ± 0.18

* Data are means ± SEM of 50 ×450 fields. ** $P < 0.05$, paired t (right compared to left lung in each treatment group). † $P < 0.05$, 2 × 2 factorial ANOVA (DMTU+CAT-right compared to saline-right lungs).

rabbits' controls' right lungs (421.97 ± 69.35, $P < 0.05$) by ANOVA. Lung DNA content was higher (expressed as milligram DNA/gram of dry lung tissue) in reexpanded lungs from the vehicle-pretreated animals of the first series ($P < 0.02$), and apparently (but not significantly) higher in reexpanded lungs from vehicle-treated rabbits from the second. Like the edema formation, the apparent increases in lung DNA were blocked by DMTU or DMTU+CAT, suggesting that they represent an influx of inflammatory cells that occurred after reexpansion (Tables 4 and 5).

Table 4
DNA and Protein Contents*

	DMTU, 500 mg/kg, $n = 7$		Saline, 5 ml/kg, $n = 7$	
	R	L	R	L
Protein, mg/g dry wt	370.70 ± 35.04	371.92 ± 35.28	412.25 ± 27.65	371.00 ± 24.73
DNA, mg/g dry wt	88.30 ± 12.09	85.49 ± 7.55	108.97 ± 10.31**	95.99 ± 13.37

* Data are means ± SEM. ** $P < 0.05$, paired t (right compared to left lung in saline treatment group).

DISCUSSION

Our results have confirmed that unilateral pulmonary edema occurs in this rabbit model of reexpansion pulmonary edema when the previously collapsed lung is rapidly reexpanded by negative intrathoracic pressure. In the experiments described, we have used this model to investigate the mechanism of

Table 5
DNA and Protein Contents*

	DMTU+CAT, (150,000 U/kg), $n = 6$		Saline, 5 ml/kg, $n = 6$	
	R	L	R	L
Protein, mg/g dry wt	421.97 ± 69.35	479.88 ± 23.18	735.26 ± 146.99**	539.91 ± 47.25
DNA, mg/g dry wt	125.96 ± 12.82	135.65 ± 6.50	181.73 ± 24.49	131.13 ± 10.37

* Data are means ± SEM. ** $P < 0.05$, 2 × 2 factorial ANOVA (DMTU+CAT-right compared to saline-right lungs).

RPE. Our data indicate that edema formation during reinflation of previously collapsed lung is due largely or entirely to partially reduced oxygen metabolites, including hydroxyl radicals and hydrogen peroxide, because its formation can be prevented by agents that eliminate these toxic metabolites. In this model, inflammatory cells enter the reexpanded lung and may themselves contribute to the observed increase in lung microvascular permeability.

Classically, explanations of RPE have centered on mechanisms that would increase fluid filtration from lung capillaries by decreasing interstitial pressures. Good evidence suggests that such mechanisms occur, but would themselves not account for increased capillary permeability to protein, nor would they be inhibited by oxygen metabolite scavengers. Warren et al. (13) clearly indicated that when dogs breathed against resistance so as to increase intrathoracic negative pressure, the flow of lung lymph increased dramatically. This occurred without an increase in cardiac output, and they attributed the increase to the influence of extravascular negative pressure on the transudation of fluid from lung capillaries into the lung parenchyma. Others have proposed that a deficiency of surfactant is casually related to edema formation in RPE, but Levine and Johnson (14) found that deflation characteristics of previously atelectatic rabbit lungs were entirely normal after one inflation. Furthermore, highly surface-active material could be obtained by alveolar lavage, which was normal when tested in a surface-tension balance. Warren et al. (13) also provided some evidence that hypoxia per se increases lung lymph flow, and eventually, of course, complete ischemia would disrupt the physical integrity of both endothelial and epithelial barriers to water and protein. However, more recent work has demonstrated no direct effect of short-term hypoxia (Po_2 12 mmHg) on alveolar capillary permeability to albumin in an isolated dog lung preparation (15). Based primarily on the concentration of ^{131}I-labeled albumin in extravascular, extracellular water of reexpanded lungs, Pavlin et al. (16) recently proposed that RPE results primarily from increased lung microvascular permeability. They asserted that mechanical forces

("stretching") applied to lung microvessels might cause loss of vasculature's normal restriction to water and protein flux by deforming preexisting endothelial pores or by creating intercellular rents.

Our data and that of Pavlin and Cheney (6) and Pavlin et al. (16) confirm that the increase in lung fluid content occurs only *after* reexpansion of collapsed lungs. We found no increase in lung wet:dry weight ratio, no changes in histologic appearance, and no changes in DNA and protein content in lungs that had been collapsed for 7 d but not reexpanded. Our examination of light microscope sections also confirmed that infection did not account for the lung weight of cellular changes that we found. Similarly, Pavlin et al. (16) reported normal lung fluid contents after 7 d of collapse, and no increase in permeability to [^{131}I]albumin occurred before reexpansion. So in this model collapse per se does not cause lung injury (as defined by these commonly accepted indicators), but reexpansion is associated with easily detected increases in lung permeability.

In addition to absent alveolar ventilation, experimental evidence indicates that atelectatic lungs are hypoperfused. Bradley et al. (17) found that blood flow to collapsed (25% of functional residual capacity) left lower lobes of dogs decreased 30% when assessed by radiolabeled microspheres, and that collapsed lobe wet:dry weight ratio was less than that of controls. The decrease in blood flow to collapsed lobes seems to occur slowly, perhaps because of delayed resorption of alveolar oxygen leading to eventual hypoxic vasoconstriction. With prolonged collapse, perfusion decreases greatly, and Aviado (18) has suggested that separate mechanisms might account for the decreases in blood flow in acute or chronic atelectasis. The available evidence indicates that blood flow decreases to collapsed lung because of the hypoxia and subsequent vasoconstriction. This would result in a change in Po_2 of alveolar capillary tissue from a normal level of 100 mmHg to one approaching mixed venous Po_2, 40 mmHg. This degree of tissue hypoxia seems sufficient to begin the events which culminate in reoxygenation injury.

McCord and Roy (19) have proposed a mechanism by which free radical damage occurs in tissue that is reperfused (reoxygenated) after a period of hypoperfusion or ischemia. In their model, tissue xanthine dehydrogenase is converted to oxygen-utilizing xanthine oxidase (probably by proteolysis) during hypoperfusion. Hypoxanthine, a degradation product of ATP, which accumulates in hypoperfused tissues, serves as a purine substrate for xanthine oxidase, being oxidized to uric acid with concomitant production of superoxide anions. The introduction of molecular oxygen into previously ischemic tissue during reperfusion causes intracellular superoxide anion production as xanthine oxidase degrades accumulated hypoxanthine. This system seems to involve transcellular calcium flux during hypoperfusion, because a calcium-dependent protease is postulated to convert dehydrogenase enzyme to xanthine oxidase, and the time course of cytotoxicity parallels the calcium paradox in Lagendorf-perfused heart preparation (20). Our evidence strongly supports a similar mechanism in collapsed lungs that are reoxygenated after a sufficiently long

period of relative oxygen deprivation.

We found that RPE can be partially prevented by DMTU, a hydroxyl radical scavenger, alone, or essentially completely prevented by the combination of DMTU+catalase, an extracellular antioxidant enzyme that eliminates hydrogen peroxide.

DMTU is the N,N'-dimethylated derivative of thiourea, which is capable of scavenging $OH^{\bullet}$ both in vitro and in vivo. The compound is nontoxic relative to other thioureas, and it has been shown to protect mice from radiation lethality and rats from pulmonary O_2 toxicity, two conditions resulting in large part from intracellular $OH^{\bullet}$ production (7). In the dose we employed (500 mg/kg), millimolar concentrations of DMTU are detectable in rat plasma within 1 h and persists for at least 8 h. The compound enters the interstitial and presumably intracellular fluid compartments readily and has been applied as a probe for $OH^{\bullet}$-mediated tissue damage. Catalase is an antioxidant enzyme (molecular weight 250,000) which remains in the extracellular fluid (essentially intravascular) space and serves as a specific probe for the effects of extracellular H_2O_2, a possible product of activated neutrophils. Lung fluid content, as assessed by wet:dry weight ratios, was significantly less in reexpanded lungs of rabbits pretreated with DMTU, and it was the same as the control in reexpanded lungs of rabbits pretreated with both DMTU and catalase. These findings were corroborated by an independent histologic assessment of edema formation. Many more alveoli contained visible edema fluid in the reexpanded lungs of vehicle-treated rabbits than in reexpanded lungs from rabbits pretreated with oxygen metabolite scavengers. In fact, there was no significant increase in alveoli containing edema in the DMTU+CAT treated rabbits' reexpanded lungs, whereas the number of alveoli with edema increased over 10-fold in the vehicle-treated pairmates. Both gravimetric and histologic assessment of edema formation indicates marked protection by free radical scavengers.

Our data also emphasize the potential importance of cell-mediated lung injury in RPE. First, on examination of light microscope sections we noted many intraalveolar macrophages and neutrophils in reexpanded lungs from vehicle-pretreated rabbits. Both 1-μm plastic sections examined by light microscopy and transmission electron microscopy of reexpanded lungs from vehicle-treated rabbits (Jackson and Herrera, unpublished observations) indicate that the alveolar interstitium contains increased numbers of inflammatory cells. Second, since extracellular CAT provides an additive protective effect compared to DMTU alone, it is possible that part of the microvascular injury is due to activated neutrophils that produce an extracellular accumulation of hydrogen peroxide. A similar mechanism might explain the partially protective effect of purely extracellular scavengers (e.g., superoxide dismutase) in the feline gut model of reperfusion injury. Finally, preliminary data from our laboratory indicate that the fraction of neutrophils in alveolar lavage fluid increases dramatically in reexpanded lungs from vehicle-treated rabbits, further suggesting the presence of chemoattractants for neutrophils (21).

This influx of inflammatory cells apparently occurs only after reexpansion and, like the edema, can be blocked by DMTU or DMTU+CAT. While very few alveoli in high power fields of contralateral (left) lungs contained ≥ 2 inflammatory cells, significantly more cell-containing alveoli were present in reexpanded (right) lungs from vehicle-pretreated rabbits. No significant increase in cell-containing alveoli occurred in reexpanded (right) lungs after adminis- tration of oxygen metabolite scavengers. This implies that biochemical events related to reoxygenation produce chemoattractant activity for neutrophils. In vitro, reaction of plasma with superoxide-generating systems produces a potent chemoattractant for neutrophils (22). This factor, which seems to be a chloroform-extractable lipid bound to albumin, apparently depends specifically on superoxide, because its appearance is inhibited by superoxide dismutase but not catalase. Similarly, another chemotactic lipid can be generated by incubation of arachidonic acid with xanthine oxidase and acetaldehyde (23). Production of superoxide by endogenous lung cells may potentiate RPE by calling inflammatory cells into the newly reexpanded lung.

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SESSION 11: POSTER PRESENTATIONS
OXYGEN EFFECT AT TISSUE LEVEL

9th International Symposium on Underwater and Hyperbaric Physiology
Undersea and Hyperbaric Medical Society, Bethesda, MD, 1987

OXYGEN AND BRAIN PHYSIOLOGIC FUNCTIONS: A REVIEW

D. Torbati

Oxygen is the key element in diving operations and in the treatment of various diseases in need of improved tissue oxygenation (1-7). Application of hyperbaric oxygenation (HBO), however, is encountered with two opposite actions: the physiologic and toxic effects of oxygen on the body's systems. The sensitivity of tissues to HBO depends on their chemical composition, the level of tissue Po_2, and the duration of exposure, as well as their antioxidant defense mechanisms and the level of their individual functional and metabolic activities (8-19). Consequently, various organs in an intact animal have different rates of development of oxygen toxicity and different characteristic signs of poisoning (2, 11, 16, 20). In most Metazoa studied, the respiratory apparatus and the central nervous system (CNS) are among the targets most sensitive to the toxic effects of oxygen, and thereby set limits on the use of oxygen for medical, diving, and aviation-space applications (2, 8, 11, 13-15, 19-21).

The purpose of this brief review is to summarize the effects of a wide range of oxygen pressures, from 7 atmospheres absolute (ATA) down to 7% oxygen at 1 ATA, on the brain's biochemical, biophysical, circulatory, and metabolic functions. At the same time, the possible pathophysiologic pathways and biochemical mechanisms involved in the development of the neurologic manifestations of oxygen poisoning will be reevaluated.

PHYSIOLOGICAL EFFECTS OF HBO ON THE CNS

Studies concerning the physiologic effects of oxygen on the CNS indicate that oxygen has both a direct and an indirect role in the regulation of blood flow, tissue oxygenation and energy metabolism, all of which are pressure dependent (2, 8, 16, 22-25). Indirectly, cellular oxygen demands may regulate

blood flow and thereby affect tissue oxygenation (26, 27). It has been suggested that oxygen has a direct effect on the control of vascular smooth muscle in the microcirculation (28-30). Acute HBO at different pressures has been shown to reduce the cerebral blood flow (24, 25, 31, 32) in man and other mammals. In experimental animals exposed to extreme limits of HBO, increases in energy metabolism in certain neuroanatomical structures (16, 33, 34), variable increases in cerebral tissue Po_2 (25, 35-37), and characteristic changes in the brain electrical activity have been demonstrated (20, 38-40). A brief account of HBO-induced alterations in these physiologic parameters and their implications in the development of the CNS oxygen toxicity is given below.

Cerebral Blood Flow (CBF) During HBO

Studies on the effect of breathing oxygen on the CBF at pressures from 1 to 5 ATA in experimental animals and up to 3.5 ATA in human subjects indicate an initial vasoconstriction that occurs during relatively short exposures (24, 31, 41, 42). In 1930, Wolff and Lennox (43) noticed that the diameter of pial arteries was slightly decreased as a result of increased oxygen content of the blood of anesthetized cats. More than a decade later, Dumke and Schmidt (44) used a flowmeter to measure the carotid artery blood flow in pentobarbital-anesthetized Macaque monkeys, and observed a decreased blood flow when 100% oxygen was inspired. In 1948, Kety and Schmidt (24) were the first to quantitatively measure the CBF in awake man breathing 85 to 100% oxygen by the nitrous oxide method. They showed a significant reduction (13%) without changes in cerebral oxygen consumption. Lambertsen et al. (31, 45) confirmed and extended these results in man, demonstrating a 15% decrease in the CBF by oxygen inhalation for 1 h at 1 ATA and a 25% reduction at 3.5 ATA. Bean et al. (25, 46) correlating CBF with cerebral tissue Po_2 and electrocorticographic (ECoG) activity in conscious rats exposed to HBO, demonstrated that the cerebral vasoconstrictive effect of oxygen failed before the appearance of the convulsive ECoG discharges, producing a large increase in regional tissue Po_2. They suggested that during HBO the initial reduction in regional CBF is changed to a secondary elevation to control levels or greater. The secondary increase in CBF will then raise the regional tissue Po_2 above the toxic levels for the CNS, precipitating overt convulsions. The vasoconstrictive effect of oxygen on the brain thus seems to be time and pressure dependent, especially for pressures higher than 2 ATA (25, 32, 45-47). This hypothesis is consistent with our data on blood flow in 10 neuroanatomic structures of conscious rats exposed to 5 ATA O_2, which showed an initial decrease in blood flow that returned to normal values before the onset of the first preconvulsive paroxysmal electrical discharge (FED) in the ECoG (32). However, exposure to an extreme pressure of 7 ATA O_2 did not produce any change either in cerebral or spinal cord blood flow before the development of the FED, indicating a pressure limit for the vasoconstrictive property of oxygen. In contrast, the regional blood flow in septum nuclei, globus pallidus,

and putamen was significantly increased during the appearance of the FED (32). At lower pressures of oxygen (i.e., 1-3.5 ATA) commonly used for HBO therapy of decompression sickness, vasoconstriction always took place. For example, 60-min exposure to 3.5 ATA O_2 in conscious rats was not adequate for the breakdown of the cerebral vasoconstrictive mechanism. Thirty-minute exposure to 5 ATA O_2 on the other hand, allowed the CBF to return to a control value (32). The secondary cerebral vasodilation during HBO may be related to the retention of CO_2 in the blood, producing a slight increase in cerebral P_{CO_2}. The increased cerebral P_{CO_2} then could oppose the vasoactive effect of oxygen (45-48).

The vasoconstrictive effect of HBO during short exposures in man (24, 31) may not last in more prolonged exposures at 2 and 2.5 ATA O_2 (49). If a vasodilatory change in cerebral vascular response to HBO is a common reaction in mammals, then this mechanism could be directly involved in the initiation of the toxic effects of oxygen by increasing the cerebral tissue P_{O_2} to toxic levels (25, 32, 36). The safety margin ("tolerance") for HBO in the brain is, therefore, determined by its capacity to maintain a constant vasoconstriction adequate to keep the tissue P_{O_2} at a level that allows the cellular antioxidant defense mechanisms to overcome the hyperoxic insult.

Cerebral Tissue P_{O_2} During HBO

At a given arterial P_{O_2}, the level of local cerebral tissue P_{O_2} is determined by local cerebral blood flow (including microcirculatory factors) as well as by the regional rate of oxygen consumption. The question of whether the magnitude of cerebral tissue P_{O_2} and the development of CNS oxygen toxicity have a cause and effect relationship has been tested in conscious and anesthetized animals. The first attempt to measure alterations in cerebral tissue P_{O_2} during HBO was made by Stein and Sonnenschein (50), using platinum oxygen electrodes. These investigators demonstrated a marked rise in cortical tissue P_{O_2} of curarized, artificially ventilated cats before the occurrence of oxygen-induced convulsions (50). In 1961, Bean (47) measured the local cerebral tissue P_{O_2} during HBO in anesthetized dogs and rats by means of platinum oxygen electrodes. In these pioneering experiments, exposure to 5 ATA O_2 raised the availability of oxygen to the brain to levels that were well maintained for a period of 22 to 25 min. The cerebral vasoconstriction that may have occurred was apparently insufficient to prevent pronounced elevation of tissue P_{O_2} to protect against CNS oxygen toxicity. Bean's data further indicated wide variations in the P_{O_2} of different regions of the brain, leading to the conclusion that some regions may serve to precipitate CNS manifestations of O_2 toxicity (47). Jamieson and Van Den Brenk (35), using large oxygen electrodes, measured cerebral tissue P_{O_2} in anesthetized rats exposed to 1 to 6 ATA O_2. They showed a linear increase in cortical tissue P_{O_2} as a function of increase in the inspired pressure of oxygen. In unanesthetized rats exposed to 5 ATA O_2, Bean et al. (25, 46) demonstrated synchronous cyclical regional cerebral blood flow changes correlated with tissue P_{O_2} that were unrelated to cardiac

or respiratory cycles. As mentioned above, in these studies the regional tissue Po_2 and blood flow were increased during preconvulsive ECoG changes and precipitation of overt convulsions. Bean et al. (46) suggested that these results were related in part to regional O_2 vascular control, involving a primary vasoconstriction followed by a secondary vasodilation, which precipitated a sharp increase in tissue Po_2.

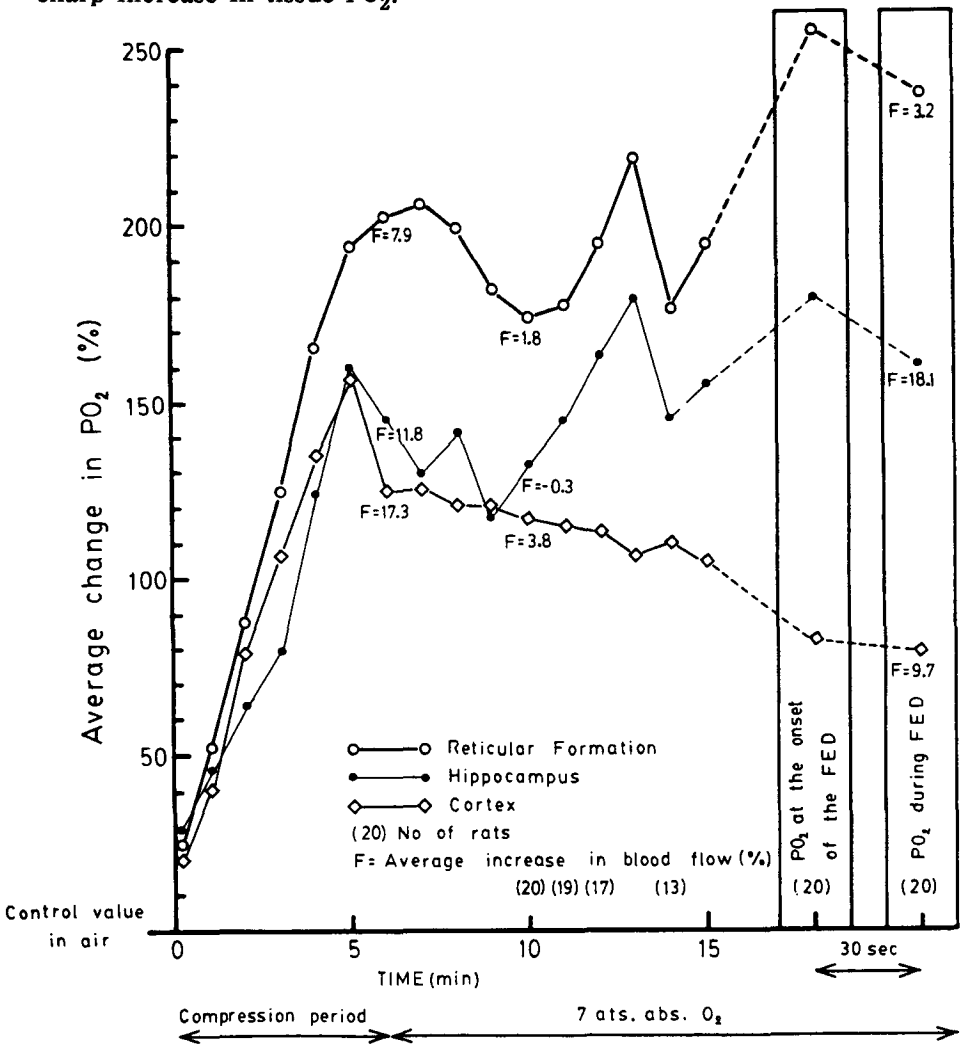

Fig. 1. The relationship between average local tissue PO_2 and regional blood flow (F) in reticular formation, hippocampus, and cortex in conscious rats exposed to 7 ATA O_2 before and during the appearance of FED. The tissue PO_2 was simultaneously recorded in three structures by platinum oxygen electrodes (5-10 u-tip) in a group of rats. Numbers in parenthesis indicate the number of data points used for calculation in each point of time. The CBF data were derived from separate groups of rats in (32).

Using bare platinum oxygen microelectrodes, we studied the correlation between changes in local tissue Po_2 and the changes in ECoG simultaneously in several neuroanatomic structures in conscious rats and rabbits (36, 37). In the study on rats, under pressure of 7 ATA O_2, 60% of the Po_2 curves, after the initial increase in tissue Po_2 at HBO, showed a secondary decrease. In the cerebral cortex this decrease continued until the onset of the FED (36). The average changes in the local tissue Po_2 of cerebral cortex, reticular formation and hippocampus in these rats are presented in Fig. 1.

The maximum values obtained for tissue Po_2 of different neuroanatomic structures in rat and rabbit, although significantly above control, were much below the theoretically predicted values (36, 37). In the absence of significant changes in local CBF of the same structures, as observed in other HBO studies in rats (32) (Fig. 1), we suggested that this relative reduction in cortical tissue Po_2 may occur as a result of an increase in oxygen consumption during the preconvulsive period of HBO exposure (32. 36, 40). Our recent studies on the alterations in regional cerebral metabolic rate for glucose (rCMRgl), which are discussed below, have provided indirect support for the above hypothesis (16, 33, 34, 51-53).

In conclusion, it is possible that these dynamic physiologic phenomena may eventually contribute to the development of early manifestations of the CNS oxygen toxicity (2, 15, 16, 25). Therefore, the effect of HBO on CNS physiologic functions must be known before it is possible to draw conclusions on the physicochemical processes leading to cellular oxygen toxicity.

TOXIC EFFECTS OF HYPERBARIC OXYGENATION

The large volume of literature concerning oxygen poisoning in various organs and organisms indicates that the toxic effects of HBO are multifactorial, and at the same time protective mechanisms are similarly numerous (2, 8-12, 14-16, 19, 21, 29, 54). A major breakthrough in understanding the possible mechanism(s) of oxygen toxicity was achieved as a result of Gerschman's "free radical theory" (55). Today, the free radical theory of oxygen toxicity is widely accepted. It postulates that an increased rate of generation of partially reduced oxygen products is responsible for the cellular toxicity of oxygen (19, 56-58).

The reactive forms of oxygen products, especially superoxide anion ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\bullet}$), can be generated as by-products of normal cell metabolism (59-62). Although the precise sources of increased free radicals during HBO exposure have not yet been identified, both mitochondria and endoplasmic reticulum are capable of increasing superoxide production as oxygen pressure is increased (6, 59-62). Theoretically, all of a cell's components can react with free radicals. The most likely pathways in generation and elimination of HBO-induced free radicals and their possible cellular targets are illustrated in Fig. 2.

The brain has a high rate of oxygen consumption and is rich in oxidizable

substrate, which can be easily targeted by an excess of oxygen free radicals (18, 26, 58). Assuming that the generalized reactions outlined in Fig. 2 can indeed occur during HBO in the brain, they will eventually lead to impairment of its delicate communicative, coordinative, and integrative functional activities (2, 11, 15-18, 21, 54, 58, 63, 64). These functions are entirely dependent on cellular energy metabolism and other biochemical pathways involved in the maintenance of the ionic balance in the membranes, which is needed for starting any communicative action. The ionic fluxes that are produced by excitatory and/or inhibitory neurotransmitters as well as by other stimulating agents conduct the communicative function. The role of neurotransmitter synthesis, release, and uptake in the development and modulation of CNS oxygen toxicity is well recognized and will be discussed below (17, 65-70). The ultimate expression of the biochemical events mediated by neurotransmitters is the biophysical phenomenon of neuronal conductivity.

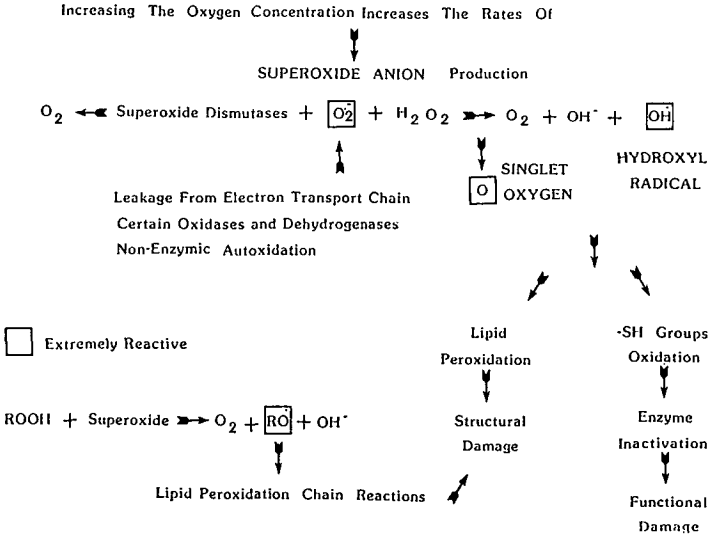

Fig. 2. Reduction products of molecular oxygen in biological systems and the major cellular components damaged by them during HBO. Sources: Chance et al. (62) and Balentine (15).

BIOPHYSICAL EFFECTS OF HBO IN THE BRAIN

The effect of HBO on the bioelectric properties of the neural elements has been studied in isolated mammalian and amphibian nerves (71, 72). Perot and Stein's (71) pioneering studies demonstrated a conduction block in the ulnar-isolated nerves of cats exposed to 13 ATA O_2. Cymerman and Gottlieb (72) showed decreases in conductivity and spike amplitude with an increase in rheobase in isolated frog sciatic nerve exposed up to 50 ATA O_2. These nerve phenomena were due to the increased Po_2 and not to pressure per se (71, 72). Correlation of the observed biophysical phenomena in isolated nerves exposed

to extreme HBO with oxygen-induced neurologic manifestation in an intact animal, however, is not always possible. On the other hand, characteristic alterations in brain electrical activity have provided a strong tool for a qualitative determination of CNS oxygen toxicity at an organismal level.

Hyperbaric Oxygenation and Brain Electrical Activity

In 1945, Cohn and Gersh (73) were the first to use changes in ECoG activity for determination of preseizure abnormalities during HBO. They described a preconvulsive slow wave activity in 5 cats exposed to 7 ATA O_2. Sonnenschein and Stein (74), using curarized cats exposed to 5 to 7 ATA O_2, demonstrated an early appearance of irregular, slow, moderately high-amplitude waves in the ECoG. The number of these waves gradually increased and was followed by occasional sharp spikes that eventually became more synchronous with a greater amplitude. Later, the slow wave and spike activity began to appear in short bursts with several sharp spikes followed by several high-amplitude slow waves. This preseizure pattern lasted from 3 to 40 min and progressed at a seizure pattern characterized by typical "grand mal" electrical activity (74). Rucci et al. (38) were the first investigators to study the preconvulsive EEG activity of the cortical and subcortical centers in conscious rats, showing spontaneous spindlelike waves with increases in the voltage and discharge rates, which were not associated with motor activity. Harel et al. (39), using curarized rabbits exposed to 4 ATA O_2, found a constant and early paroxysmal electrical discharge consisting of desynchronization, fast, sharp, and spike activity of high amplitude, which were simultaneously developed in several brain structures. The onset of the first cortical paroxysmal electrical discharge (FED) in conscious rats was defined by Torbati et al. (75, 76) to be an obvious preconvulsive neurologic sign of oxygen toxicity. However, Raday et al. (77), analyzing the preconvulsive ECoG activity in conscious rats exposed to 6 ATA O_2, demonstrated a significant reduction in the mean frequency and amplitude before the onset of the FED. Torbati et al. (40) characterized the quantitative ECoG frequency changes in conscious rats before, during, and after the appearance of the FED at 3, 4, and 5 ATA O_2. The pattern in ECoG frequency alteration was similar during exposure of 3, 4, and 5 ATA O_2, with marked elevation of slow wave activity before the onset of the FED (40). The sequence of changes in ECoG in conscious, unrestrained rats exposed to HBO is illustrated in Fig. 3.

Thus, the changes in ECoG can be a useful tool for more accurate determination of signs of CNS oxygen toxicity that are not visible or readily detectable (40, 76, 77). The underlying mechanisms of these HBO-induced changes in the brain electrical activity should be sought in the primary biochemical events taking place during development of CNS oxygen toxicity (16, 40). However, these preconvulsion ECoG findings may not occur in all species, as evidenced in conscious rabbits (37) and in pig (78) in which myoclonic fits, resembling oxygen-induced convulsions, precede the ECoG abnormalities.

Safe or "Asymptomatic" Period	Elevation in EEG Slow Wave Activity	Successive Paroxysmal Electrical Discharges	Successive Overt Convulsions
Pre-Convulsive Segments Variable Duration in Each Segment			Convulsive Segment Variable Duration

Fig. 3. Sequence of changes in ECoG in conscious, unrestrained rats exposed to HBO, showing several stages in the development of the neurological signs of oxygen toxicity. Sources: Simon and Torbati (20), Torbati et al. (40, 75, 76), and Raday et al. (77).

The sequence of the visible signs of oxygen toxicity in man and animals indicates that the CNS is usually affected earlier than the respiratory system at pressures equal to or higher than 3 ATA O_2, whereas the latter is primarily affected at lower pressures of oxygen (2, 8, 21). Oxygen in this low-pressure range, however, is extensively used for diving and therapy purposes (4, 7). Due to outstanding respiratory manifestations of HBO at pressures lower than 3 ATA O_2, the possible overlapping neurologic effects of oxygen toxicity may not be easily identified. Therefore, using the ECoG changes as an index for CNS oxygen toxicity, we also tried to detect possible CNS effects of oxygen at 1 and 2 ATA O_2, during which the dominant oxygen-induced respiratory distress phenomena could cover up toxicities possibly occurring in the brain and/or other organs (20, 79). The ECoG records in conscious rats exposed to 1 ATA O_2 did not show significant changes during the first 24 h of exposure (20). Starting the 2nd d of hyperoxia, however, a gradual increase in ECoG slow wave activity, accompanied by reduction in the amplitude, was observed. At this stage, respiratory distress and cardiac arrhythmias were also noticed frequently. The increased ECoG slow wave activity was visually obvious after 48 h of hyperoxia, and reduction in the amplitude became noticeable about 8 h before death or after 72 h of exposure, whichever occurred earlier (20). Recently, we recorded ECoG along with several respiratory and circulatory parameters in conscious rats exposed to 2 ATA O_2 for 12 h (Torbati and Floris, unpublished data). Our preliminary results indicate that either or both ECoG slow wave activity and extensive paroxysmal electrical discharges actually occur at this relatively low pressure only after substantial increase in respiratory distress. The overall changes taking place at this pressure in a conscious rat are illustrated in Fig. 4.

BIOCHEMICAL EFFECTS OF HBO IN THE CNS

Since World War II, many studies have been conducted on the biochemical

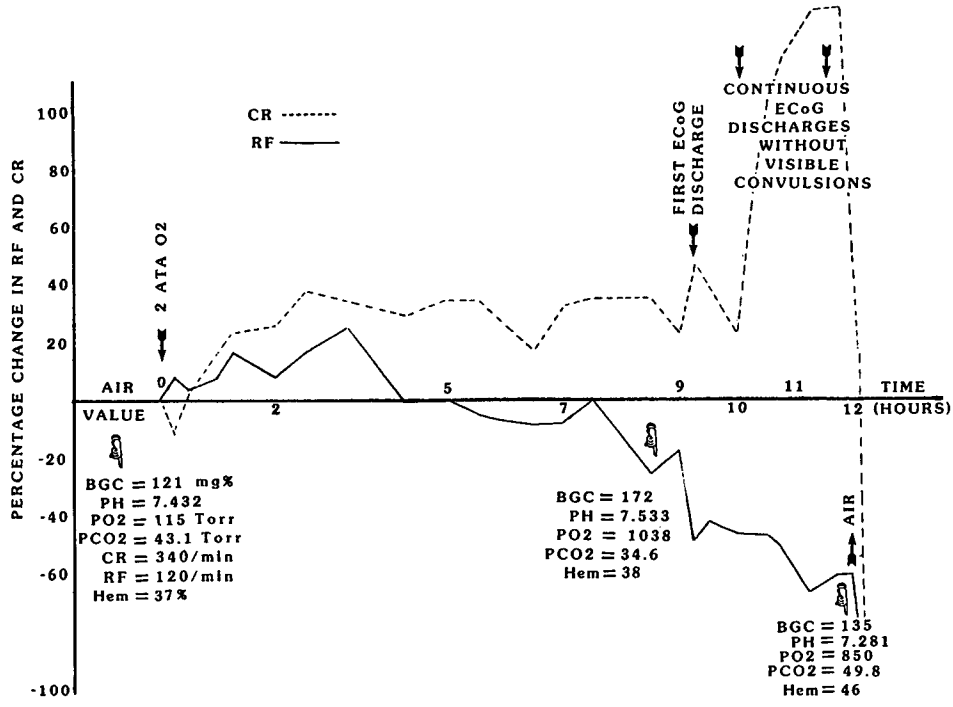

Fig. 4. Multiparameter monitoring of ECoG, ECG, respiratory frequency (RF), hematocrit (Hem), blood glucose (BGC), and blood gas composition in a conscious, unrestrained rat during continuous exposure to 2 ATA O_2 for 12 h. Successive paroxysmal electrical discharges in the ECoG can develop at this pressure during development of severe respiratory distress, without visible motor manifestation of CNS oxygen toxicity. Respiratory frequency and respiratory distress were continuously monitored by a conductive gel-in-rubber gauge secured around the rat thorax (Dan Torbati and D. Floris, unpublished data). For sequential changes in the above parameters at 1, 3, and 5 ATA O_2 see ref (20).

correlates of HBO exposure, and excellent reviews have been published (10, 12, 14, 54, 63, 80-82). A great number of functional compounds, especially enzymes involved in carbohydrate metabolism and structural components such as membrane unsaturated fatty acids, are among the sensitive targets of HBO (80-84).

Many biochemical studies concerning the effect of HBO on the brain have been focused on the relationship between energy metabolism and development of CNS oxygen toxicity on the one hand (14, 54, 61-65, 80-82, 85-88), and the effect of HBO on the neurotransmitters on the other hand (17, 63, 65, 70, 89, 90). In this report, only the main conclusions and controversies will be discussed briefly.

ENZYME INHIBITION THEORY OF THE CNS OXYGEN TOXICITY

The classic works on the effects of HBO on metabolism of brain and other organs were carried out simultaneously by Stadie et al. (9, 82) and Dickens (80, 81) in 1945. Stadie and coworkers found loss of metabolic activity in several organs, especially the brain. They pointed out however that the loss of metabolic activity under HBO develops slowly and therefore plays little or no role in the acute phases of oxygen poisoning or in the early death of intact experimental animals (85). One of the striking findings by Stadie et al. (9) regarding the brain oxidative metabolism was the lack of change in the rate of oxygen uptake in brain slices taken from rats preexposed to 8 ATA O_2 for 30 min. In these experiments, the pressure and duration of exposure were sufficient to produce many neurologic manifestations of oxygen toxicity, and in some cases, death.

Dickens (80, 81) worked on brain metabolism and tissue enzymes using brain slices at different oxygen pressures. He demonstrated that in brain slices exposed to HBO, glucose, lactate, and pyruvate oxidation is almost abolished, whereas the oxidation of succinate and glutamate was not changed. Dickens concluded that all stages of glucose oxidation, with the exception of the succinate stage, and specifically the enzyme pyruvate oxidase stage are poisoned, and that respiration as well as glycolysis are affected. However, these enzymatic changes failed to indicate a sufficiently rapid time course to explain a much more rapid incidence of visible manifestations of in vivo oxygen toxicity. These classic studies led to the formulation of the "enzyme inhibition theory" of oxygen toxicity (12, 54).

More than a decade later, Kaplan and Stein (91, 92) investigated the effect of HBO on the uptake of potassium, sodium, and glutamate in guinea pig brain and kidney cortical slices. They found that at pressures exceeding 4 ATA O_2, the brain cortical slices lost considerable quantities of K^+ and their ability to remove glutamate was impaired. Based on these observations they suggested that HBO exerts its toxic effect on tissue by reducing the energy available for the establishment of chemical gradients across cell membranes that are essential for normal functioning of these cells (93).

Sanders et al. (93) studied the effect of 1, 3, and 5 ATA O_2 on ATP concentration in the rat brain, liver, and kidney. The concentration of ATP in the brain was reduced by 57% after 90 min exposure at 1 ATA O_2; by 34% at 3 ATA; and by 55% at 5 ATA. In these experiments the results at 5 ATA O_2 were achieved during oxygen-induced convulsions, with the animals frothing at the mouth and gasping deeply (93). Sanders et al. supported the suggestion of Kaplan and Stein (91) that HBO exerts its toxic effect by reducing available energy. In another study, Sanders and Hall (94) found that cerebral hemisphere from animals exposed to 5 ATA O_2 for 90 min showed no decrease, but rather an increase in oxidative phosphorylation when succinate and α-keto-glutarate were used as substrates.

Brue et al. (95) studied glucose metabolism in rat and guinea pig brain

and heart sections and in bovine retinas under 1, 3, 6, and 10 ATA O_2 using $^{14}CO_2$, confirming "the classical inhibition of glucose utilization." They also noted an increase in pentose cycle activity in all tissues, while glucose consumption increased only in retinal tissue. These investigators concluded that neither the stimulation of glycolysis nor the phosphate pentose cycle can be considered the mechanism of cellular oxygen toxicity (95).

ENZYME MODULATION THEORY OF CNS OXYGEN TOXICITY

In 1965, Gottlieb (11) suggested that oxygen toxicity does not necessarily have to be due to enzyme inhibition. This idea was later supported by experiments by Gottlieb et al. (96, 97) and by Hemrick and Gottlieb (98), demonstrating that in vitro as well as in vivo oxygen, depending on its partial pressure, exerts activating or inhibitory effects on Na-K-ATPase derived from several tissues. Based upon these experiments, Gottlieb and co-workers (97) proposed an alternative metabolic hypothesis for CNS oxygen toxicity involving induction of Na-K-ATPase activity at some levels of HBO. Since the primary role for maintaining electrolyte balance is assigned to the Na-K-ATPase, or sodium pump, modulation of its activity might affect neural conductivity. Furthermore, it is recognized that a large proportion of cellular energy production is used for membrane transport system through Na-K-ATPase (99).

Studies by Chance et al. (100) provided a biochemical basis for the short-term effects of oxygen in terms of its influence on the intracellular oxidation-reduction states of pyridine nucleotide. Using mitochondria from several sources, they demonstrated an early oxidation of the reduced pyridine nucleotide by exposure to HBO. The observed oxidation of pyridine nucleotide in in vitro systems was also detected in in vivo systems in brain, kidney, and liver of anesthetized rats.

Mayevsky et al. (101) measured pyridine nucleotide oxidation fluorometrically in unanesthetized rats exposed to 6 ATA O_2. They found an increase in pyridine nucleotide oxidation of greater than 14%, which may be a manifestation of increased energy metabolism. The waves of increased oxidation were observed between bursts of seizure activity and were similar to those seen when KCl was applied to the surface of the cortex. They are thought to be due to K^+ leakage from the cells into the extracellular space. In another study, Mayevsky (102) used a multiparameter monitoring approach in awake rats exposed to HBO. The cortical tissue Po_2, NADH redox state, extracellular K^+, and ECoG were recorded simultaneously. The main events that occurred during the preconvulsive stage were activation of ECoG, oxidation of NADH, and a small increase in extracellular K^+. During the convulsive period, the high extracellular K^+ was pumped back into the cells, indicating that the ATPase system was activated rather than inhibited (102).

Nolan and Faiman (103) studied the effect of HBO on cerebral cortical ATP, phosphocreatine, lactate, pyruvate, and glucose. They found no changes in ATP concentration, and neither lactate nor pyruvate levels differed from

control levels before or during the onset of convulsions. They concluded, therefore, that oxygen-induced convulsions are not the result of either a fall in high-energy phosphates or an inhibition of their synthesis (103). Faiman et al. (63) also studied the correlation between development of oxygen toxicity and alteration in ATP, phosphocreatine, GSH-GSSG, and GABA. They concluded that oxygen-induced convulsions do not seem to be correlated with changes in pyridine nucleotide oxidation, ATP, or phosphocreatine. Furthermore, cerebral GSH-GSSG is not altered in oxygen-exposed mice, lipid peroxidation does not occur, and brain GABA levels per se do not seem to influence the susceptibility of mice to oxygen convulsions. Dircks and Faiman (57) investigated also the in vitro effect of HBO on free radical formation, lipid peroxidation and K^+, Na^+, and Ca^{++} ion flux in rat and mouse cerebral cortex exposed to 1, 3, 6, 9, 12, or 14 ATA O_2. Although the levels of lipid peroxidation in mouse cortex were significantly higher than those in rat cortex at each oxygen pressure, the degree of peroxidation became significant only at extreme pressures of 9 ATA O_2 or more. These investigators concluded that it was impossible to correlate free radical formation and lipid peroxidation with onset of seizure in intact animals.

In contrast, Kovachich and Mishra (104) have demonstrated an extensive lipid peroxidation in brain slices even under standard incubation conditions of 1 ATA O_2. Noda et al. (18), studying in vivo lipid peroxide formation and distribution in the brain of rats exposed to 5 ATA O_2 for 20 min, indicated a significant lipid peroxidation in various neuroanatomic structures. The pentose shunt producing NADPH may oppose lipid peroxidation (95). If these processes and other antioxidant defense mechanisms are not adequate to prevent extensive lipid peroxidation, the plasma membrane integrity could be damaged, ultimately affecting the Na-K-ATPase activity. However, Kovachich et al. (105) and Kovachich and Mishra (106), unlike Hemrick and Gottlieb (98), have demonstrated only an inhibitory effect of O_2 on Na-K-ATPase derived from outer layers of rat cerebral cortex in in vitro and in vivo systems at 1 and 4 ATA O_2, respectively. In conclusion, it is conceivable that this membrane-bound, pressure-sensitive, and SH-containing key enzyme could be either stimulated, inhibited, or modulated, depending on Po_2 levels and location in the brain (16). The results of our in vivo experiments on regional cerebral metabolic rate of glucose (rCMRgl), obtained at various pressures and durations of HBO exposures, may provide some indirect support for such a variable activity as described below.

SEQUENTIAL ALTERATIONS IN GLUCOSE METABOLISM DURING HBO

Many of the above discrepancies may be because studies were often carried out in different species or locations in the brain, using various pressures and durations of HBO. Furthermore, the gross and visible manifestations of CNS oxygen toxicity, such as convulsions, were usually chosen as

the indicators of toxicity. Oxygen-induced convulsions however, are actually the final functional expression of the physicochemical disorders that have already taken place in the brain and/or are in progress (2, 39, 51). The biochemical changes observed during HBO, in both in vitro and in vivo studies, actually occur at different times, pressures, and space. The molecular bases of oxygen toxicity should be sought in cellular or organelle levels; however, for practical purposes, determination of sequence of the harmful toxicity events is extremely important in intact and integrated biological systems (16).

To trace the sequential effect of oxygen on CNS metabolic functions at the organismal level, we developed an awake, unrestrained rat model in which the rates of glucose utilization could be measured simultaneously in various neuroanatomic structures during ongoing exposure to HBO or normoxic pressure (51). In the experiments described below we measured rCMRgl at a wide range of oxygen pressures (7% at 1 to 5 ATA pure oxygen and 5 ATA normoxia), during "asymptomatic" periods of exposure, and during different stages in the development of neurologic and respiratory manifestations of oxygen toxicity.

GLUCOSE UTILIZATION DURING CNS OXYGEN TOXICITY

As illustrated in Fig. 1, a gradual reduction in cortical tissue Po_2, without changes in cortical blood flow, may exist before the development of the ECoG manifestations of CNS oxygen toxicity. Such a relationship between cortical tissue Po_2 and blood flow, in the absence of CNS or pulmonary signs of oxygen toxicity (possible effect on Pa_{O_2}), could be interpreted as the result of an increase in tissue oxygen consumption (32, 36, 40). To test this hypothesis, the rCMRgl as an index of metabolic rate for oxygen was determined before, during, and after development of CNS oxygen toxicity in conscious rats exposed to 3 and 5 ATA O_2 (51-53). The rCMRgl was measured using [^{14}C] 2-deoxyglucose technique (107) and was correlated with changes in ECoG, as shown in Fig. 3. A summary of the rCMRgl data is presented in Tables 1 and 2.

The results of measurement of rCMRgl during two consecutive preconvulsive periods (before and during successive FED) indicated that the metabolic response of various neuroanatomic structures to HBO varied and was time dependent (Table 1). During 30 min of "asymptomatic" exposure at 5 ATA O_2, a significant increase in rCMRgl was observed in the majority of the brain structures investigated, while no obvious changes in ECoG were recorded (51). On the other hand, significant increases and decreases in rCMRgl were observed concurrently in different neural structures during preconvulsive successive paroxysmal electrical discharges and oxygen-induced convulsions (51, 52). This extreme redistribution of glucose utilization rates is illustrated in Fig. 5.

The outstanding changes in rCMRgl during neurologic signs of oxygen toxicity included significant increases in the globus pallidus and substantia

Table 1
Summary of Changes in Brain Glucose Utilizations and ECoG in Several Groups of Conscious Unrestrained Rats Before, During, and After Development of the Neurologic Signs of Oxygen Toxicity at 5 ATA O_2 and During Normoxic Exposures at 1 and 5 ATA. Data Derived from Torbati et al. (51).

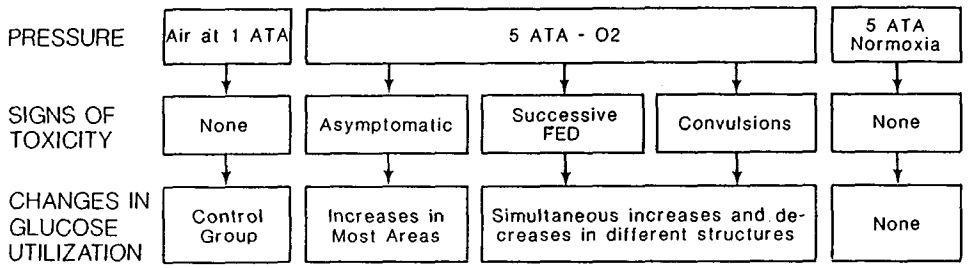

Table 2
Summary of Changes in Brain Glucose Utilization, ECoG, and Respiratory Distress in several Groups of Conscious, Unrestrained Rats Before, and During Development of the Neurologic Signs of Oxygen Toxicity at 3 ATA O_2.*

Exposure time, h	1	2	3.5 "Resistant"	3.5 "Sensitive"
Significant increase in rCMRgl	5	1	0	8
Respiratory distress	None	None	None	Decreased Respiratory Frequency
ECoG Changes	None	None	None	Slow Wave Activity Electrical Discharges

* No changes in any of the measured parameters was found in normoxic groups at 1 and 3.5 ATA for an equivalent period of time. Data derived from Torbati et al. (33), Torbati and Lambertsen (34, 53). No significant decreases in rCMRgl were found in any of the 28 structures examined during these preconvulsive periods of exposure. See text for definitions.

nigra, and significant reductions in cortical structures and auditory nuclei (51, 52) (Fig. 5). The large increases in the globus pallidus and substantia nigra are consistent with some pathologic changes observed in these structures after

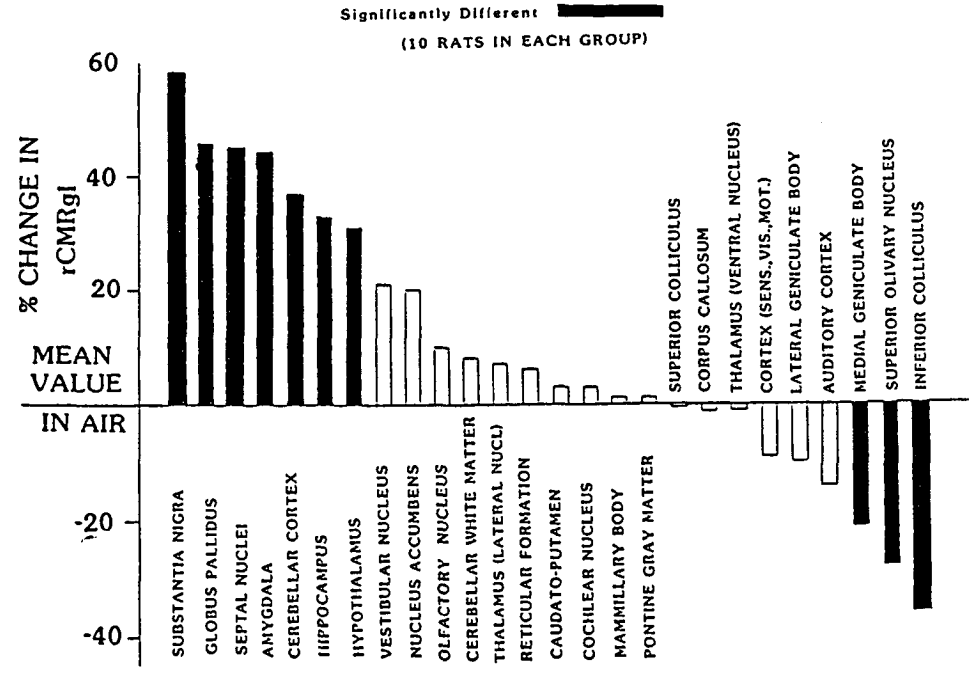

Fig. 5. Average changes in the rCMRgl of several neuroamatomic structures during appearance of the preconvulsive successive paroxysmal electrical discharges in a group of conscious, unrestrained rats exposed to 5 ATA O_2. Data derived from Torbati et al. (51).

severe CNS oxygen toxicity (108). The HBO-induced alterations in rCMRgl were rapidly reversible if the exposure to 5 ATA O_2 was interrupted immediately after the appearance of the FED (51). This may also indicate that the decompression had no apparent effect on rCMRgl.

Assuming that the preconvulsive increase in rCMRgl represents increased brain energy metabolism, these results are consistent with our prediction of a possible increase in oxidative metabolism of the brain during HBO (32, 36, 40). Furthermore, the lack of decrease in rCMRgl before the onset of the FED may indicate that the appearance of the electrical manifestations of oxygen toxicity in the brain are not due to a reduction in brain energy metabolism (51). The mechanism of changes in rCMRgl during development of CNS oxygen toxicity could be related to the membrane lipid peroxidation (18, 104), resulting in increased membrane permeability (109). These events may lead to increased extracellular K^+ accumulation (91, 92, 101, 102), with resultant activation or modulation of Na-K-ATPase (96-98, 105, 106).

The results of measurement of rCMRgl at 3 ATA O_2, which requires longer exposures to produce toxic manifestations of oxygen toxicity than do exposures at 5 ATA O_2, enabled us to look at energy metabolic changes in a much slower rate, especially during "asymptomatic" periods of HBO exposure.

Thirty-to-sixty minute exposure to 3 ATA O_2, which has on average a preconvulsive period of 4 h (40), produced a significant increase in rCMRgl in 5 of the 28 neuroanatomic structures examined (34). Most of these increases disappeared during a longer exposure of 120 to 150 min at the same pressure; only the thalamic nuclei still showed a significant increase in glucose utilization under these conditions (33). Since alterations in ECoG activity in conscious rats are likely to occur about the 3rd h of exposure at 3 ATA O_2 (40), the rCMRgl was also measured during 180 to 210 min of exposure (53). According to the ECoG responses the oxygen-exposed rats fell into two different categories: a) rats that did not show changes in ECoG pattern before or during rCMRgl measurement (i.e., no increase in slow wave activity and/or occurrence of FED), considered to be "oxygen-resistant"; b) rats that showed either increased ECoG slow wave activity or developed paroxysmal electrical discharges before and/or during rCMRgl measurement. This category was regarded as "oxygen-sensitive" (Table 2). The "oxygen-resistant" rats did not show significant changes in rCMRgl in any investigated structure, whereas the "oxygen-sensitive" rats showed significant increases in 8 of the 28 neuroanatomic structures as compared to "oxygen-resistant" and normoxic groups at 1 and 3 ATA for the same period of time (53). The lack of change in rCMRgl of the "oxygen-resistant" rats during a prolonged exposure at 3 ATA O_2 may indicate that the occurrence of an earlier increase in glucose utilization should be considered only a transient physiologic reaction. The changes in rCMRgl in the "oxygen-sensitive" rats at 3 ATA O_2 were generally similar to those observed during the preparoxysmal electrical discharge period at 5 ATA O_2, in which only increases were observed in the same structures (51). The distribution patterns of rCMRgl during these early preconvulsive signs of CNS oxygen toxicity, however, were different from those observed during post-FED, as well as during oxygen-induced convulsions at 5 ATA O_2 (51, 52). The simultaneous occurrence of increases and decreases in rCMRgl during advanced periods of CNS oxygen toxicity (Fig. 5) may imply that the overt oxygen-induced convulsions are not the results of either an increase or decrease in brain energy metabolism, but a functional disorder created by both.

rCMRgl CHANGES DURING SHORT AND LONG EXPOSURES TO 1 AND 2 ATA O_2

The changes in the rate of glucose utilization during "asymptomatic" exposure before development of CNS oxygen poisoning led us to investigate systematically the possible changes in rCMRgl at lower pressures of 1 and 2 ATA O_2, which are extensively used for HBO therapy and diving. The rCMRgl at these pressures was measured for durations in which no obvious signs of any type of oxygen toxicity were present, as well as during prolonged exposures with mild respiratory manifestations of oxygen toxicity (33, 34, 79, 110-112). A summary of rCMRgl data obtained during various durations of exposure and conditions at 2 ATA O_2 is presented it Table 3.

Table 3

Summary of Changes in rCMRgl in Several Groups of Rats Exposed to 2 ATA O_2 During Asymptomatic Exposures After Development of Respiratory Distress, Following Intermittent Exposures, and During Pentobarbital Anesthesia. Data derived from Torbati et al. (33), Torbati and Lambertsen (34), Torbati and Lambertsen (110), and Torbati (111, 114)

EXPOSURE (HOURS)	0.5	1	4	8	4-INTERMITTENT 1-HOUR O 2 3-HOURS AIR INTERVAL	1 ANESTHETIZED PENTOBARBITAL
INCREASES IN rCMRgl	NONE	14/28 LIMBIC, AUDITORY, VISUAL & CORTICAL STRUCTURES	2/28 INFERIOR COLLICULUS & SUPERIOR OLIVARY NUCLEUS	NONE	NONE	NONE
SIGNIFICANT DECREASES IN rCMRgl	NONE	NONE	NONE	NONE	2/28 INFERIOR COLLICULUS & SUPERIOR OLIVARY	1/28 SUPERIOR OLIVARY NUCLEUS

The first 30-min exposure at 2 ATA O_2 produced no significant changes in the 28 neuroanatomic structures examined (111), indicating that HBO has no immediate effect on brain glucose metabolism. A consecutive 30 to 60 min exposure at this pressure, on the other hand, produced significant increases in rCMRgl in 50% of the neuroanatomic structures investigated (34). Most of the rCMRgl values obtained after longer asymptomatic exposure of 4 h had reversed to control levels, showing significant increases only in two auditory nuclei (33) (Table 3). Thus, at this pressure, as at 3 ATA O_2, a transient increase in rCMRgl exists without any obvious neurologic signs of oxygen toxicity. It is worth mentioning that four 1-h intermittent exposures to 2 ATA O_2, with 3-h air intervals, which are known to significantly increase tolerance and survival to oxygen toxicity (113), entirely abolished the rCMRgl increases observed in single exposures of 1 and 4 h, producing even some reduction in auditory nuclei (110).

The pentobarbital anesthesia also had the same effect in eliminating any increases in rCMRgl that occurred after 30 min exposure to 2 ATA O_2 (114). A prolonged 8-h exposure to this pressure, which was capable of producing significant reduction in respiratory frequency and initiating respiratory distress, did not produce any change in rCMRgl as compared to normoxic groups at 1 and 2 ATA for the same period (79). These negative findings may indicate that the respiratory system at 2 ATA O_2 is affected independently from CNS in conscious rats. However, as mentioned before, electrical manifestations of CNS oxygen toxicity could also occur at this pressure in much longer exposures with severe respiratory distress (Fig. 3).

The effect of 6 h breathing oxygen in conscious rats exposed to 1 ATA O_2 was negligible, increasing the rCMRgl only in the superior olivary nucleus (33). Twenty-four hour exposure at this pressure, which produced characteristic reduction in the respiratory frequency did not create any significant changes in 29 neuroanatomic structures examined, including those possibly involved in the generation of respiratory rhythm and pattern (112).

EFFECT OF HYPOXIA ON rCMRgl

The involvement of oxygen radicals has also been suggested in hypoxic and ischemic brain trauma (58). To compare the changes in rCMRgl during hyperoxic exposure with those during hypoxia, conscious rats were exposed to 7% O_2 with nitrogen at 1 ATA for 30 min (115). Statistically significant increases in rCMRgl of 39 to 95% were observed in 25 of the 28 neuroanatomic structures examined. Unlike the results found with hyperoxic exposure, the highest increases in rCMRgl during hypoxic exposure were obtained in cerebral and cerebellar white matter (95 and 60%, respectively). The distribution patterns of rCMRgl, at least in 50% of structures, was also different from those observed during development of CNS oxygen toxicity or during "asymptomatic" exposure at different pressures. These differences may indicate that both metabolic changes and the origin of the neuroanatomic structures involved

in hyperoxic and hypoxic exposures are probably different. The increases in rCMRgl during hypoxia are known to be due to an increase in glycolysis (116), whereas the increases and decreases obtained in several periods of HBO could be related to changes in oxidative metabolism (16, 36). In summary, although the oxygen free radicals might be generated during both hypoxia and HBO, their effect on energy metabolism of different neuroanatomic structures is not the same.

EFFECT OF HBO ON NEUROTRANSMITTERS

Among the vast variety of HBO-sensitive compounds in the CNS, the neurotransmitters that are responsible for transferring stimuli among neuronal elements themselves and with other reflectory systems have been regarded as sites sensitive to the toxic effects of oxygen (17, 65-70, 89, 117-121). Extensive physiologic, pharmacologic, and biochemical studies have been focused on the effect of HBO, especially on amino acid neurotransmitters that involve both inhibitory and excitatory functions of the CNS. The process of chemical transmission involves synthesis, degradation, storage, release, reuptake, and receptor binding (17). The normal functioning of any of these systems requires the integrity of the membrane structure and function. The toxic effect of HBO on any of these processes may eventually lead to malfunctioning of the entire CNS by producing imbalance between the excitatory and inhibitory neurotransmission (17, 117, 120). The implications of such oxygen-induced damage to neurotransmitter systems have been investigated by several groups (17, 58, 67, 68, 89, 120) and will be discussed briefly below.

EFFECT OF HBO ON AMINO ACID NEUROTRANSMITTERS

Amino acid neurotransmitters, both in brain tissue and in nerve endings, include glutamate, aspartate, GABA, glycine, and taurine (17). Among these neurotransmitters, the level of GABA concentration has been shown to be correlated with the onset of oxygen-induced convulsions (117). as an inhibitory transmitter, GABA is necessary for maintaining a proper balance of excitatory and inhibitory reactions in certain neuroanatomic structures. It is therefore, reasonable to assume that HBO-induced damage to the GABA system and reduction in its synthesis will produce hyperexcitability, leading to seizures (17). GABA is formed by the GABA shunt, a bypass in the citric acid cycle. Glutamic acid as a precursor is transformed by action of GAD to GABA (122). Studies on the enzymes involved in biosynthesis and degradation of GABA indicate that GAD is among the components of this neurotransmitter system that are most sensitive to HBO-induced inhibition (68, 119, 123, 124). It is postulated that HBO causes oxidation of -SH groups in the GAD molecules, resulting in inactivation of its biosynthetic function (17). The experimental evidence for the above hypothesis shows that a decrease in GABA in different species is associated with an increased susceptibility to the toxic effects of

oxygen as tested by shortening of the preconvulsive interval (65, 66, 117, 120, 125). However, it has been shown that oxygen-induced convulsions can also develop when GABA levels are higher than normal (126). Faiman et al. (63) were unable to show a clear relationship between GABA content and oxygen-induced convulsions. Although these controversial data cannot yet exclude a direct role of GABA content in the generation of the neurologic manifestation of oxygen toxicity, they do at least indicate that GABA concentration is related to an overall derangement in CNS metabolism by HBO (14, 16), and certainly has a role in prevention of the late motor manifestations of CNS oxygen toxicity.

The involvement of other neurotransmitters such as catecholamines in susceptibility of the CNS to oxygen toxicity has also been investigated (118, 121). It has been suggested that an increased sensitivity to HBO, as evidenced by increased levels of plasma and brain catecholamines, is related to an overall enhancement of metabolism and stressful reactions (16).

MODIFICATION OF CNS OXYGEN TOXICITY

Many hemodynamic, pathophysiologic, endocrinologic, environmental, and pharmacologic factors are known to affect the time course of development of oxygen toxicity (2, 8, 21, 127-134). In general, the variation in natural resistance to oxygen toxicity among different species is wide and is influenced by such factors as age, sex, diet, body temperature, and the level of basal metabolism (2, 8, 127-129, 135).

Hemodynamic and Pathophysiologic Factors

The role of arterial CO_2 retention in the etiology of CNS oxygen toxicity was discussed in a previous section (45, 47, 48). HBO exposure is usually associated with an acute increase in arterial blood pressure and a decrement in cardiac output (8, 22, 136, 137). Due to cerebral circulatory autoregulation, these hemodynamic factors may not immediately affect the sensitivity of the brain to HBO. However, a characteristic increase in hematocrit level and oxygen-induced hypercoagulability (138) may modify the time course of CNS oxygen toxicity, as demonstrated in our studies using heparin as an anticoagulant agent (139).

Among other important biochemical changes that may take place during HBO is an increased concentration of ammonia and histamine in the blood and brain of intact animals (125, 140, 141). These compounds are known to change the permeability of the blood-brain barrier (BBB) (142-144). Changes in the BBB may affect the naturally protected microenvironment of the neuronal elements, thereby negatively contributing to the development of the neurologic manifestations of oxygen toxicity. Recent studies, however, indicate that the BBB in conscious rats exposed to HBO was not affected, at least up to the development of the FED (145).

Endocrinological Factors

Hormonal changes have a significant effect on tolerance to oxygen toxicity (75, 76, 146-152). The effect of thyroid hormones in enhancing the susceptibility of the animals to oxygen-induced convulsions is well documented and is thought to be related to an increased level of oxygen consumption (146). Lack of hypophyseal and adrenocortical and adrenomedullary hormones in hypophysectomized and adrenalectomized rats was shown to reduce susceptibility to HBO, as determined by the increase in the time required for the appearance of the motor manifestations of CNS oxygen toxicity (147-152). Using changes in ECoG as a more precise criterion for the determination of the early signs of CNS oxygen toxicity, we were able to demonstrate only a transient resistance in conscious, adrenalectomized rats subjected to single or repeated HBO exposure (75, 76, 151). In these experiments, the temporary resistance to the toxic effects of HBO in the brain lasted for 2 to 3 d postoperatively, and the same rats became gradually more sensitive up to 22 d after adrenalectomy (151). It has been suggested that the early protection afforded by adrenalectomy is probably due to elimination of adrenocortical and especially adrenomedullary hormones (148). Hence, the effects of stressful conditions in accelerating the time course of CNS oxygen toxicity can be attributed to increased secretion of adrenomedullary catecholamines as well as to activation of the hypothalamo-hypophyseal and adrenocortical systems (147, 148, 150, 153). The protective effect of beta blocker against early and advanced neurologic manifestations of oxygen toxicity provides indirect evidence on the possible role of medullary hormones in the development of brain oxygen toxicity (129, 154, 155).

Environmental Factors

Among the environmental factors affecting CNS tolerance to HBO, the effects of light, noise, temperature, and stressful situations such as handling of animals are well known (128, 129, 135, 156). Although most of these environmental factors may exert their influence through the general stress reaction, some of them could also be related to the effect of sensory stimulation in the brain (156).

Pharmacologic Agents

Many drugs and natural antioxidant agents are known to delay or modify the duration of tolerance to HBO (125, 131-134, 140, 154, 155, 157-160). The effect of these agents is generally based on protecting sensitive enzymes, reducing basal metabolism, or preventing lipid peroxidation (131-135, 157-161). In any case, if the invisible signs of CNS oxygen toxicity are not properly monitored, such as by ECoG recording (16, 40, 75-77) and/or biochemical measures (68, 162), the physicochemical process of toxicity could proceed to irreversible stages without revealing any external warning signs (16). The ordinary, visible manifestations of oxygen toxicity in experimental animals may be undetected due to a masking effect of some "protective"

agents. In man, however, several well-defined, preconvulsive signs and symptoms of oxygen toxicity, such as nausea, mental confusion, dizziness, twitching, etc., do exist; they all should be considered as signals to terminate HBO exposure (2, 163). In some cases, the overt generalized convulsions could be the first sign of CNS oxygen toxicity (2). So far, the safest method for extension of tolerance to the CNS effects of HBO seems to be intermittent HBO exposures with normoxic intervals (113).

HBO THERAPY AND THE CNS

Oxygen is not only the key element in any future diving technology, but it also is used for treatment of decompression sickness, especially with type II which involves neurologic disorders (164, 165). Many other neural diseases have also been treated by HBO with varying degrees of success (166-170). The acute physiologic effect of HBO of producing vasoconstriction (24, 31), and at the same time improving tissue oxygenation (35-37) and probably brain glucose utilization (33, 34), could be useful in reducing brain edema (144, 169, 171) and treating spinal cord injuries (167, 168), multiple sclerosis (170), and other neurologic problems (7, 166).

SUMMARY AND CONCLUSION

The physiologic and toxic effects of oxygen on the CNS during hyperbaric exposure at a wide spectrum of pressures and durations in man and other mammals were reviewed.

Alterations in cerebral, circulatory, metabolic, biochemical, and electrical activity before, during, and after progressive development of oxygen toxicity in the CNS were described and their involvement in the appearance of the neurologic signs of oxygen toxicity was evaluated.

The biochemical changes in the energy-producing pathways and neurotransmitter systems that may be involved in the basic mechanisms of neural oxygen toxicity were reevaluated.

The "oxygen free radical" theory was explained as a tentatively acceptable explanation for molecular oxygen toxicity, and the metabolic and neurotransmitter hypotheses of the cellular mechanisms of CNS oxygen toxicity were compared.

New data that were provided differentiated between oxygen effects in the CNS and in the respiratory system during prolonged exposure to 1 and 2 ATA O_2 in conscious rats.

The energy metabolic changes in the brain at various durations and pressures of HBO exposure were compared with those obtained during a hypoxic exposure of 7% O_2 at 1 ATA and no similarity between them was found.

The internal and external factors modifying the tolerance to HBO were described, and the application of HBO therapy for CNS disorders was

summarized.

It has been concluded that although the molecular basis of oxygen toxicity should be sought at cellular and organelle levels, for practical purposes integrative studies in intact organisms are required. Hence, simultaneous monitoring of cerebral, electrical, circulatory, and energy-producing functions is a useful tool for determining safety margins of HBO, as well as for tracing the primary mechanisms of oxygen toxicity in the CNS.

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OCULAR HYPERURICOSIS INDUCED BY HYPERBARIC OXYGEN

W. J. Ehler, C. H. Bonney, and K-W Lam

Following multiple exposures to hyperbaric oxygen (HBO) the eyes of many patients have been reported to develop myopia, whereas others have developed myopia and nuclear cataracts (1, 2). The myopia is an immediate response and is considered to be due to reversible changes in the lens. The nuclear cataract development is a delayed (6 mo. to a year) phenomena (1). Experimentally, exposure to HBO has produced changes in corneal endothelium in guinea pigs (3), and lens epithelial changes in both guinea pigs and mice (4).

Maintenance of intraocular pressure and nutrition of the cornea and lens are dependent on the formation and composition of the fluid filling the anterior segment, the aqueous humor. In this study, aqueous humor was taken from rabbits after exposure to HBO and analyzed for levels of ascorbate and uric acid.

METHODS AND MATERIALS

New Zealand white rabbits in a weight range of 2 to 3 kg were used in this study. Twelve rabbits were used in the control group and were exposed to ambient air only. Fifteen rabbits were used in the treatment group. The treatment group was exposed to 2.4 ATA of 95 to 96% oxygen for 90 min for a total of 60 dives.

The rabbits, in individual wire cages, were placed into a Rheem diving chamber. No medications were administered. The chamber was flushed with 100% oxygen until an oxygen concentration of 95 to 96% was reached. It took approximately 25 to 30 min to attain this concentration. When the final oxygen concentration was reached the chamber pressure was increased to 45 fsw (13.72 m), and the timing of the dive began. Oxygen concentration within the

chamber was monitored with a Beckman oxygen analyzer. Carbon dioxide levels within the chamber were also monitored using a Beckman carbon dioxide analyzer. The relative humidity within the chamber was kept in the range of 35 to 60%. After a 90-min exposure to HBO, the chamber was depressurized in approximately 10 min. This procedure was repeated daily, 5 d/wk, until a total of 60 dives had been conducted. No animal received more than 1 dive/d.

Collection of aqueous humor samples was conducted in the following manner: anesthesia was induced with an intramuscular injection of ketamine (200 mg/kg), topical anesthetic was applied to the corneas (1% proparacaine). Samples were collected by paracentesis, under a Zeiss surgical microscope at ×25, using a 30-gauge needle attached to a 1-ml syringe. Aqueous humor was taken from each eye, immediately placed on ice, and maintained separately for analysis. Thus, the statistical analysis of the control group was based on 24 samples and the treatment group on 30 samples.

The aqueous humor was analyzed for uric acid and ascorbate levels using high performance liquid chromatography (HPLC). The column was a Micro-Bondapak-NH_2 column for the separation of biochemical constituents in aqueous humor. The column was equilibrated in 10 m*M* $NH_4H_2PO_4$ and eluted with the same buffer. One microliter of standard ascorbate solution (1-2.0 mmol/liter) was injected into the column to identify the retention time of ascorbate, and the relationship of UV absorption peak height to ascorbate concentration. Based on experience, the UV absorbance is directly proportional to ascorbate concentration between 0.05 and 10.0 m*M*. An aliquot of 1 μl of aqueous humor was injected into the column and eluted in the same way as the standard. The eluate was analyzed at 254 and 280 nm by a UV monitor (Waters Associates, model 441). The UV absorption peak height in the location of ascorbate was compared to the peak height of the standard ascorbate solution to calculate the ascorbate concentration of the sample.

RESULTS

No animal was found to have lens opacities as viewed at the time of aqueous humor sample collection.

The control animals showed a mean ascorbate level of 22.83 mg/dl (± 5.48) as compared to the treatment group value of 19.46 mg/dl (± 6.75). The mean uric acid level for the control group was 0.12 mg/dl (± 0.103) and 0.30 mg/dl (± 0.19) for the treatment group. The control and treatment group means were analyzed using the two-tailed *t* test. No significant difference was found between the ascorbate levels for the control and treatment groups. The mean uric acid levels were found to be significantly different ($P < 0.001$).

DISCUSSION

The study was not designed to look for nuclear cataracts. HBO exposures, as used in this study, might show such changes if the animals were held for a

number of months.

Ascorbate

It is thought that the eye maintains its concentration of ascorbic acid through blood flow to the ciliary processes (5). In this study there was no significant change in the ascorbate level, indicating that the blood flow to the ciliary processes was not reduced appreciably.

Uric Acid

In man, uric acid is the final metabolic product of the metabolism of the purines, adenine, and guanine. These compounds may be found within cells linked to deoxyribose-5-phosphate, forming two of the four basic units of chromosomal DNA, and they combine with ribose-5-phosphate to form two of the structural units of RNA. Hormonal effects are mediated by cyclic AMP, which also contains these purines. Purines may be liberated by enzymatic degradation of tissue (6).

Normally, aqueous humor contains only trace amounts of uric acid (7). The initial report of significant levels of uric acid in aqueous humor was from eyes with glaucoma that had been treated medically for years before surgery (8). The questions raised by the discovery of uric acid in aqueous humor addressed the source of uric acid and its role in either the physiology of the eye, or the pathology of glaucoma. In an experimental model of hyperuricemia, Yonetani et al. (9) found evidence that production of uric acid was associated with stimulation of alpha adrenergic receptors by the systemic administration of epinephrine. Similar data for alpha receptors in the eye do not exist. The source of intraocular uric acid has been addressed in two experimental studies. In the first, hyperuricemia was produced in the rat and aqueous humor was sampled. No significant elevation of uric acid was found (10). A second study evaluated epinephrine topically applied to the cornea of rabbits (11). The rabbit aqueous humor samples were found to have a significant elevation of uric acid. These studies indicate a blood-aqueous barrier to uric acid and that the uric acid arises from reactions within the eye.

Uric acid within eyes of rabbits treated with HBO is a new finding. Aqueous samples were taken several hours after the last dive. The mean values reported in this study may represent lower figures than actually produced. A diminished value could be the result of either enzymatic activity of uricase, an enzyme not found in man, or from intraocular turnover. Washout of the uric acid by the constant formation and outflow of aqueous humor would produce a decay of the uric acid unless it was being added to the aqueous humor on a continual basis. In the case of HBO, it is not known if the appearance of uric acid is related to the increase in tissue oxygenation, or to a regional ischemia secondary to the oxygen vasoconstrictive effects on blood vessels and resulting decreased perfusion (12). Nor is it yet known if the production of uric acid is from altered biochemistry or from cell death. If it is the former, it is suspected that it would be a transient event but with perhaps permanent

ocular tissue changes.

In neither the pharmacologically produced nor HBO-produced ocular hyperuricosis is the presence of uric acid understood in terms of ocular physiology. The known changes, myopia and nuclear cataracts, may be related to changes in the composition of the aqueous humor.

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CENTRAL NERVOUS SYSTEM OXYGEN TOXICITY IN A CLINICAL SETTING

G. B. Hart and M. B. Strauss

Unfavorable reactions to a particular medical therapy should be avoided where practical, and reduced in occurrence when possible. The grand mal epileptiform seizure occurring with oxygen under pressure as initially studied by Bert (1) is distracting and avoidance is highly desired at this time.

The isolation and identification of oxygen was first reported by Priestly in 1775 (2), and independently by Scheele in 1777 (3). The first toxic effect was noted by Scheele in 1782 (4) on peas, which sprouted early in oxygen but failed to thrive. The first report of oxygen being toxic to animals was by Sequin and Lavoisier in 1789 (5).

This report reflects the incidence of grand mal epileptiform seizures in patients presented for treatment of certain disease states from December 1967 to January 1 1986 (Naval Regional Medical Center, Long Beach, CA), December 1967 to 1 August 1975, and Memorial Medical Center, Long Beach, CA, 1 December 1974 to 1 July 1986. The senior author started this clinical experience with protocols of treatment that were less than 3.0 atmospheres absolute (ATA) oxygen, as seizures are unlikely in exposures of 2 h or less (1, 6, 7).

The first applicant to be considered for the position of a full-time hyperbaric nurse in 1968 was administered an appropriate oxygen tolerance test of 2.8 ATA oxygen and developed a grand mal seizure after a 10-min oxygen exposure. She was found to be a known epileptic — having required anticonvulsants from childhood. This single occurrence stimulated the following study of all seizures occurring with oxygen under pressure at the above facilities.

SEIZURE PROTOCOL

Each seizure was studied by the following method: Patient was immediate-

ly compressed in the monoplace chamber (excepting past 4 yr, gas source was changed from oxygen to air) and the oxygen delivery system was removed. The patient was removed from the chamber and the following observations were made: blood pressure, pulse rate, respiration, and temperature.

These laboratory tests were performed on an emergency basis: complete blood count, arterial blood gases, and chemical profile (with particular emphasis on blood sugar levels in diabetics). The radiologic examination was limited to the chest unless there was a remaining neurologic deficit; then a CT scan of the brain was performed.

An electrocardiogram and an EEG were obtained immediately, and neurology consultation was obtained before further pressurization.

EXPERIENCE

Three thousand one hundred and sixty patients (2041 males and 1119 females) (average age 50 yr ± 22) were treated with an appropriate protocol for their respective diseases. There were 44 seizures in 32 patients (24 males, 8 females), average age 37 ± 18 (Table 1). The average seizure occurred during the eighth compression considering all seizures. Nine patients had seizures on the first compression. Predisposing factors are shown in Table 2.

Twenty-two seizures (50%) in 16 patients occurred in the 313 (10%) patients receiving HBO at greater than 2.0 ATA, namely 2.5 to 3.0 ATA. The remaining seizures, 22 in 16 patients, occurred in the remaining 2847 (90%) patients receiving HBO at 2.0 ATA or less. The majority of the seizures (35) occurred in the first half of this clinical experience (Fig. 1) in the initial 1000 patients treated.

Table 1
CNS Oxygen Toxicity

Disease	No. Patients/No. Treated	No. Seizures	Pressure ATA O_2
Clostridial myonecrosis	9/166	11	2.5
Burns	2/256	3	2.5
Air embolism	4/107	7	3.0 - 2.5
Anoxic encephalopathy	4/ 30	10	2.0
Radiation injury	2/367	2	2,0
Osteomyelitis	2/404	2	2.5 - 2.0
Spinal cord injury	3/156	3	2.0
Peripheral vascular insufficiency	2/326	2	2.0
CVA	1/244	1	2.0
Tay Sachs	1/ 1	1	2.0
Sickle cell disease	2/ 26	2	2.0

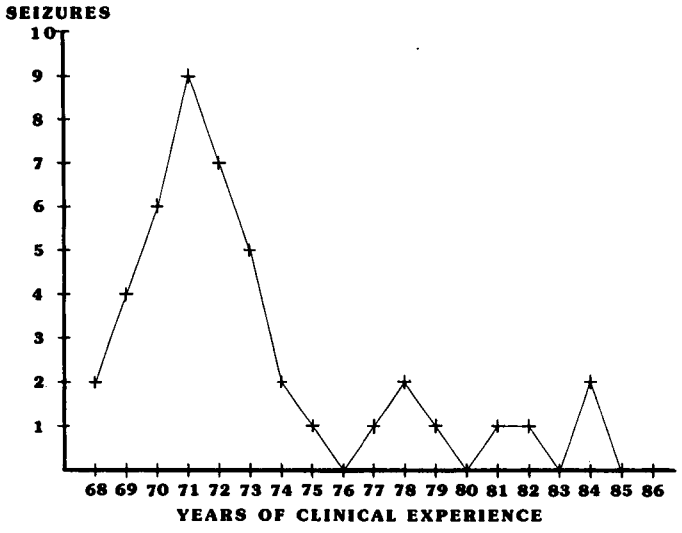

Fig. 1. CNS oxygen toxicity.

Six patients were febrile with temperature (rectal) of 102°F or greater. Five were suffering from gas gangrene and the other from burns. These six had tachycardia and tachypnea but were not hypotensive. Four patients, two with air embolism and two with anoxic encephalopathy, were bradycardic and hypertensive after seizures. These 4 demonstrated signs of increased intracranial pressures before and after treatments.

The chest x-rays and hemograms performed after seizures did not demonstrate any abnormalities that were not observed before seizures.

The EEG's revealed 38 of the seizures occurring in 26 patients to be true oxygen seizures, i.e., slowing of the wave forms bilaterally across the cerebral hemispheres; 6 patients had epileptic foci (3 were known epileptics and 3 were covert). Of 24 patients who were retreated, 12 additional seizures occurred in 12 patients. The 12 suffering a second seizure had been placed on dilantin 100 mg 3 times/d to control seizures. The other 12 patients that did not suffer a second seizure had received dilantin 100 mg 3 times/d and 65 mg phenobarbital 3 times/d before compression.

Eight patients were discovered to be acidotic (arterial pH less than 7.2) upon withdrawal from the chamber.

There were 315 patients with insulin-dependent diabetes mellitus and 18 of these suffered insulin reactions while undergoing HBO. Ten of these reactions occurred in the first 30 in the experience and were classically (15 of 18) in the late afternoon. In each case the patient became tachypneic, disoriented, and quickly (within 5 min) proceeded to a semicomatose or comatose state. After leaving the chamber, each was found to have tachypnea, tachycardia, and hypotension. Each was found to be hypoglycemic (lowest blood sugar, 30 mg/dl) and each responded to administration of a hypertonic glucose

Table 2
Seizures During First Compression

Disease	No. Patients	Predisposing Medications	Predisposing Condition
Air embolism	3	steroids	0
Anoxic encephalopathy	2	steroids	0
Gas gangrene	2	1-apresoline	1-acidosis
Sickle cell disease	1	acetazolamide	0
Peripheral ischemia	1	0	epileptic

solution. The insulin reactions occurred on the 8th d of treatment, ± 2 d. Care is now taken to time HBO after alimentation. This group is not included in the seizure group.

There were eight focal seizures occurring in faces, arms, or legs which are not included in this series because they occurred before and after HBO. They all occurred in patients with anoxic encephalopathy or cerebrovascular accidents.

CHARACTER OF SEIZURES OCCURRING WITH HBO

Seizures occur abruptly with a very short aura or none at all. Those with aura usually shout before the seizure. It is a grand mal epileptiform seizure and in 8 patients it commenced with a Jacksonian March in one or another extremity. They usually evacuate bowel and bladder (n = 32). Each has had retrograde amnesia after the seizure, but is fully aware that something exceptional has occurred. The postictal state was primarily one of somnolence. Thirty-two of the seizures occurred in the afternoon or evening.

MEASURES TO AVOID SEIZURES

The following preventive measures have been found to minimize the risk of HBO seizures.

1. Avoid treating patients who are febrile (>101°F[0]).
2. Avoid administering drugs that might lead to an increase of tissue CO_2, i.e., carbon anhydrase inhibitors, decarboxylase inhibitors, or high levels of narcotics.
3. Avoid the use of aspirin, ascorbic acid, and steroids.
4. Avoid photostimulation—fluorescent lights are not used directly on the patient.
5. Avoid the use of stimulants such as caffeine.
6. Avoid vasodilators where practical.

DISCUSSION

One patient with Tay-Sachs disease was accepted for HBO at the pleading of a local researcher in the field. However, HBO seemed to accelerate seizure activity rather than reduce it.

Four patients were felt to have had aurae, and the seizures were aborted by the chamber operators. Three patients had visual or auditory hallucinations and one had an anxiety reaction. The latter was consuming 20 cups of coffee per day and upon cessation had no further reactions. The 3 prior patients were retreated without incident, except that auditory hallucinations in 1 patient continued to occur, and upon investigation it was noted that freak electronics transmitted music into the audio system of the chamber. The 2 patients with visual hallucinations had previously used hallucinogenic drugs.

This review of our experience does not represent any concerted effort to achieve a controlled study. We initially used dilantin or dilantin plus phenobarbital to control seizures in the early patients (before 1975) who needed additional HBO. Since 1973, alpha tocopherol has been given with each HBO treatment.

Seizure activity per chamber compression has diminished over the past 18.5 yrs (Table 3). This change is attributed to careful screening of each patient and avoiding conditions or medications that place them at risk for CNS oxygen toxicity.

Table 3
Seizure Activity Per Patient Compressions

Year	Seizure/No. Patient Compressions
1970	1/ 385
1975	1/ 4285
1980	1/ 7500
1985	1/12253

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EVALUATION OF A SAFETY PROTOCOL FOR HYPERBARIC OXYGEN THERAPY BY MEANS OF THE ESTIMATION OF HYDROXYL RADICAL PRODUCTION

C. Akiba, K. Makino, A. Hyugaji, M. Kono, M. Shibayama, T. Nakayama, N. Doi, N. Takano, and Y. Mano

Interest in free radical metabolites of xenobiotics as possible toxic intermediates dates to the early 1940s. Most early speculations concentrated on the fact that some physical carcinogenic processes, such as ionizing radiation and UV light, produce free radicals. The first report about oxygen free radicals came from Gerschman et al. (1).

Although in recent years investigators have made considerable progress in resolving the properties of the enzymes superoxide dismutase (SOD), catalase, and glutathione peroxidase, the relationship between scavenger systems and oxygen free radicals in mammalian lung damage caused by hyperoxia is still imperfectly understood.

These enzymatic reactions are indirect evidence for the presence of the oxygen free radical species. Cellular damaging oxygen free radicals such as hydroxyl radical are probably produced in the epithelial membrane of the lung and caused by exposure to hyperbaric oxygen (HBO) (2), and the free radicals leak from the membrane to alter blood flow and lung permeability.

Recently, the method of spin trapping has been developed to identify short-lived free radicals. In this method, primary reactive oxygen free radicals react with the diamagnetic compound 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) to form stable nitroxide radicals (spin adducts) which can be detected and identified by electron spin resonance (ESR) spectroscopy (3). The spin-trapped adducts of DMPO are characterized by known hyper-fine coupling constants.

In our study we have observed hydroxyl radical spin adducts (DMPO*OH), DMPO*6 line spin adducts, as well as SOD activity on in vivo experiments with

exposure to 100% O_2 at 3 ATA and 4 ATA for 60 min by comparison with room air control. In addition, we report here in situ experiments with exposure to 100% O_2 at 5 ATA for 60 min.

Our results strongly suggest that the activation of hydroxyl radical generation during HBO exposure with lipid peroxidation, and that cellular membranes, particularly plasma membranes, exhibit behavior after suffering from oxygen free radicals.

MATERIALS AND METHODS

Animals, HBO Exposures, and Blood Sampling

New Zealand white rabbits weighing between 2.0 and 2.5 kg were used. Rabbits were exposed to 100% O_2 at 3 ATA ($n = 7$) and 4 ATA ($n = 9$) for 60 min in a specially designed chamber. The chamber was flushed with air at the end of the exposure. A control group and the animals were returned to room air after the exposure and were kept in altering 12-h light and darkness cycles. Arterial blood sampling was performed as follows: Precontrol samples were collected from each animal before starting the experiment, zero time means the time when the animal was returned to room air, and postexposure samples were prepared by holding the samples for 1, 3, 6, 12, 24, 36, 48, and 72 h before analysis. The volume of arterial blood samples was 1.5 ml. Plasma was obtained by centrifugation at 500 *g* for 10 min at 4°C, immediately after the blood collection. All samples were stored in a freezer at -40° ± 2°C until ESR measurement.

ESR Measurement

The spin trap DMPO was purchased from Sigma Chemical Inc. (St. Louis, MO). ESR spectra were measured on a JES-FE 2 XG model (JEOL, Tokyo). DMPO (20 μl) was added to plasma (200 μl) and ESR measurement was started 30 s after the preparation. The hyperfine coupling constants obtained were as follows: $A_N = A_H = 14.9$ G for DMPO*OH spin adduct, and $A_N = 15.8$ G, $A_H = 22.6$ G for DMPO*6 line spin adduct. ESR conditions: microwave power, 8 mW; modulation amplitude, 2 G; scanning field, 3350 ± 50 G; amplitude, 2000: sweep time, 30 and 60 s.

The Detection of SOD by ESR

Xanthine oxidase, hypoxanthine, SOD, dimethylenetriamine-pentaacetic acid (DETAPAC) were purchased from Sigma. All other chemicals were of reagent grade. The superoxide generating system using xanthine-xanthine oxidase contained 2 m*M* hypoxanthine (50 μl), 5.5 m*M* DETAPAC (30 μl), plasma sample (50 μl), DMPO (20 μl), and 0.272 U/ml xanthine oxidase (50 μl). ESR spectra were recorded 45 s after the initiation of the reaction (4). 50 U/ml standard solution of SOD was inserted into every 10 plasma samples.

In Situ Experiment

Three normal subjects were recruited from the staff at our laboratory. No one received drugs that could affect plasma cholesterol levels or lipid metabolism. Forty milliliter of blood was drawn from the antecubital v. in the fasting staff, and anticoagulated using heparin sodium (Novo Industry, Denmark). Thirty milliliter of venous whole blood was centrifuged at 500 *g* for 10 min at 4°C, and a 10-ml plasma sample was obtained. A precontrol sample was obtained just before starting the experiment. Ten milliliter of venous whole blood and of plasma alone was put into disposable plastic culture dishes. The exposure condition was 100% O_2 at 5 ATA for 60 min. The venous whole blood samples were centrifuged immediately after the exposure to obtain plasma. All samples were separated and contained in a 5-ml vacuum tube, and were kept at 4°C over each time course.

Analysis of Cholesterol by High Performance Liquid Chromatography

The estimation of the fraction of damaged LDL in the samples was determined by high performance liquid chromatography (HPLC) using commercial enzyme kits. Determiner TC "555" was purchased from Kyowa Medex, Tokyo. Lipoprotein subfractions separated by size-exclusion column were monitored by postcolumn staining with the reagents (550 nm). The HPLC condition for cholesterol measurement was as follows: column, TSK guard-column PWH + TSK gel G 4000 SW + G 3000 SW, 7.5 mm i.d. × 7.5 cm + 7.5 mm i.d. × 60 cm × 2; eluent, 50 m*M* Tris-HCl buffer + 0.1 *M* NaCl (pH 7.4); enzyme solution, determiner TC "555," flow rate and pressure of eluent, 0.8 ml/min, 42 kg/cm^2; reaction mixture 0.48 ml/min, 5 kg/cm^2; temperature of the reactor (0.4 mm i.d. × 15 m Teflon tube), 50°C; detector, o.d. 550 nm, 0.16 AUFS (5). Apparatus was HPLC CCPM type, Toyo Sodo Chemical Industries, Tokyo.

RESULTS

Effects on Hydroxyl Radical and DMPO*6 Line Spin Adducts by Hyperbaric Exposure, In Vivo Experiments

The diving profile of animals at 4 ATA is shown in Fig. 1. The changes of hydroxyl radical in plasma samples at various times after the exposure to 100% O_2, either 3 ATA or 4 ATA, for 60 min are illustrated in Fig. 2. The relative percent of DMPO*OH spin adducts was calculated as follows: total peak high of 4 lines (DMPO*OH spin adducts) was divided by the peak high of standard manganese oxide. Each value of the time course was finally divided by the precontrol values of each animal.

A significant increase to 176.6 ± 47.2% ($P < 0.05$) of hydroxyl radical occurred at 24 h postexposure, after exposure at 4 ATA. The difference began to decrease at 36 h postexposure to a value higher than control values by 34.2 ± 21.0%, and there were no significant differences from control after 48 h postexposure. The changes of the time course of hydroxyl radical at 3 ATA

and air control groups were not significant. The relative value of air controls was 105.3 ± 9.6%.

The data of DMPO*6 line spin adduct showed that exposure at 4 ATA

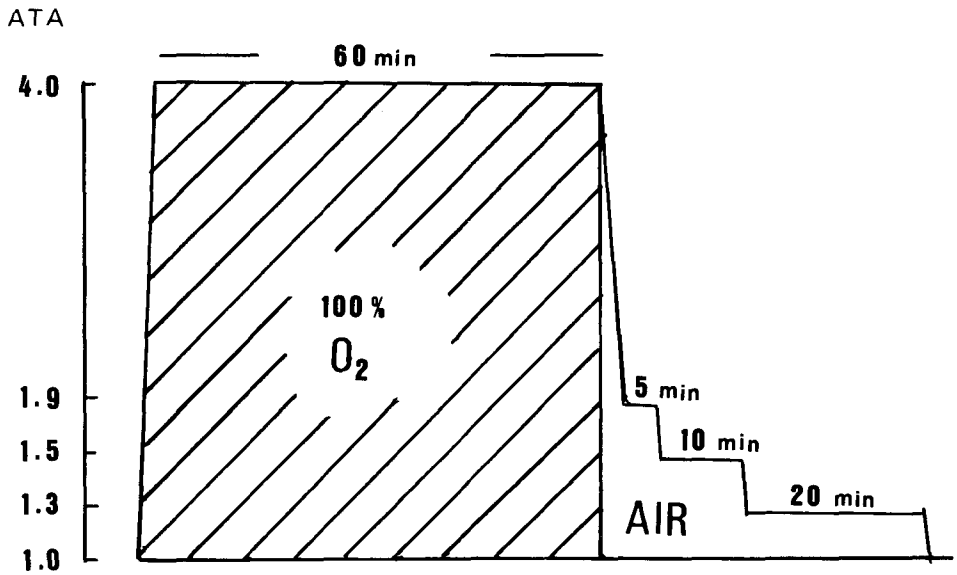

Fig. 1. Diving profile to exposure to 100% oxygen at 4 ATA for 60 min.

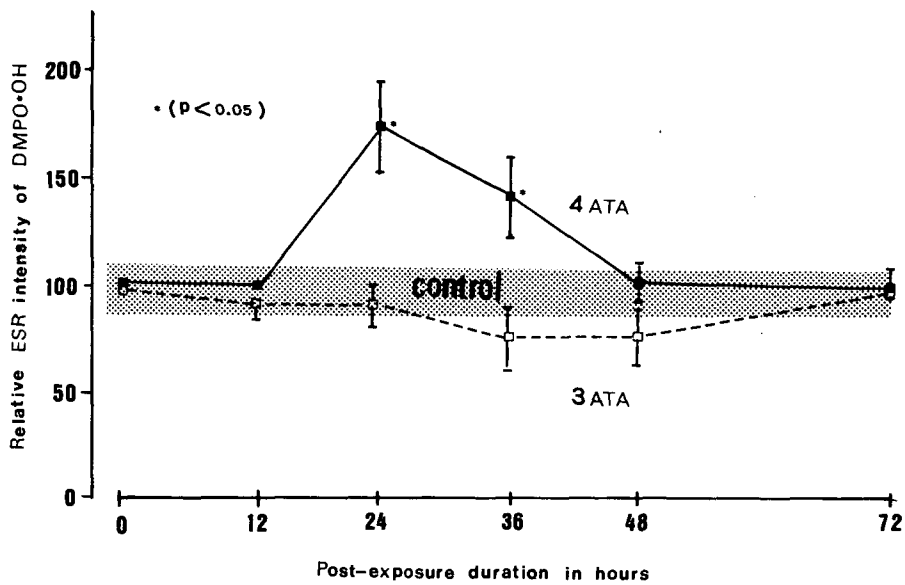

Fig. 2. Relative ESR intensity of DMPO*OH

resulted in a marked increase to 245.7 ± 67.1% ($P < 0.05$) at 24 h of postexposure. Conversely, the sample from exposed animals at 3 ATA revealed a significant decrease below control levels to 36.8 ± 13.9% ($P < 0.05$) at 48 h postexposure. The air control values were 101.1 ± 12.4%.

Effect on SOD Activity of Exposure to 100% O_2 at 4 ATA for 60 Min

Superoxide dismutase activity decreased from 47 ± 4 U/ml as the control value to 33 ± 7 U/ml at zero time. No significant changes occurred at 12, 24, and 48 h postexposure. Significant differences, however, were seen at 72 h postexposure, increasing to 61 ± 6 U/ml, a 45.9% change ($P < 0.05$) (data not shown).

Effect on Cholesterol Content of Plasma Membrane by Exposure to 100% O_2 at 5 ATA for 60 Min, In Situ Experiments

The relative area percent of the fraction of damaged low density lipoprotein (LDL) vs. native LDL was observed. Exposed plasma alone showed a slight increase in the peak area as a function of time and reached a maximum value at 48 h postexposure. The value at the maximum point increased by 2.85%. Control value was 2.04 ± 0.13%. Postcontrol value at 72 h used as monitors of the untreated plasma sample remained constant within 0.13%, and therefore the data obtained from the treated plasma alone show the effect of the HBO exposure on the samples. Thus, damaged LDL in whole blood had a maximum value at 24 h postexposure showing a 2.3-fold elevation. Moreover, the high level of damaged LDL remained at this value for 72 h postexposure.

DISCUSSION

Numerous papers suggested that pulmonary oxygen toxicity in animals is mediated by oxygen free radicals. However, no direct evidence has been shown for oxygen cytotoxicity, which would be responsible for simultaneous production of superoxide, hydroxyl radical, and hydrogen peroxide. The identification of hydroxyl radicals using DMPO was established by ESR (6) and we measured hydroxyl radicals and DMPO*6 line spin adducts in the same plasma samples over the same time postexposure. We have direct evidence that both hydroxyl radicals and DMPO*6 line spin adducts are implicated. It is very interesting that these spin adducts had the maximum yield at 24 h postexposure after exposure to 4 ATA oxygen. Additionally, no major changes of these spin adducts were observed, either during exposure to 100% O_2 at 3 ATA or at least 12 h postexposure when exposed to 4 ATA. It seems unreasonable to assume that oxygen free radical species are not produced under such exposure conditions, or could not be detected because of the low levels of spin adducts. Nevertheless, we could not identify the DMPO*6 line spin adducts, but our data from this ESR line provide several reasons supporting the above considerations (data not shown).

We measured enzymatic activities of catalase and glutathione peroxidase,

however, and our data did not show significant differences, either during exposure or postexposure.

The changes in SOD activity, determined by the new sensitive and specific method using ESR spectroscopy, seem not to support the results of spin adducts. Our data, however, suggest that the decrease of SOD activity at zero time may be due to the generation of superoxide during the exposure to 100% O_2 at 4 ATA. Additionally, a significant increase of SOD activity at 72 h postexposure is probably a sign of preventive reactions to cytotoxicity. Although the generation and the rate of production of superoxides have been predicted with the estimation of SOD activity, our data did not show a clear-cut correlation between hydroxyl radical and superoxide.

The differences in the yield of damaged LDL by oxidation between whole blood and plasma alone exposed to 100% O_2 at 5 ATA for 60 min are evidence that lipid peroxidation on membranes is advanced slowly by continuous chain reactions, especially in blood cells. The present data do not show whether blood cells are the only cells that demonstrate progressive lipid peroxidation. Our data support the suggestion by Rosen and Freeman (7) that significant lipid peroxidation was caused by extracellularly generated oxygen radicals. Acute oxygen toxic effects have been shown at our university hospital in 5 patients (4.35%), among 115 patients during a 5-yr period from 1980 to 1985, when we used a Laerdal open mask. Three of the 5 patients had early manifestations of oxygen poisoning at oxygen pressures at 2.8 ATA and 2 patients at 3.0 ATA.

The oxygen percentage of respiratory gas used for HBO at our laboratory was 69% in a Puritan-Bennett open mask, 80% in the Laerdal open mask, and 86% in a demand-type mask. These differences in oxygen values were due to differences in the mechanical characteristics of breathing mask units, i.e., dead space, face fitting, and mechanical resistance at high pressure (8). Therefore, the onset of acute oxygen toxicity during HBO treatment seems to be prevented at least in clinical use. However, our data suggest that more medical attention should be given to post-HBO exposure when the exposed pressure is less than 3 ATA.

In summary, important evidence is shown for the correlation of oxygen free radicals and lipid peroxidation. Hydroxyl radical, DMPO*6 line spin adducts, and the amount of damaged LDL in plasma were dramatically and simultaneously increased at 24 h postexposure. These findings suggest that a principle role of the oxygen free radical is not only to cause cellular damage directly but to initiate lipid peroxidation with a long duration.

As part of our study, an attempt was made to prospectively identify spin adducts entrapped by DMPO and behavior of LDL after the HBO exposure; this requires further experimentation.

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ON THE USE OF HYPERBARIC OXYGEN THERAPY IN SUDDEN DEAFNESS AFTER DIVING

M. Pilgramm, H. Lenders, G. Frey, and B. Fischer

SHORT COMMUNICATION

We report on observations that were made in 4 patients treated at the Federal Armed Forces Hospital in Ulm and at the LVA Hospital in Klausenbach, West Germany. All 4 patients were sport scuba diving in the Maldives for the first time.

Patient 1: male, 54-yr-old

On his 5th d of diving, dived to 30 m with scuba apparatus, surfaced without any problems within the prescribed time. When he reached the surface he experienced a feeling of deafness and pronounced tinnitus in the right ear. There was no vertigo and no other sign of decompression sickness. After irrigation of the right ear because of questionable cerumen, the findings remained unchanged. After waiting for 2 d he broke off his holiday and flew home. On arrival in Germany the audiogram shown in Fig. 1 was recorded.

Hearing was normal in the left ear; there was no vestibular dysfunction nor any neurologic symptoms. The patient received 10 d of hyperbaric oxygen (HBO) therapy for 1 h/d at 2.5 ATA with 100% O_2, plus simultaneous infusion therapy with low-molecular dextran.

Result: No change in auditory findings under this therapy, and the exceedingly disturbing high-pitch tinnitus also persisted. According to his history, the patient had normal hearing before his holiday.

Patient 2: female, 38-yr-old

On the 2nd d of diving, dived to some 25 m with scuba apparatus, and

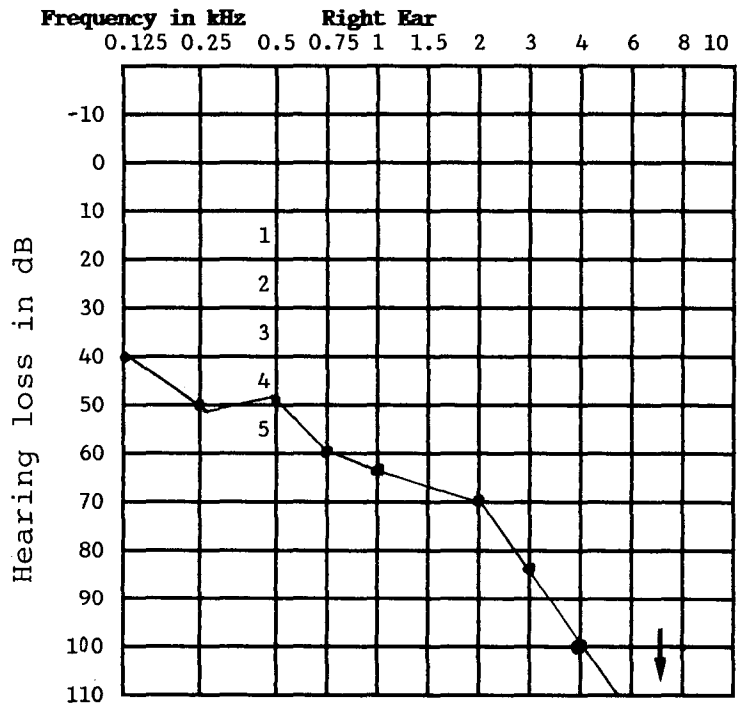

Fig. 1. Audiogram of patient 1 upon admission to hospital.

surfaced without any problems within the prescribed time. Shortly before reaching the surface, she experienced pronounced tinnitus on the right with a cotton-wool feeling in the right ear. There was no vertigo and no other sign of decompression sickness. The patient immediately broke off her holiday. The audiogram shown in Fig. 2 was recorded upon her arrival for treatment in Germany. Hearing is normal in the left ear. There are no vestibular or neurologic symptoms. The patient was immediately given the same therapy as patient 1.

Result: No effect on auditory findings or on the tinnitus. According to her history, the patient had normal hearing before her holiday.

Patient 3: male, 41-yr-old

On the 8th d of diving, dived to some 15 m with scuba apparatus, and surfaced without any problems. When removing his apparatus on board the boat, the patient suddenly experienced tinnitus on the right with a slight loss of hearing. There was no vertigo and no other sign of decompression sickness. After waiting 2 d, during which there was no change in the findings, he broke off his holiday. The audiogram shown in Fig. 3 was recorded upon arrival for treatment in Germany.

Hearing is normal in the left ear. There are no vestibular or neurologic

symptoms. Recompression therapy was started immediately, using the U.S. Navy tables, and was given for 2 consecutive d. This was followed by the same treatment as for patients 1 and 2.

Upon completion of therapy, the audiogram in Fig. 4 was recorded.

Result: Improved audition, but no effect on the tinnitus. According to his history, the patient had normal hearing before the holiday.

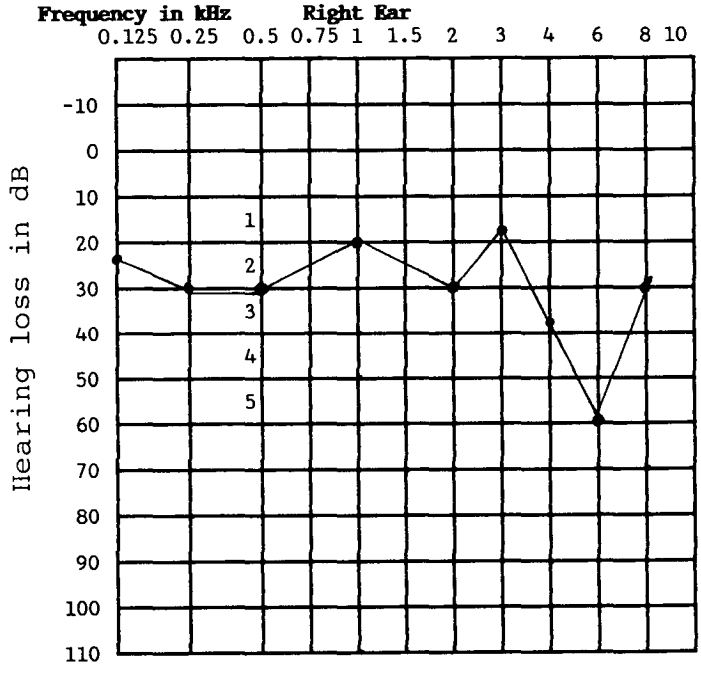

Fig. 2. Audiogram of patient 2 recorded upon her arrival for treatment in Germany.

Patient 4: female, 31-yr-old

On the 10th d of diving, dived to 40 m, and surfaced within the prescribed time. Some 5 m below the surface the patient experienced a muffled feeling in the right ear, accompanied by slight vertigo. As she came out of the water rotatory vertigo increased to the right together with a total loss of hearing on the right and pronounced tinnitus. The patient immediately broke off her holiday. The audiogram shown in Fig. 5 was recorded upon arrival in Germany. Apart from a known, long-standing, minimum high-pitch deafness, hearing in the left ear is normal. In ENT and neurologic terms, the patient also suffers irritable nystagmus to the right. Therapy as patient 3. The audiogram after 12 d is shown in Fig. 6.

Result: Enormous improvement in hearing. No change in the tinnitus. Low-level residual vestibular symptoms.

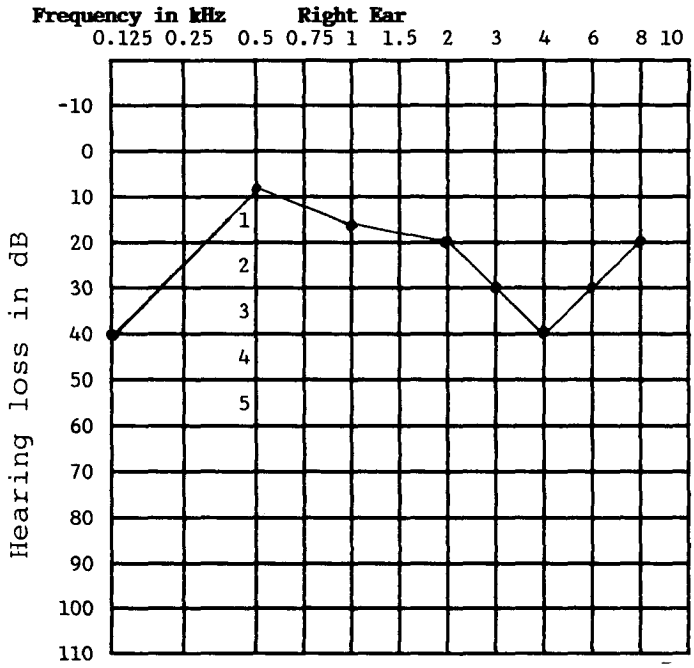

Fig. 3. Audiogram of patient 3 upon admission to hospital.

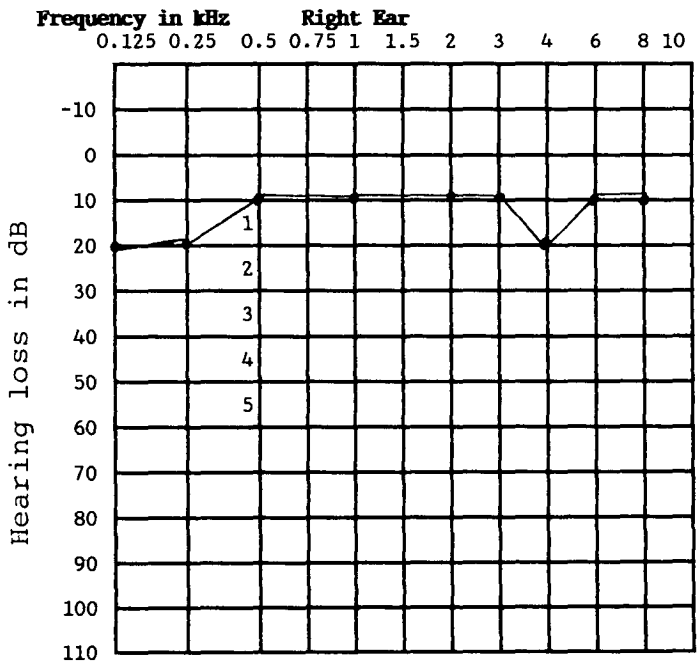

Fig. 4. Audition of patient 3 after therapy.

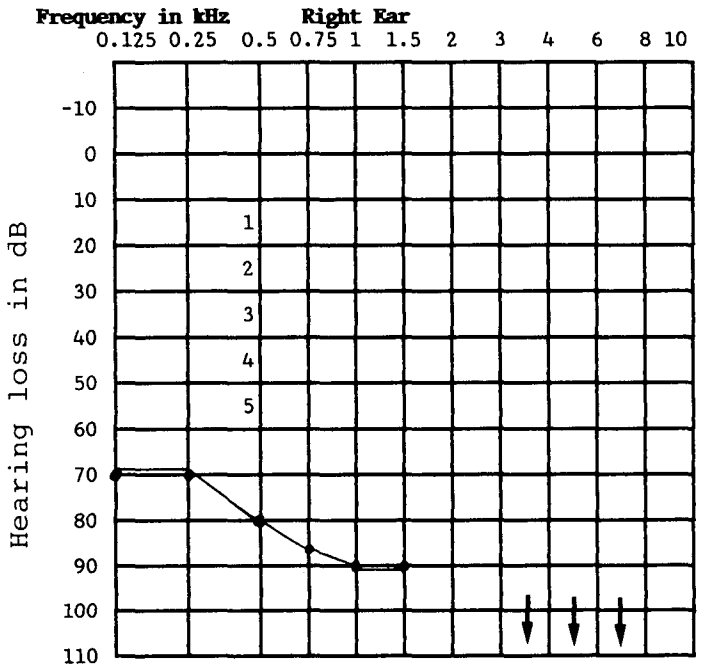

Fig. 5. Audition of patient 4 before therapy.

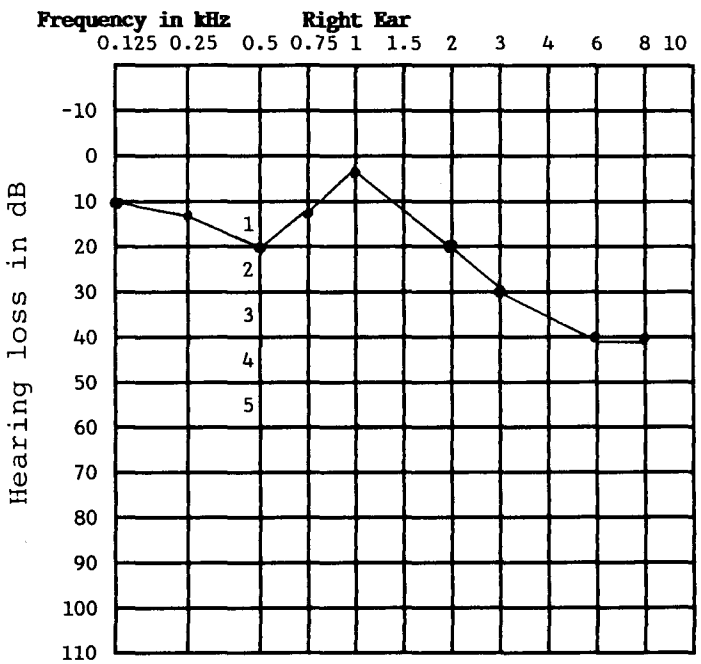

Fig. 6. Audition of patient 4 after therapy.

CONCLUSION

On the basis of experience gained in 4 cases it is not yet possible to derive any universally valid rules. What is interesting, however, is that no change was seen during HBO at 2.5 ATA in patients without recompression, while very pleasing improvements in hearing were recorded in the other 2 patients (treated with U.S. Navy Table 6). The tinnitus remained therapy-resistant in all cases.

All 4 patients commenced therapy no later than 4 d after their last dive. No caissonlike symptoms were detected in any of the patients which would have pointed to general decompression damage. On the thin-section computer tomogram of the petrous bone no change could be detected with the available resolution. None of the patients reported any sensation of pain in the ear region directly after surfacing to indicate that they had experienced a barotrauma.

The reason for the one-sided inner ear damage thus remains unclear for the moment. An accumulation of small bubbles of nitrogen in the capillaries of the inner ear with the subsequent undersupply and resultant loss of hair cell function is no more than speculation at present. It is likewise not impossible that the sudden deafness happened purely by chance at the time of surfacing, and that it has a completely different origin from what could have been over-rapid decompression. It must be stated, however, that 2 of the patients who had suffered sudden deafness in conjunction with a dive achieved better results in terms of hearing gain after 2 recompression sessions followed by HBO than were achieved with HBO in isolation.

SESSION 12: OXYGEN TOLERANCE IN MAN

9th International Symposium on Underwater and Hyperbaric Physiology
Undersea and Hyperbaric Medical Society, Bethesda, MD, 1987

DEFINITION OF TOLERANCE TO CONTINUOUS HYPEROXIA IN MAN: AN ABSTRACT REPORT OF PREDICTIVE STUDIES V

C. J. Lambertsen, J. M. Clark, R. Gelfand, J. B. Pisarello, W. H. Cobbs, J. E. Bevilacqua, D. M. Schwartz, D. J. Montabana, C. S. Leach, P. C. Johnson, and D. E. Fletcher

This abstract report has a dual purpose. One purpose is to provide the overall goals, design, and procedures of Predictive Studies V. The subordinate purpose is to describe specific elements of neural effects produced by prolonged hyperoxia. Summary observations on other functions are considered in related papers in this proceedings and elsewhere (1-5).

Predictive Studies V is a multiyear, collaborative investigation of oxygen poisoning in normal men, during uninterrupted exposures to oxygen over the range of hyperbaric oxygen exposure most useful in diving, treatment of gas lesion diseases, and general hyperbaric medicine.

The investigation followed approximately 20 yr of prior investigations of oxygen effects in animals and human subjects, during which necessary concepts of design selection of critical target functions, methods, and safety procedures accumulated to a degree that justified the extensive searches into unknown, essentially unexplored aspects of human oxygen toxicity.

The investigation was designed to determine rates of development and recovery for oxygen-induced effects on multiple selected organ, tissue, or cell functions. It was expected that the nature of specific toxic effects, rates of development, and rates of recovery would differ at different pressures of hyperoxic exposure. Therefore the systematic overall studies were carried out to include exposure to several different pressures of inspired O_2. The pressures selected for 100% breathing selected were 3.0, 2.5, 2.0, and 1.5 ATA. At each pressure a duration of exposure greater than previously systematically investigated was selected. The design thus provided the basis for definition of oxygen "dose-response" in terms of pressure, duration, and effect. It provided

for the analytic derivation of oxygen tolerance tables for multiple critical organ functions over the probable range of usefulness for continuous oxygen exposure.

In this fundamental design, the resting condition and continuous oxygen exposure were chosen to allow determinations of *maximum tolerance* of tissue or organ functions at each level of inspired oxygen pressure. The results would thereby provide the necessary (and presently unavailable) baselines for important medical uses of oxygen in general hyperbaric oxygen therapy, and in hyperoxic treatment of gas lesion diseases in undersea and aerospace activity. The results would also provide the essential baselines for subsequent investigations in man of adverse or advantageous modifying factors relating to rate of development of oxygen poisoning or its effects (e.g., interrupted oxygen exposures, work, drug administration, carbon dioxide inhalation or retention, narcosis, respiratory gas density).

In the investigation emphasis was given not only to pulmonary oxygen poisoning, but to functions for which information concerning rates of development and recovery have been essentially unexplored in man at toxic oxygen pressures greater than 1 atm (e.g., neurologic, respiratory, cardiovascular, endocrine, renal).

Background

This program had its origins in the awareness that, in most uses of oxygen for therapy, the essential life element oxygen combines desirable effects critical to relief of hypoxia in some tissues, with potentially lethal toxic effects of hyperoxia in other tissues. In the absence of adequate information concerning human oxygen tolerance, fear of oxygen poisoning has for many years limited the therapeutic and operational use of hyperoxia to its full potential. This information concerning actual tolerance is needed to replace the arbitrary and nonoptimal patterns of diver and patient oxygen exposure employed over the past several decades. The required detailed definition of toxic effect and recovery encompasses organ systems and functions beyond the occurrence of pulmonary "irritation" and the occurrence of a convulsion.

At the onset of the Predictive Studies V program, the status of detailed information concerning development and recovery in human organ oxygen poisoning is grossly indicated by Fig. 1. Such studies of human pulmonary oxygen poisoning had not been accomplished at oxygen pressures greater than 2.0 atm or even between 1.0 and 2.0 atm.

Except for symptomatic, brain circulatory, and EEG observations (6-10) no systematic investigation of human CNS oxygen tolerance had been accomplished at pressures greater than 1 ATA. While *physiologic* effects of oxygen have been investigated to pressures as high as 4.0 ATA (10-14), systematic determinations of onset and recovery of *toxic* effects of oxygen on cardiovascular and other critical organ functions had not been made at pressures greater than 2.0 ATA.

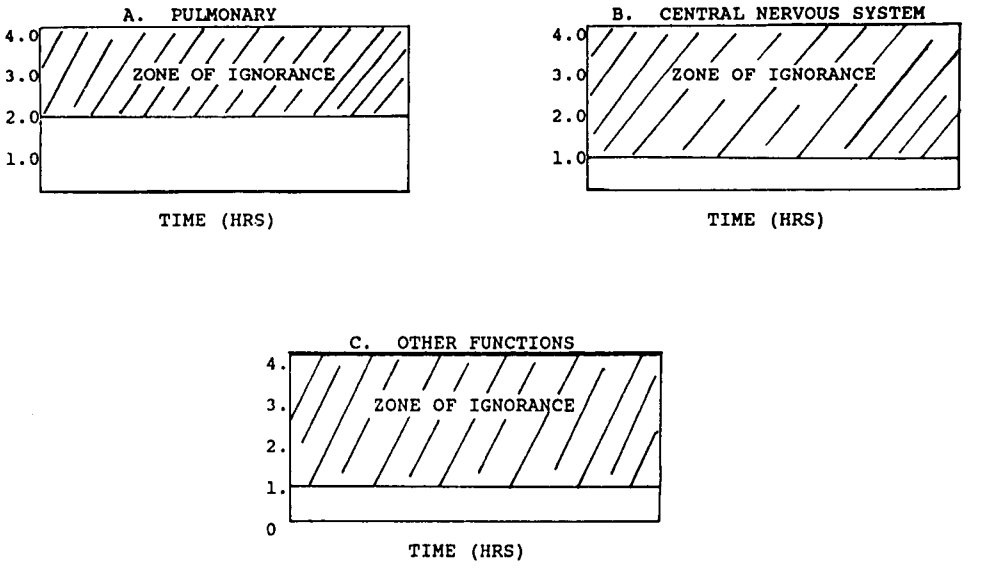

Fig. 1. Status of information concerning human tolerance to continuous hyperoxia.

Against the background of prior investigations, it was recognized that no organ or system (e.g., the brain, the eye, the heart, or even the lung) has a single function, single oxygen dose, or single oxygen tolerance in any hyperoxic exposure (6) (Fig. 2). It was therefore a goal of the program to measure not only effects on multiple organ systems, but discrete effects within the system being investigated.

Moreover, it was considered philosophically that development of cytochemical oxygen poisoning began from the moment of exposure to hyperoxia, and progressed at a rate related to the absolute pressure of oxygen at the discrete sites of the molecular process involved. Functional expressions of these myriad loci of oxygen poisoning in any tissue or organ system could be expected to develop at different rates, and in much of the mass of body tissue the progressive poisoning will not become physiologically obvious and must be sought by selective chemical searching.

Because any observed effects of oxygen poisoning must be recognized as consequences of a pathologic process, it was considered that measurements of *rates of recovery* had even greater significance than the operationally important measures of *rates of development* of adverse effects.

For these several reasons cited, the overall Predictive Study of Human Oxygen Tolerance combined measurements of rates of development and recovery of measurable physiologic changes, with measurement of corresponding chemical changes reflected in blood.

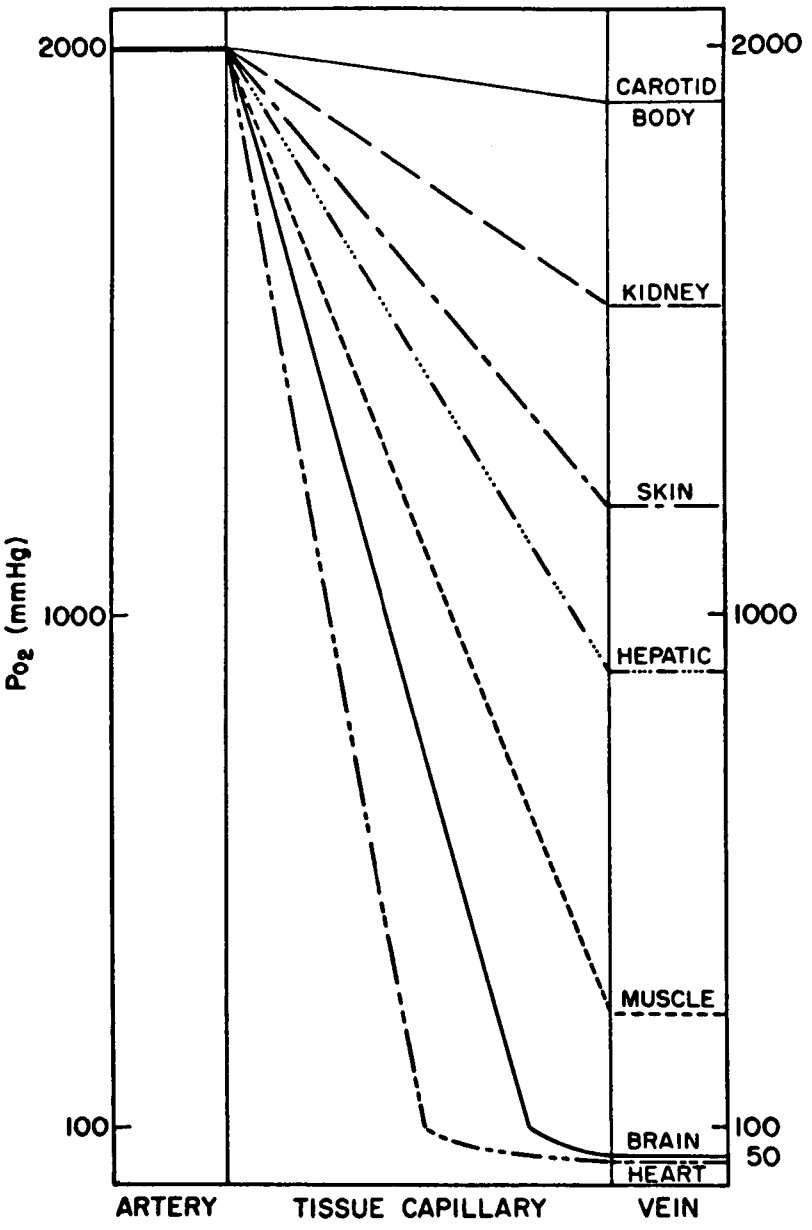

Fig. 2. Fall in PO_2 across the capillary bed in various tissues during oxygen breathing at 3.5 ATA. From Lambertsen (6).

Goal of Program Design

In our previous investigations aimed at defining human pulmonary tolerance to hyperoxia, a system of *pulmonary oxygen tolerance curves* was derived (6, 15, 16) (Fig. 3). For these, based on decrements in vital capacity, measurements obtained in studies by several investigators at oxygen pressures of 2.0, 1.0, 0.8, 0.7, and 0.5 ATA were combines (15, 16). This subsequently allowed predictive estimation of *cumulative pulmonary toxic dose* (CPTD) for oxygen, in terms of a *unit pulmonary toxic dose* (UPTD) defined as that producing the toxic effect of 100% O_2 for 1 min at 1 ATA (17, 18).

An applied goal of Predictive Studies V has been to obtain the measurements necessary to improve on these previously derived pulmonary oxygen tolerance curves, and to generate the information required for equivalent oxygen tolerance tables for other critical organ or tissue functions, including brain, sensory, neural, muscle, cardiac, blood, and endocrine.

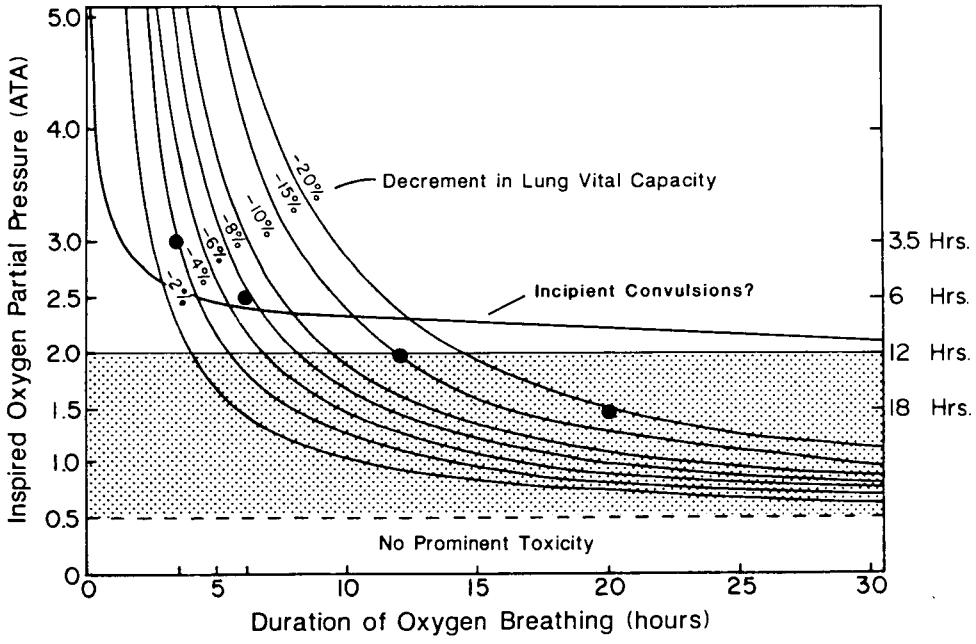

Fig. 3. Progression of pulmonary oxygen toxicity during continuous exposure to hyperoxia. Shaded area represents levels of inspired PO_2 at which investigations had been carried out before Predictive Studies V. Black dots indicate pressure/durations for oxygen exposures in present program. Modified from Lambertsen (6).

PROCEDURES AND METHODS

Oxygen exposure pressure and duration limits planned, and approved by the formal university procedures for research involving human beings, are as outlined in Table 1.

Young men were selected as subjects from volunteers, following the successful completion of detailed clinical medical examinations including special examinations of the systems under study (e.g., auditory/vestibular, neurologic, ophthalmologic, cardiac, pulmonary, hematologic). Numbers of subjects to be used at each pressure were determined by needs and practicality of statistical evaluation, as a particular toxic effect was encountered.

Table 1
Planned Pressures and Durations of Hyperoxic Exposures

Ambient Pressure, ATA	P_{O_2}, ATA	Duration, h
1.0	0.21	24
1.5	1.5	18
2.0	2.0	12
2.5	2.5	6
3.0	3.0	3.5

To accomplish the fundamental and applied goals cited required repeated measurements of multiple functions (Table 2) before, during, and after exposures of the normal subjects to each of the several different increased oxygen pressures (Fig. 4). It is clearly not possible or necessary to measure each of these functions continuously over the entire period of an oxygen exposure. Modules of measurement performance were therefore used, as in previous Predictive Studies (19, 20) (Fig. 5). These were repeated before, at planned times during, and after the oxygen exposures. At the highest oxygen pressure (3.0 ATA), measurements were repeated at short intervals. At the lower degrees of hyperoxia, intervals between measurement modules could be longer (Fig. 4).

The study was begun with investigations at 3.0 ATA to assure detection of any neurologic or other effects not encountered or searched for in prior studies at 2.0 or 1.0 ATA. This emphasis provided guidance in final design and safety for the exposures at lower pressures. On the same basis the study at the intermediate pressure of 2.5 ATA was then performed last, to assure awareness and preparation for investigations of potential interactions of neural, pulmonary, cardiovascular, and other effects.

Details of subject instrumentation, measurement, and analysis will be provided in the full documentation of major study components. The methods employed will be cited below in describing specific observations.

SUMMARY OF OBSERVATIONS

Throughout the study the most striking observations related to effects on visual function, on the lung, and probable interactions of preconvulsive neural

Table 2
Measurements During Continuous Oxygen Exposure in Man

Electroencephalography
clinical interpretation
spectral analysis
response to photic stimulation
Visual function
visual evoked cortical response
electroretinography (dark and light adapted)
fields (rodenstock perimeter)
pupillary reaction
acuity
accommodation
color vision
Auditory-vestibular function
audiometry (air conduction)
brainstem auditory evoked response
high frequency audiography
eye tracking, nystagmography
caloric stimulation
Muscle power (skeletal, respiratory)
Performance (perceptual, cognitive, and psychomoter)
Pulmonary function
flow-volume loops
spirometry
density dependent flow rates
closing volumes
peak inspiratory and expiratory pressures
airway resistance and conductance
frequency dependence of compliance
carbon monoxide diffusing capacity
arterial blood gases and acidity (PCO_2, PO_2, pH)
Cellular and chemical composition of bronchoalveolar lavage fluid
Respiration-respiratory gas exchange
Temperature

Table 2 Cont.

Cardiovascular function
- electrocardiography
- cardiac output, rate, stroke volume
- mean thoracic impedance
- blood pressure, systemic vascular resistance
- orthostatic reflex responses

Liver blood flow and function

Renal function

Endocrine activity, via plasma hormone levels
- insulin
- cortisol
- aldosterone
- vasopressin
- growth hormone
- beta-endorphin
- adrenocorticotropic hormone
- thyroid stimulating hormone

Hematologic effects

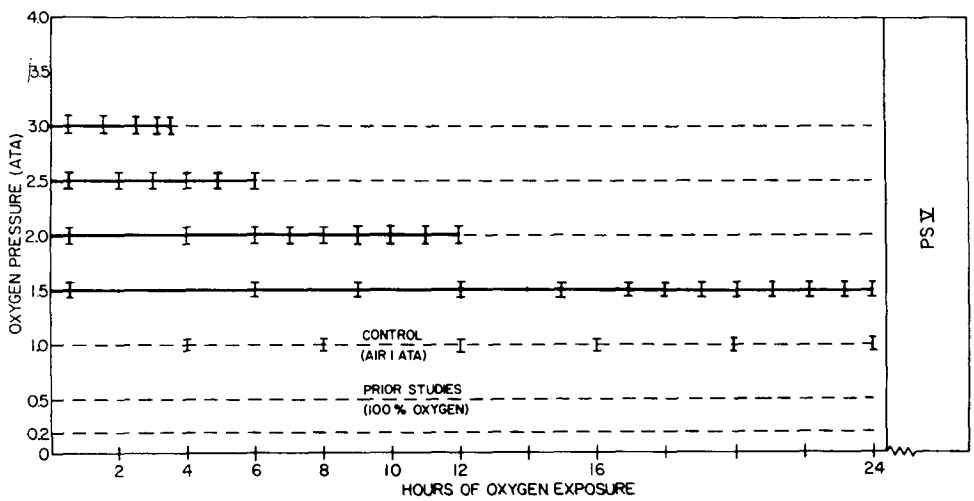

Fig. 4. Plan for continuous oxygen exposures in man.

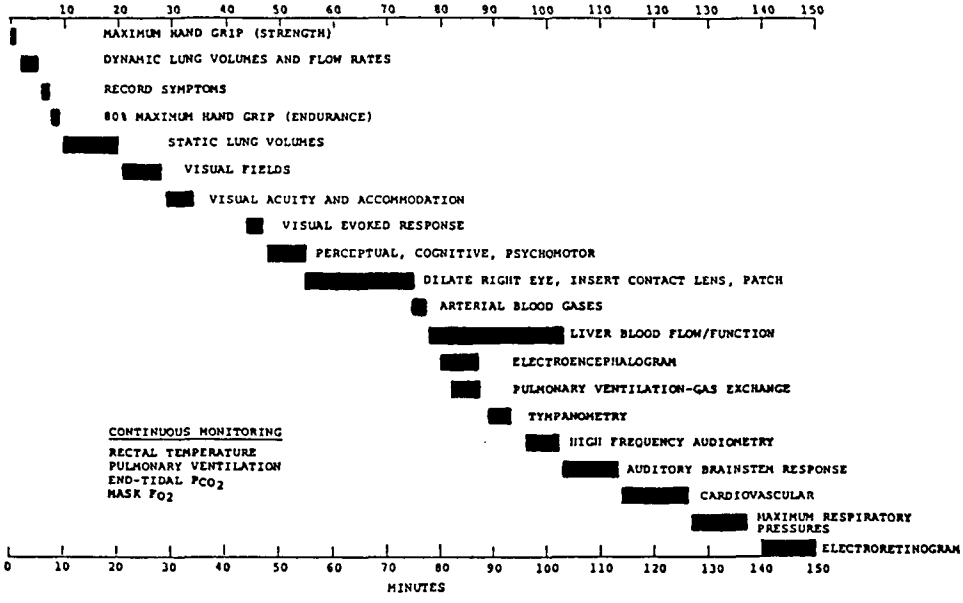

Fig. 5. Predictive Studies V measurement module for 2.0 ATA and 1.5 ATA studies.

activity with effects on cardiovascular and respiratory-pulmonary functions.

Table 3 indicates in simple form the presence or absence of an oxygen-related effect for major components of the overall study. Relations of these effects are summarized below. Details of specific observations and circumstances will be provided elsewhere.

Mental and Psychomotor Function

Consciousness was maintained throughout each oxygen exposure, even up to the moment of occurrence of a convulsion at 3.0 ATA in 1 subject. The selected measures of mental and psychomotor function (short-term memory, visual reaction time, finger dexterity, and general reasoning, all administered by the IFEM Performance Measurement System) have been remarkably stable at each of the oxygen pressures investigated. While no significant decrements occurred, comparisons of mental and psychomotor performance during oxygen exposure at 3.0 ATA and air at 1.0 ATA indicate that small "learning trends" in air exposure may have been masked during this highest oxygen exposure.

Convulsion and Associated Measurements

Only 1 convulsion has occurred during the entire sequence of the Predictive Study V exposures. This followed 3.0 h of oxygen breathing at 3.0 ATA.

Table 3
Summary of Specific Toxic Effects Observed

Function	O_2 ATA 1.5	2.0	2.5	3.0
Brain cortical				
Convulsion	o	o	o	•
Perception, cognition, psychomotor	o	o	o	o
Pre-convulsive cortical electrical activity (EEG)	o	o	o	o
Somatosensory evoked potential				o
Thermal	o	?	?	•
Respiratory	•	?	?	•
Neuromuscular power				
Hand grip	o	o	o	o
Inspiratory	o	o	o	o
Expiratory	o	o	o	o
Cardiovascular	?	•	•	•
Pulmonary	•	•	•	•
Hearing/vestibular				
Hearing thresholds	o	o	o	o
Auditory brainstem potentials	o	o	o	o
Vestibular response	o	o	o	o
Visual				
Central vision acuity	o	o	o	o
Peripheral vision	o	•	•	•
Retinal electrical response (ERG)	•	•	•	•
Pattern evoked cortical potential	o	o	o	o
Color vision	o	o	o	o
Accommodation	o	•	•	•
Pupil size, reactivity	o	o	•	•
Hepatic	*	*	*	*
Renal	*	*	*	*
Endocrine	*	*	*	*
Blood	*	*	*	*

Key: o = Measured, no change; • = Change observed; * = Measured, not reported here; ? = Change observed. May be physiologic or mixed effect.

Normal mental and psychomotor performance measurements were obtained 30 min before the convulsion. An observation of large significance was a normal subject-investigator cooperative performance of audiometric measurement at 3.0 ATA of oxygen, during the 4-min period before the occurrence of convulsion. This confirms the equivalent observation in open-sea diving that capability for intelligent performance is retained up to the moment of abrupt electrical disorganization that initiates the convulsion (21).

Respiratory and thermal changes associated with the single convulsion, during oxygen exposure at 3.0 ATA, are shown in Figs. 6 and 7. Respiratory components and end-tidal gases were monitored because each is related to the neural or neurochemical control of respiration, which were therefore considered in the program design as potential targets of neurologic oxygen poisoning, rather than having primary relation to direct pulmonary oxygen poisoning. The findings represent the first such tracking of preconvulsive respiratory events, and complement equivalent preconvulsive measurements of brain circulation and metabolism (10).

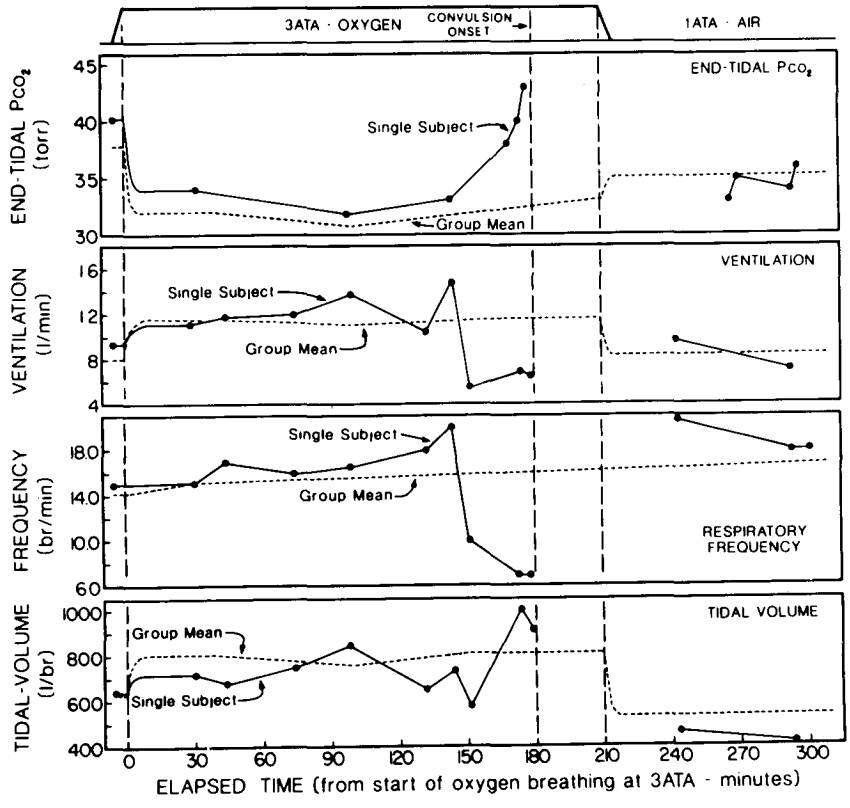

Fig. 6. Effects on ventilation of O_2 breathing at 3 ATA for 3.5 h. Comparison of 1 subject who convulsed with group of 12 subjects.

A progressive decrease in respiratory frequency occurred before the convulsion. An associated rise in end-tidal P_{CO_2}, in spite of increased tidal volume, indicated a decrease in alveolar ventilation. The special significance of the rise in alveolar P_{CO_2} is the inevitable effect of hypercapnia on brain blood vessels, with a cerebral vasodilatation, increased blood (oxygen) flow, and consequent exposure of brain tissue to further increase in oxygen partial pressure. A vicious cycle of accelerated progression of brain oxygen poisoning should result (6, 10, 11).

The fall in deep body temperature before convulsion resembles that observed in toxic oxygen exposures of small animals (22). The rise following the oxygen convulsion reflects the vigorous physical tonic and clonic "exercise" of the generalized convulsion (21).

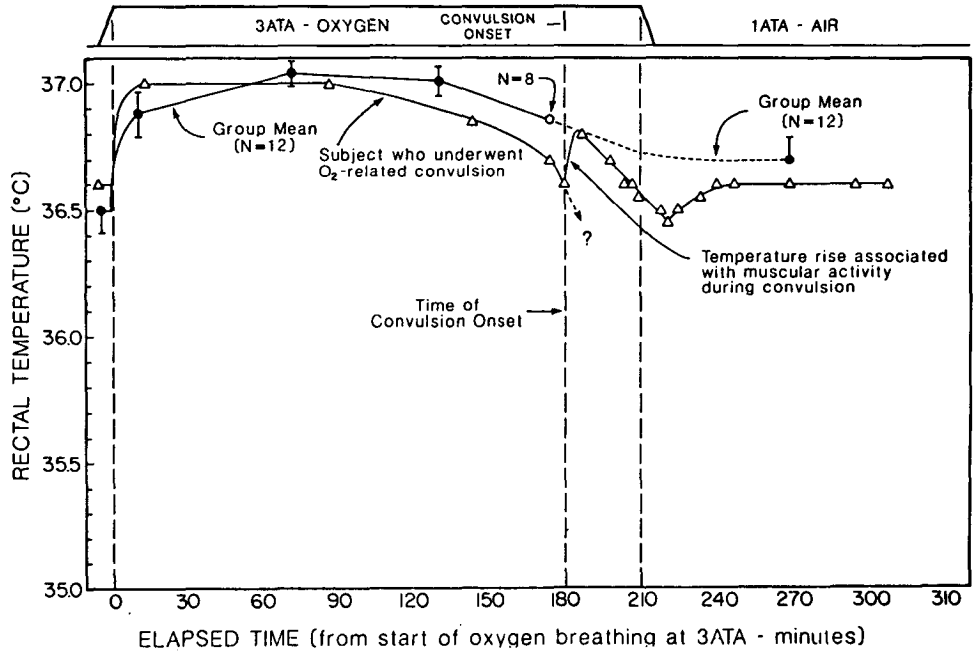

Fig. 7. Effects on body temperature of O_2 breathing at 3 ATA for 3.5 h. Comparison of 1 subject who convulsed with group of 12 subjects.

Recovery of consciousness was characteristic of previous experience in oxygen convulsions (21), with a time course equivalent to that in recovery from an episode of grand mal epilepsy. Monitoring of EEG, ECG, impedance cardiograph, and temperature were continuous during and following the convulsion. Other postoxygen exposure measurements were begun 1 h after the onset of the convulsion.

Brain Cortical Electrical Activity (ECG-EEG)

Change in brain cortical electrical activity (measured from 12 scalp electrodes placed according to International 10-20 System) was not evident in continuous clinical recordings during oxygen exposure at rest, even at 3.0 ATA for 3.5 h. Definite EEG changes were found at 3.0 ATA in only 2 subjects. One, cited above, had classical EEG manifestations of the seizure at 3.0 h of exposure, and postictal phases of a typical oxygen convulsion. No preconvulsive EEG changes were observed. The other subject had an approximately 10-s interval of flat EEG in association with a 20-s period of hypotensive loss of consciousness due to an episode of extreme bradycardia immediately after a 2.5 h exposure. Return to consciousness was accompanied by a mild clonic seizure and a 30-s interval of disorganized EEG activity, followed by resumption of normal EEG activity.

No clinically evident EEG changes were observed in any subjects during 5 to 6-h exposures at 2.5 ATA, 8 to 12-h exposures at 2.0 ATA, and 16 to 19-h exposures at 1.5 ATA. Detailed energy spectral analyses of the taped EEG records obtained at 3.0, 2.5, 2.0, and 1.5 ATA are not complete at this stage.

Somatosensory Evoked Cortical Response

No evident changes in somatosensory cortical evoked response (stimulus to median nerve at wrist) were observed in any subject at 3.0 ATA oxygen exposure. The measurement was not utilized at the lower exposure pressures.

Temperature Regulation

Deep body temperature (rectal) was continuously monitored, along with repeated measurements of carbon dioxide production, in search for toxic neural or metabolic disruptions of temperature regulation. Following an onset lag after start of hyperoxia, progressive hypothermia was found at 3.0 ATA in the single subject who experienced an oxygen convulsion (Fig. 7), in most subjects at 2.5 and 2.0 ATA, but in none of the subjects at 1.5 ATA.

Respiration and Respiratory Reactivity

Details of ventilatory changes in prolonged oxygen exposures are described in this symposium (3). This investigation of respiratory control was emphasized for its vital importance as a neurologic control system, the normal functions and reactivity of which could be quantitatively examined for evidence of toxic disruptions.

Previously described physiologic increases in ventilation, with associated decreases in end-tidal P_{CO_2} (6), were observed in all hyperbaric exposure conditions of this study. No clear indication of diminished effectiveness of respiratory control function occurred except in the subject who experienced a convulsion at the 3.0 ATA oxygen pressure. An important finding related to safety in hyperoxic exposures was that respiratory reactivity, either to carbon dioxide or to hypoxic stimuli, was not diminished in spite of observation of reduced carotid chemoreceptor response in cats following extreme oxygen

poisoning at 1 ATA (23).

Cardiovascular-Neurologic Interactions

Recognizing the dual central neurologic control of cardiac activity (vagal, sympathetic) and the importance of baroreceptor reflex regulation of blood pressure, cardiac electric activity (ECG) was monitored continuously, with periodic measurement of orthostatic reflex function. Effects on cardiovascular function were observed at all oxygen pressures investigated (4). These ranged from the previously documented bradycardia (8, 12), to a single episode of exaggerated bradycardia resulting in loss of consciousness and a gross diminution of the normal tachycardial response to standing. Details of each of these observations are provided in a companion report in this proceedings (4). Their special significance is the indication of oxygen-induced neurologic (presumably vagal) influence on cardiac rate function, with the additional possibility of oxygen effect on intrinsic cardiac electrical functions.

Pulmonary Functions

The extensive previous studies of pulmonary oxygen poisoning at 0.5 to 2.0 ATA (12, 24) were successfully expanded to include oxygen exposures at 1.5, 2.0, 2.5, and 3.0 ATA. Descriptions of measurements provided in this proceedings (2) reveal differences in rates of onset and rates of recovery at the several different levels of hyperoxic exposure, and contribute the first empirical data establishing pulmonary oxygen tolerance guidelines at oxygen pressures greater than 2.0 atm. Special significance in this component of the Predictive Study is given to the observations during oxygen exposure at 2.5 ATA of an abrupt and large decrease in vital capacity, superimposed on the progressive decrement characteristic of that induced by lower oxygen pressures. This abrupt respiratory handicap, as for the above-mentioned exaggerated bradycardia, requires consideration and investigation of a possible neurologic influence on lung structures directly poisoned by oxygen.

Neuromuscular Power

Potential neural or neuromuscular effects of prolonged hyperoxia were investigated in relation to respiratory and pulmonary functions, and to function of purposeful muscular activity. The observations indicate no serious handicap to motor, respiratory, or pulmonary functions.

Skeletal muscle force and endurance were measured by handgrip dynamometer, with maximum force and duration of maintenance of 80% of maximum forces recorded electrically. Maximum force showed less than 10% increment or decrement from preexposure control levels. Endurance changes were within 20% of controls. For both force and maintenance of force, improvement occurred in exposure to oxygen at 3.0, 1.5 ATA, and 0.2 ATA, whereas small decrement was associated with exposure to 2.0 and 2.5 ATA O_2.

Maximum Respiratory Pressures

Maximum inspiratory pressures at residual volume of functional residual capacity were not consistently decreased during and after oxygen breathing at 1.5, 2.0, 2.5, or 3.0 ATA. Maximum expiratory pressures at both total lung capacity and functional residual capacity were decreased most consistently by a mean value of about 17% near the end of the prolonged exposures at 1.5 ATA O_2. However, similar decrements also occurred during prolonged periods of air breathing at 1.0 ATA.

Auditory-Vestibular Function

Auditory-vestibular function was determined under controlled conditions (Hearing Laboratory) before oxygen exposure, and repeated about 10 h after termination of oxygen exposure. These measurements were supplemented by performance of extended high frequency audiometry during oxygen exposures (Fig. 5), along with monitoring of brainstem auditory evoked responses. No significant difference in any of the measured hearing functions was found in comparison with control exposures to 0.2 ATA O_2. The 1 subject who convulsed after 3.0 h of oxygen exposure at 3.0 ATA performed normally on a full-range, high-frequency audiometric test that was in progress at the onset of the convulsion.

Electronystagmographic responses to caloric stimulation were subnormal in many subjects after oxygen exposures at 2.5, 2.0, and 1.5 ATA. Since this measurement requires maintenance of an alert mental state and can be influenced by subject fatigue, the finding may not be specifically due to hyperoxia. Similar changes were found in some control subjects after 20-h air exposures at 1.0 ATA.

Effects on Visual Functions

The initial observation of oxygen-induced decrease in peripheral visual fields was made by Behnke et al. (12) in a subject exposed continuously to oxygen at 3.0 ATA for 3.5 h. This essentially unexplored neural effect of hyperoxia required measurements of multiple components of visual function at each of the oxygen pressures of this Predictive Study. The effects observed thus far in continuous oxygen exposure at 3.0, 2.5, 2.0, and 1.5 ATA are summarized in Table 4 and Fig. 8.

SIGNIFICANCE

In this integrated investigation of oxygen tolerance in normal men permitted detailed examination and quantitation of oxygen effects in a manner not possible without the cooperative and intelligent intercommunication between subject and investigator. The information obtained therefore is not only more detailed and extensive than could be obtained through the preliminary research in various lower animals; it is of direct and permanent relevance to the expanding importance of hyperoxygenation in diving,

Table 4
Effects of Continuous Oxygen Exposure on Visual Function in Man

Pressure	Acuity	Accommodation	Color	Pupils	VER	ERG	Fields
3.0 ATA	17 of 18 showed no change	11 of 18 had lengthened nearpoint	no change	6 of 18 had small increases in diameter	no change	3 of 10 had decrement in B-waveamplitude (16-30%)	mean decrement 50% in 18 at 3.5 h (range 8-91%) onset at 2.5-3.0 h
2.5 ATA	no change	1 of 8 had lengthened nearpoint	no change	1 of 8 had oscillating pupils	no change	1 of 8 had 39% amplitude loss during exposure (recovery by 0.7 h post). 1 other had 0.14% amplitude loss at end of exposure followed by postexposure loss of 62% (recovery by 12.3 h)	2 of 8 had decrement (35%) during exposure (recovery by 0.6 and 1 h)
2.0 ATA	no change	1 of 7 had lengthened nearpoint	no change	no change	no change	4 had a mean amplitude decrement of 50%. Recovery within 12-16 h postexposure	definite decrement in 1 subject (35%) Recovery delayed
1.5 ATA	no change	no change	no change	no change	no change	1 of 9 subjects showed amplitude decrement of 31%	no change

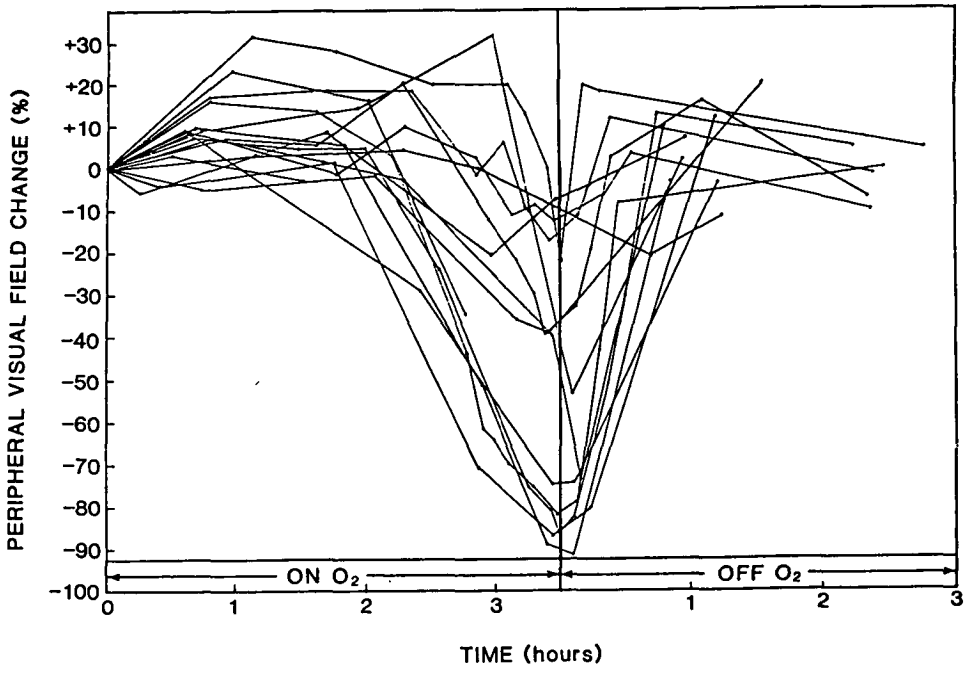

Fig. 8. Peripheral visual field changes in man during and after oxygen exposure at 3.0 ATA.

aerospace, hyperbaric, and general medicine.

Details of quantitative measurements made on multiple organ systems establish function, tissue, and chemical targets which can be most rationally and profitably investigated at each different level of oxygen pressure/duration exposure. The overall program is further intended to provide the essential baselines for both basic and applied research related to extension of oxygen tolerance by overcoming the limits posed by oxygen poisoning. This topic will remain at the forefront of research endeavor related to life, medicine, undersea activity, and ultimate aerospace operations.

The present study in resting subjects is a prerequisite to investigation of oxygen effects in active physical states, in exposures to external environmental stresses, or in attempts to modify oxygen effect by pharmacologic means. It is for the present considered that basic chemical processes of oxygen poisoning, which generated the functional changes observed in resting subjects, will also occur in situations of large practical importance in nonresting states such as underwater swimming or performance of other physical work. However, it is considered that the oxygen dose (Po_2) experienced in some tissues will be modified by carbon dioxide (11), whereas the oxygen dose in other tissue sites (e.g., lung) will not. It is also considered that functional influences of certain of these chemical changes will be modified by factors such as exercise, and other influences will not. The availability of baseline information in the resting

state provides the opportunity to investigate these considerations, and makes it possible to investigate the influences of factors modifying oxygen tolerance, including intermittent exposure and drugs.

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PULMONARY TOLERANCE IN MAN TO CONTINUOUS OXYGEN EXPOSURE AT 3.0, 2.5, 2.0, AND 1.5 ATA IN PREDICTIVE STUDIES V

J. M. Clark, R. Gelfand, N. D. Flores, C. J. Lambertsen, and J. B. Pisarello

This summary report is part of a comprehensive study of specific organ oxygen tolerance (Predictive Studies V). Oxygen effects on pulmonary function were measured in normal, resting men who breathed oxygen continuously at 3.0, 2.5, 2.0, and 1.5 ATA to predefined limits of CNS, cardiac, or pulmonary tolerance (1-3). Pulmonary-related measurements obtained before and after exposure included arterial blood gases while breathing air at rest and during exercise; carbon monoxide diffusing capacity; spirometry; flow-volume loops on air and helium-oxygen; nitrogen closing volumes; airway resistance; and lung compliance. Arterial blood gases on oxygen and maximum respiratory pressures were measured early and late in the oxygen exposure period, whereas flow-volume maneuvers and spirometry were repeated at regular intervals during oxygen exposures. Comparisons of data obtained over the range of oxygen pressures will be emphasized in this presentation. Parameters selected for comparison include lung volumes and flow rates, lung compliance, and CO diffusing capacity.

In preparation for this series of experiments, it was anticipated that oxygen tolerance would be limited by neurologic effects of oxygen toxicity at 3.0 ATA and by pulmonary effects at 2.0 and 1.5 ATA, with oxygen exposure at 2.5 ATA in a transitional zone between neurologic and pulmonary limitations (1, 4, 5). Based on all the information that was available at the outset of this study, maximum exposure durations were established for each oxygen pressure and, as part of a comprehensive system to ensure safety of the subjects, criteria were established for exposure termination before the predetermined limits. The selected limits of continuous exposure duration were 3.5 h at 3.0 ATA, 6 h at 2.5 ATA, 12 h at 2.0 ATA, and 20 h at 1.5 ATA.

Our previously derived pulmonary oxygen tolerance curves (4, 5) were used to predict degrees of pulmonary effect for the maximal durations of exposure at each pressure (Fig. 1). The curves indicated that continuous oxygen exposures of 12 h at 2.0 ATA and 20 h at 1.5 ATA would both produce average vital capacity (VC) decrements of 15 to 20% and would therefore be limited by pulmonary effects of oxygen toxicity. On the other hand, it did not seem likely that pulmonary intoxication would be limiting at 3.0 and 2.5 ATA, where predicted VC decrements for maximal exposure durations were about 4 and 8%, respectively.

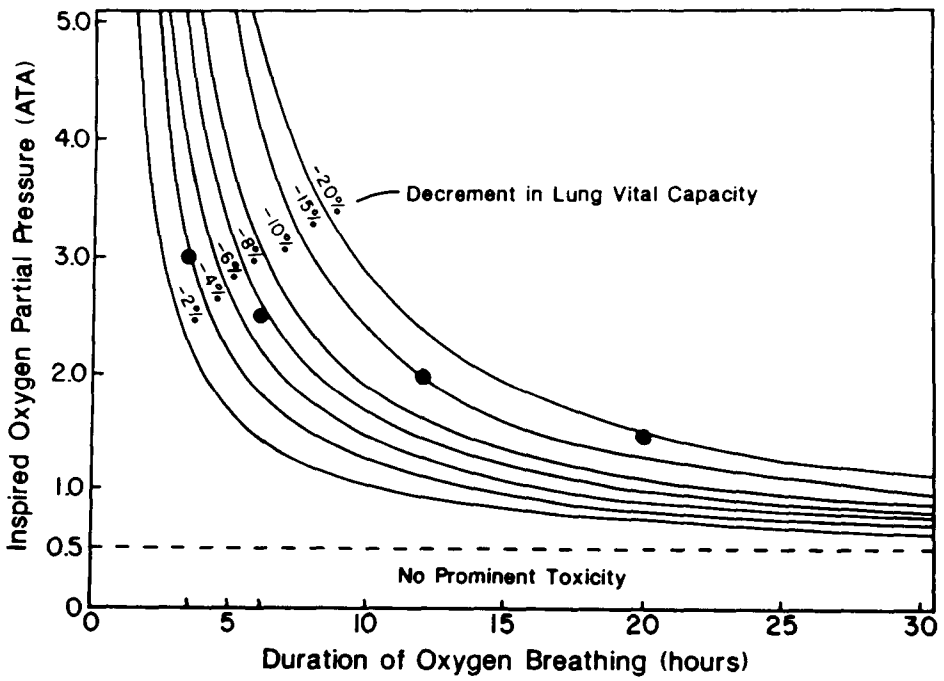

Fig. 1. Predicted pulmonary oxygen tolerance in man. Points indicate predicted changes in VC for maximum exposure durations at 3.0, 2.5, 2.0, and 1.5 ATA.

Progression of Subjective Pulmonary Symptoms During Oxygen Exposure at 3.0, 2.5, 2.0, and 1.5 ATA

At regular intervals during oxygen exposure, each subject was given a list of symptoms that are known to be caused by pulmonary or neurologic oxygen toxicity (4). The symptoms were rated as absent (0), mild (1+), moderate (2+), or severe (3+), and average ratings for the entire subject group were calculated. An overall "pulmonary symptom" rating was derived by combining average ratings for chest pain, cough, chest tightness, and shortness of breath. Pulmonary symptom ratings were then plotted against duration of exposure for each oxygen pressure (Fig. 2). Pulmonary symptoms were moderate (2+) on the

average by the end of oxygen exposure at 1.5 and 2.0 ATA, and mild (1+) before exposure termination at 2.5 and 3.0 ATA. Rates of symptom development increased progressively, as expected, with increase in oxygen pressure. However, the increment in the rate of symptom progression for the transition from 1.5 to 2.0 ATA was much greater than that observed for comparable transitions to 2.5 and 3.0 ATA. It is possible that longer oxygen exposures at 2.5 and 3.0 ATA, beyond the tolerance limits found at these pressures, would be associated with a wider separation of pulmonary symptom curves than that observed for mild symptoms.

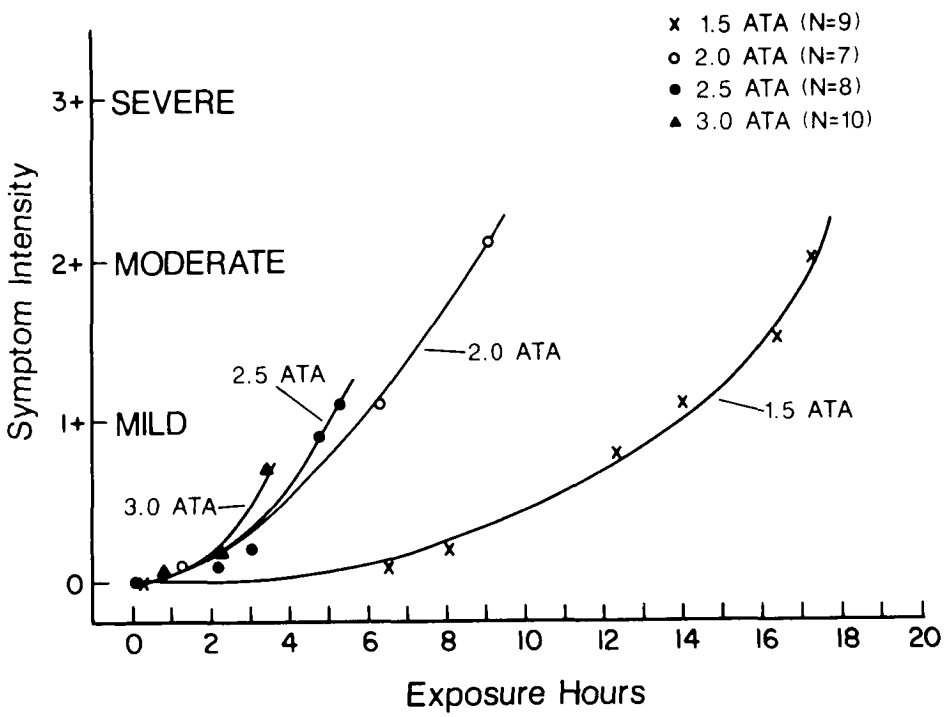

Fig. 2. Pulmonary symptoms during oxygen exposure at 3.0, 2.5, 2.0, and 1.5 ATA. The overall pulmonary symptom curve for each oxygen pressure combines average ratings for chest pain, cough, chest tightness, and shortness of breath.

Rates of Development of Pulmonary Intoxication During Oxygen Exposure at 3.0, 2.5, 2.0, and 1.5 ATA

In parallel with periodic assessment of symptoms, rates of development of pulmonary intoxication during oxygen exposure at 2.5, 2.0, and 1.5 ATA were monitored by repeated performance of flow-volume maneuvers and spirometry. Pulmonary function was evaluated objectively only before and after the 3.5 h exposures at 3.0 ATA. Of the many lung volumes and flow rates that were measured, VC was selected for comparison across different pressures, because

it decreased consistently and significantly at each of the oxygen pressures that were studied (Fig. 3). Data shown for the 1.5 and 2.5 ATA exposures represent average measurements in 9 and 8 subjects, respectively. The 2.0 ATA curve describes average data obtained previously in 11 subjects (6) and more recently in 7 additional subjects. All 3 curves were derived by fitting regression lines to the data for each pressure on probability-linear coordinates and then translating the lines to linear—linear coordinates. The single point for 3.0 ATA is an average of measurements obtained in 13 subjects about 2 to 4 h postexposure.

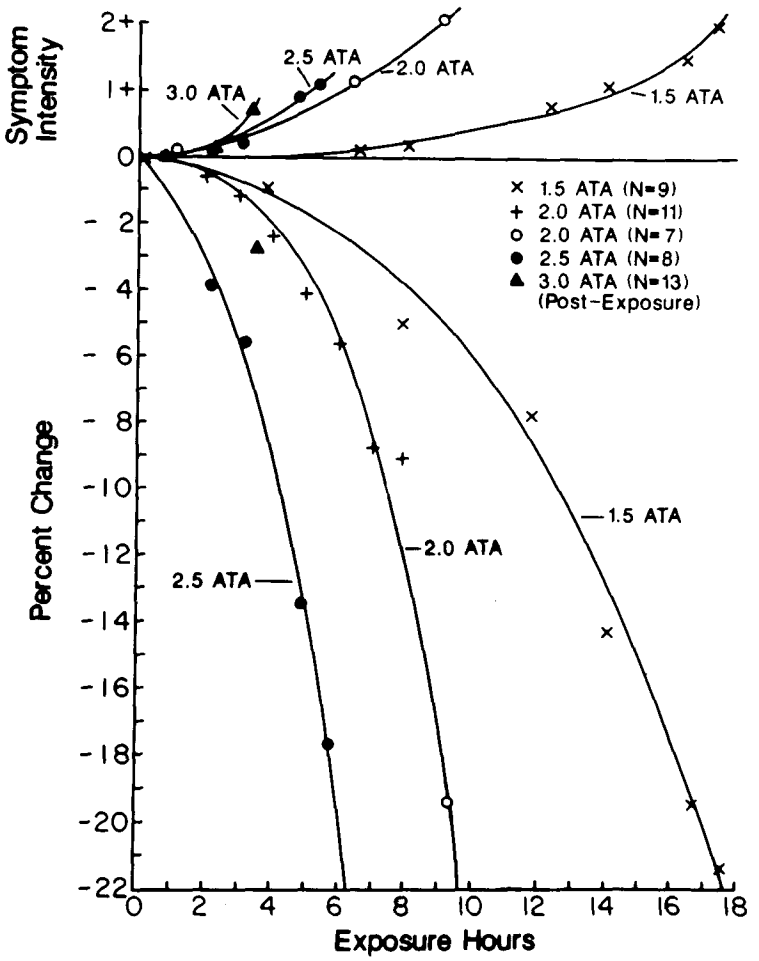

Fig. 3. Pulmonary symptoms and VC changes in man during oxygen exposure at 3.0, 2.5, and 2.0, and 1.5 ATA. Vital capacity measurements during the oxygen exposures at 1.5 and 2.5 ATA were obtained in the present series of experiments. The 2.0 ATA data represent 2 groups of subjects. The last exposure point (0) is the average value for 7 subjects in the present series of experiments. All other points at 2.0 ATA are average data from an earlier group of 11 subjects (6).

The curves for 1.5, 2.0, and 2.5 ATA all show a progressive fall in VC with continued duration of exposure, and the rates of fall are greater at higher oxygen pressures. Location of the 3.0 ATA point between the 2.0 and 2.5 ATA curves is not inconsistent, because these measurements were obtained after 2 to 4 h of recovery from the previous exposure. At the outset of this study, the rapid rate of vital capacity recovery after exposure to oxygen pressures above 2.0 ATA was not expected. Rates of recovery from similar vital capacity decrements at different oxygen pressures are compared below.

Figure 3 also compares rates of decrease in VC at 2.5, 2.0, and 1.5 ATA with progression of pulmonary symptoms at the same pressures. The data show that prominent decrements in VC were concurrent with mild symptoms and are therefore consistent with the conclusion that pulmonary symptoms did not contribute significantly to the observed changes in lung volumes and flow rates. This confirms the overall impression of investigators who remained inside the chamber that the subjects were highly motivated and delivered maximal effort even when symptoms were prominent.

Abrupt Exacerbation of Pulmonary Effects at 2.5 ATA

The 8 subjects who breathed oxygen at 2.5 ATA for an average duration of 5.7 h had a VC decrement of nearly 18%. This observed change was more than twice the prediction of about 8% from our previously derived pulmonary oxygen tolerance curves (Fig. 1). However, if the data for 2 subjects who had very large changes in lung volumes and flow rates are excluded from the group, the average decrease in vital capacity at the end of oxygen exposure for the remaining 6 subjects is 9.3%, which agrees well with the predicted change of 8%.

Percent changes in VC and maximal mid-expiratory flow rate (FEF_{25-75}) during oxygen exposure at 2.5 ATA and during the early recovery period at 1.0 ATA for 1 of the 2 subjects with large changes are shown on Fig. 4. Both parameters decreased during the early exposure period, reversed partially, and then decreased prominently near the end of a 5.1 h exposure. Percent changes in VC and FEF_{25-75} at the end of oxygen exposure were 44 and 67%, respectively. During the first 1.5 h of recovery at 1.0 ATA, VC remained at about the same level, but FEF_{25-75} fell further to a decrement of 87 to 88%. Vital capacity recovered to a level 22% below the preexposure control value at 2 h of recovery and was decreased by only 5% at 5 h postexposure. Mid-expiratory flow rate increased only slightly at 2 h and then rose sharply to within 2% of the control value by 5 h of recovery. During the period of maximal pulmonary function impairment at 1 to 2 h of recovery, the subject had no chest pain and only mild cough and dyspnea. He was not wheezing and felt that he could inspire fully, but rapid expiration was very difficult for him.

The observed rapid rates of development and reversal of oxygen effects in this subject are more commonly associated with neurologic rather than pulmonary effects of oxygen toxicity and are consistent with an interaction of

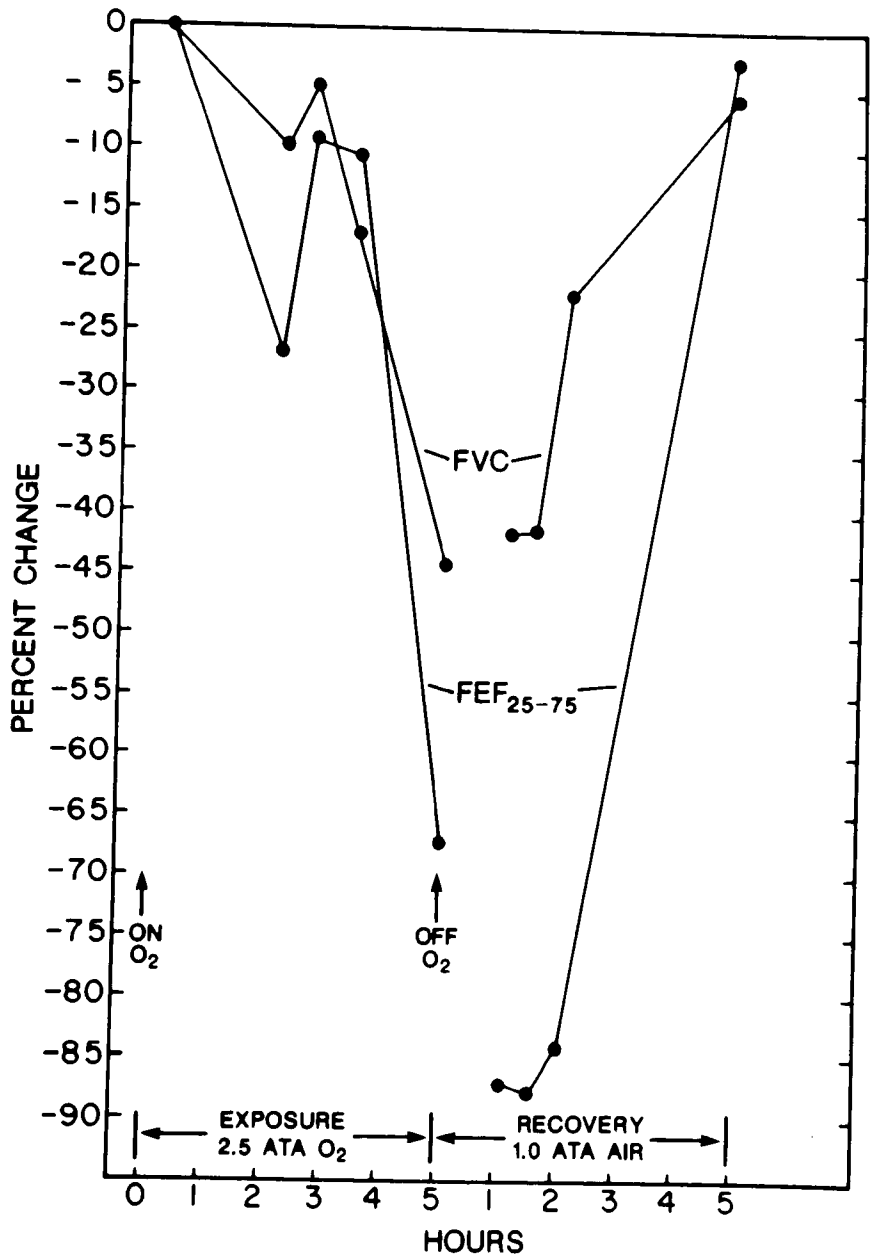

Fig. 4. Changes in VC and maximal FEF_{25-75} in 1 man during and after oxygen exposure at 2.5 ATA. Values obtained during a 5.1 h exposure and the first 5 h of recovery are shown. Control measurements at 2.5 ATA were obtained during early oxygen exposure. Preexposure control measurements at 1.0 ATA were used for the postexposure data.

toxic effects on the CNS and lungs (4, 7). Such an interaction could explain the observation that 2 of 8 subjects at 2.5 ATA developed pulmonary effects of oxygen toxicity much more rapidly than predicted from pulmonary tolerance curves derived from measurements at oxygen pressures of 2.0 ATA or less.

Patterns of Oxygen Effects on Lung Volumes and Flow Rates at Different Oxygen Pressures

Comparison of relative changes in selected lung volumes and flow rates after oxygen exposure at 3.0, 2.5, 2.0, and 1.5 ATA revealed that the changes observed after exposure at 3.0 and 2.5 ATA had a different pattern than those found after exposure at 2.0 and 1.5 ATA (Fig. 5). The measurements selected for comparison are the forced vital capacity (FVC), 1-s forced expired volume ($FEV_{1.0}$), FEF_{25-75}, and peak expiratory flow rate (PEFR). Although the changes observed after exposure at 3.0 ATA are generally the smallest in magnitude, their interrelationships are most consistent with an increase in small, peripheral airway resistance as a cause of the associated restriction in expiratory flow rates (8, 9). Large airway resistance, as measured in a body plethysmograph (10), was not significantly altered after any of the oxygen exposures.

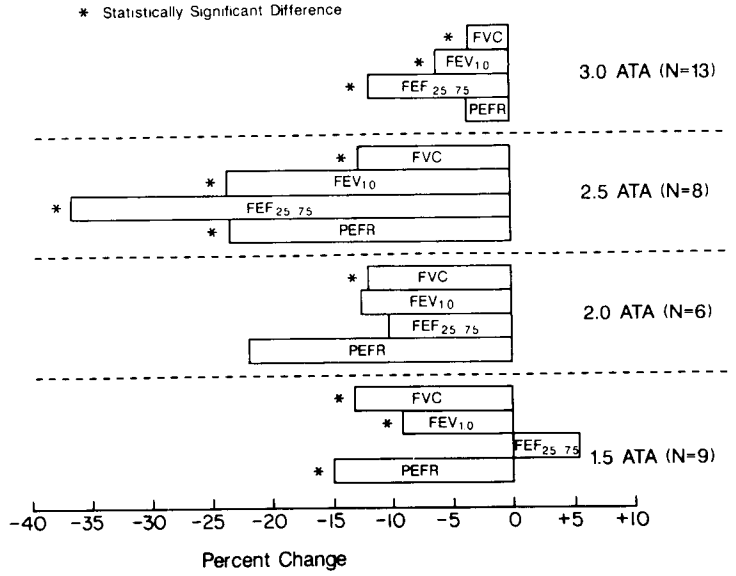

Fig. 5. Patterns of effects on lung volumes and flow rates after oxygen exposure at 3.0, 2.5, 2.0, and 1.5 ATA. Average changes in selected lung volumes and flow rates measured at comparable postexposure intervals after oxygen exposures at 3.0, 2.5, 2.0, and 1.5 ATA are shown. Changes in the same parameters measured near the end of oxygen exposure at 2.5, 2.0, and 1.5 ATA were larger, but the smaller postexposure values were used for comparison with the data for 3.0 ATA, where pulmonary function was not evaluated during exposure to allow more time for measurements of neurologic functions.

After exposure at 3.0 ATA, PEFR known to be highly effort dependent (11), was not significantly changed, whereas FVC had a statistically significant but quantitatively small reduction. The largest change was in the FEF_{25-75}, which is effort independent (11). In addition, the $FEV_{1.0}$ was reduced more than FVC. A similar pattern of effects was found after oxygen exposure at 2.5 ATA except that all changes were larger and PEFR was also significantly reduced.

It is of interest that FEF_{25-75} were not significantly reduced either during or after oxygen exposure at 2.0 and 1.5 ATA, despite the fact that these exposures caused the most prominent pulmonary symptoms and VC decrements. The observed patterns of effects are consistent with the interpretation that small airway function is impaired more selectively by oxygen exposure at 3.0 and 2.5 ATA than at 2.0 and 1.5 ATA.

Recovery of VC After Oxygen Exposure at 2.5, 2.0, and 1.5 ATA

Vital capacity was measured repeatedly at 1.0 ATA after oxygen exposure at 2.5, 2.0, and 1.5 ATA to determine rates of recovery (Fig. 6). The most rapid recovery occurred after exposure at 2.5 ATA, where an average VC decrement of 4.4% at 2.2 h postexposure was not statistically significant. Recovery of VC after exposure at 1.5 ATA was also relatively rapid. The average value was still significantly reduced by 7.4% at 13 h postexposure, but was slightly above the preexposure control at 24 h. Despite the similarity of pulmonary symptoms and VC changes at the termination of oxygen exposures at 2.0 and 1.5 ATA (Fig. 3), recovery was slower after the 2.0 ATA exposure. An average decrement of 6.6% at 24 h postexposure was statistically significant, but a 3.6% reduction at 48 h was not.

Performance of bronchoalveolar lavage at about 9 h after both the 2.0 and 1.5 ATA exposures provided additional evidence of different recovery states. This procedure is known to cause transient reductions in lung volumes and flow rates (12). The 2.0 ATA subjects had a reversal of recovery, as indicated by pre- and postlavage VC values of -12.9 and -20.3%, respectively, whereas the 1.5 ATA subjects continued to recover with pre- and postlavage changes of -12.7 and -7.4%, respectively.

Effects on Lung Compliance

Dynamic and static lung compliance were measured in a body plethysmograph with an intraesophageal balloon (13) before and after oxygen exposure at 2.5, 2.0, and 1.5 ATA. Dynamic lung compliance at breathing rates of 15, 30, and 60 was not reduced after oxygen exposure at any pressure.

Static lung compliance was not altered after oxygen exposure at 2.5 ATA, but it was significantly reduced after the 2.0 and 1.5 ATA exposures (Fig. 7). Again, exposure at 2.0 ATA for an average time of 9.7 h produced greater changes than exposure at 1.5 ATA for 17.6 h. Both overall lung compliance and specific lung compliance (compliance per liter of lung volume) were significantly reduced by 30.8 and 36.8%, respectively, after exposure at 2.0 ATA.

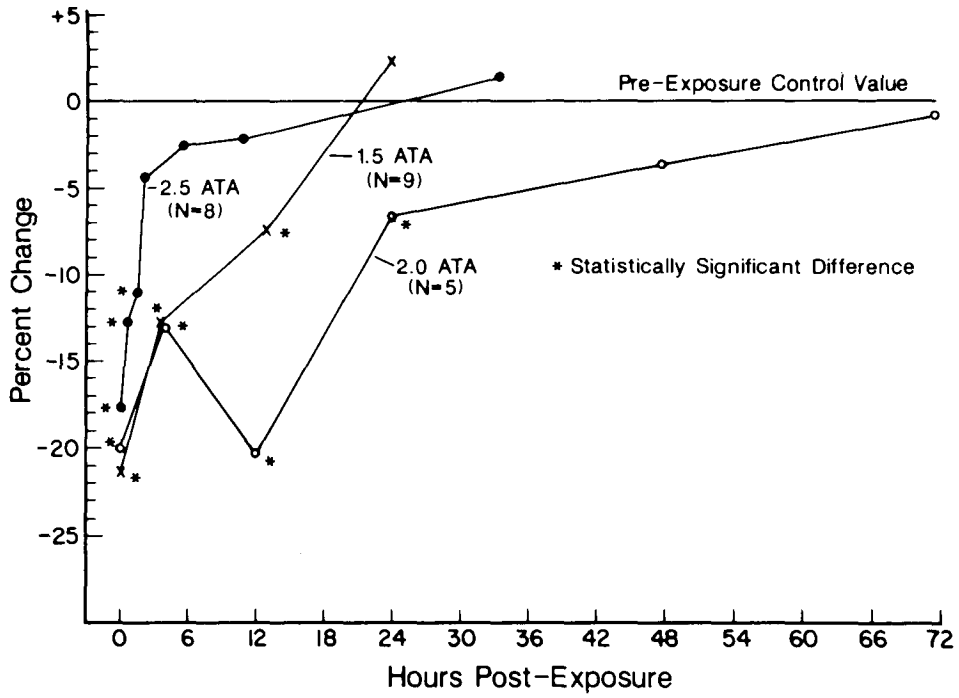

Fig. 6. Recovery of VC after oxygen exposure at 2.5, 2.0, and 1.5 ATA. Average percent changes in VC relative to preexposure control values are shown, and statistically significant differences are indicated. Recovery from pulmonary oxygen poisoning was followed more closely in these subjects than in any previous group.

Following the 1.5 ATA exposure, only the 25.7% reduction in specific lung compliance was statistically significant.

Effects on Pulmonary Diffusing Capacity for Carbon Monoxide

Average values of pulmonary diffusing capacity for carbon monoxide, measured by the single breath method (14), were significantly reduced after oxygen exposure at 2.5, 2.0, and 1.5 ATA, but not after the 3.0 ATA exposure (Fig. 8). The 3.0 ATA data indicate that oxygen effects on pulmonary gas exchange occur later than effects on lung volumes and flow rates during the progression of pulmonary intoxication at that pressure (15). Average carbon monoxide diffusing capacity after oxygen exposure at 1.5 ATA was significantly reduced only at 13 h postexposure, but after exposure at 2.0 and 2.5 ATA, average decrements, though small, were statistically significant for at least 8 to 9 d. The magnitude of change after exposure at 2.0 ATA exceeded that after exposure at either 1.5 or 2.5 ATA.

Because average values measured at 8 to 18 d postexposure had not fully returned to preexposure control levels, extended follow-up measurements were obtained from 2 wk to 5 mo. after oxygen exposure. Average values for the

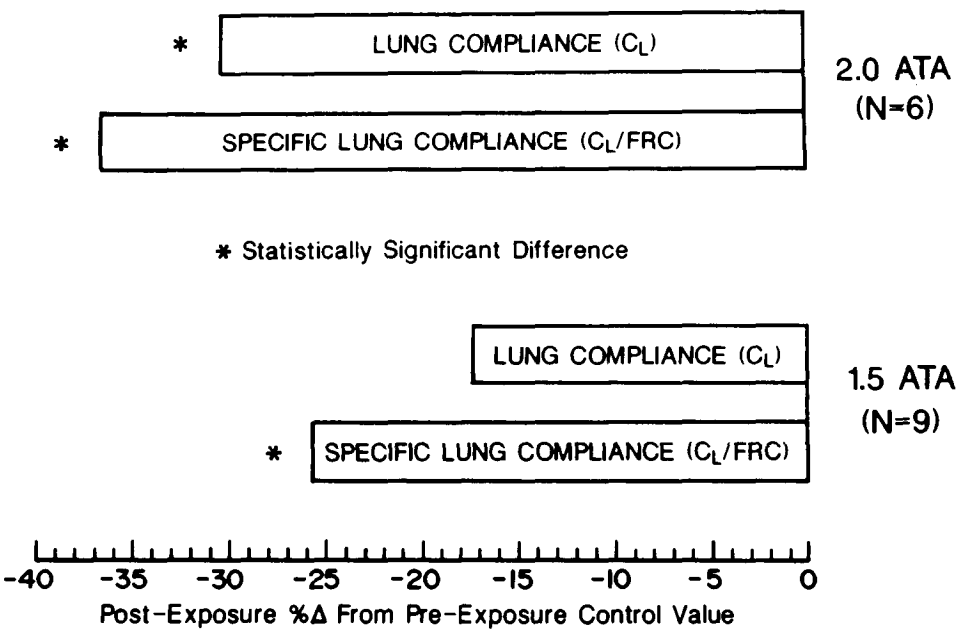

Fig. 7. Effects of oxygen exposure at 2.0 and 1.5 ATA on lung compliance. Static lung compliance was calculated from a plot of lung volume against intrathoracic pressure during slow, controlled inspiration. Measurements were obtained about 4 h postexposure in both series of experiments. Follow-up measurements at 3 to 5 wk postexposure were normal in all subjects.

subject groups exposed at 1.5 and 2.5 ATA had fully returned to the preexposure control level, whereas the diffusing capacity for the 2.0 ATA group was reduced by only 0.6 $ml \cdot min^{-1} \cdot mmHg^{-1}$ or less than 2% of the control value. Four of the 7 subjects in this group were not available for long-term follow-up, and it was necessary to use values obtained earlier in the postexposure period. Pulmonary diffusing capacity for carbon monoxide may prove to be a sensitive index of complete recovery from pulmonary oxygen poisoning.

SUMMARY AND CONCLUSIONS

Rates of pulmonary symptom intensification and decrease in VC are progressively increased with elevation of inspired oxygen pressure. Although VC decrements occur concurrently with symptoms, the lung volume changes become prominent when symptoms are still mild. In contrast to the progressive fall in VC with increasing pulmonary intoxication at each oxygen pressure that was studied, patterns of associated changes in other lung volumes and flow rates are not the same at each oxygen pressure. The observed patterns of effects are consistent with the interpretation that small airway function is impaired more selectively by oxygen exposure at 3.0 and 2.5 ATA than by exposure at 2.0 and 1.5 ATA.

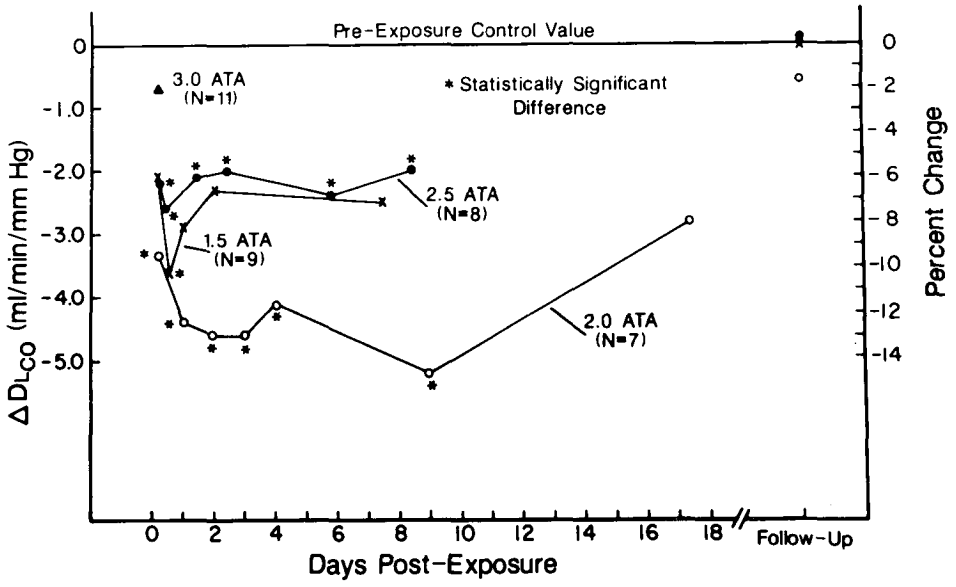

Fig. 8. Lung carbon monoxide diffusing capacity in man after oxygen exposure at 3.0, 2.5, 2.0, and 1.5 ATA. Average changes in carbon monoxide diffusing capacity relative to preexposure control values are shown, and statistically significant differences are indicated. Subsequent measurements of diffusing capacity in all subjects who were available for follow-up equaled or exceeded the preexposure control value.

Magnitude of decrement in vital capacity does not always reflect overall degree of pulmonary intoxication or subsequent rate of recovery. Despite similar VC changes after oxygen exposure at 2.0 ATA for nearly 10 h and exposure at 1.5 ATA for almost 18 h, the 2.0 ATA exposure caused greater impairment of pulmonary function and required a longer recovery period. Conversely, the rapid occurrence of large decrements in VC during oxygen exposure at 2.5 ATA was followed by an equally rapid rate of recovery after exposure termination. Rapid onset and reversal of oxygen effects on the lung at high pressures are consistent with interaction of neurologic and pulmonary effects of oxygen toxicity.

No single measure of pulmonary function is ideally satisfactory for monitoring rate of pulmonary intoxication during oxygen exposure or for tracking rate of recovery after exposure termination. In the absence of a single measure that accurately monitors progression and reversal of pulmonary oxygen poisoning, one possible approach involves the development of a composite index of pulmonary intoxication that incorporates multiple measures of pulmonary function. A second approach that can be used in lieu of or along with the first involves selective applications of one or more toxicity indices to specific conditions of exposure or recovery. An important objective in the continuing analysis of pulmonary data obtained during and after continuous

oxygen exposures at 1.5 to 3.0 ATA consists of testing the accuracy and precision of such methods for defining pulmonary oxygen tolerance in man.

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EFFECTS ON RESPIRATORY HOMEOSTASIS OF PROLONGED, CONTINUOUS HYPEROXIA AT 1.5 TO 3.0 ATA IN MAN IN PREDICTIVE STUDIES V

R. Gelfand, J. M. Clark, C. J. Lambertsen, and J. B. Pisarello

Acute hyperoxia at 1.0 to 3.0 ATA produces well-known effects on respiratory homeostasis and on the respiratory response to CO_2 (1) which are rapid in onset following administration of oxygen. These effects are mediated by a direct action of hyperoxia on peripheral respiratory chemoreceptors, and by an indirect action of hyperoxia on CNS acid-base balance. During spontaneous breathing of 100% oxygen, even at 1 ATA, electrical activity of the carotid body is diminished (2). Central venous P_{CO_2} rises due to reduction in the CO_2 buffering capacity of hemoglobin (1). The result of these two opposing actions on respiratory drive (decrement due to reduced peripheral input to the CNS; increment due to elevated P_{CO_2} in the CNS) is a net increase in ventilation and reciprocal decrease in arterial CO_2 tension (1). Furthermore, suppressed electrical activity of the peripheral chemoreceptors in hyperoxia contributes to a reduction in slope of the ventilatory response to CO_2 (respiratory CO_2 reactivity) (3). These "physiologic" effects of hyperoxia are considered modifications of otherwise intact physiologic processes, not the result of its "toxic" effects (1).

The several phases of Predictive Studies V (4) have involved prolonged exposures of men to continuous hyperoxia at 3.0, 2.5, 2.0, and 1.5 ATA for definition of CNS oxygen tolerance and for investigation of effects of prolonged hyperoxia on CNS and other organ functions (4-6). Its design provided an opportunity to search for toxic effects of supranormal oxygen pressures that might develop in respiratory functions only during extended exposures. Before these investigations there was virtually no information concerning such effects of prolonged toxic exposures to hyperoxia on respiratory functions in man. There is little current information even in animals (7, 8).

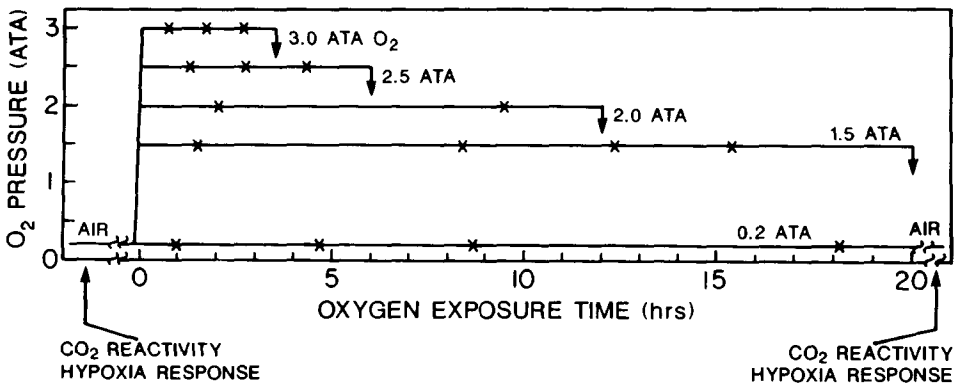
Ventilation and related parameters were measured in this program for several purposes. They were to: a) monitor the subject's respiratory status for safety, b) investigate potential changes in these parameters as possible indicators of hyperoxic effects on the integrated neural and neurochemical mechanisms of respiratory control, c) investigate changes in these parameters as potential indicators of hyperoxic effects on neuromechanical functions of the airways, lungs, and respiratory muscles, and d) investigate changes in ventilatory responses to hypercapnia and to hypoxia as potential indicators of hyperoxic effects on chemoreception of CO_2 and O_2.

Since changes in body temperature can alter ventilation (9), and hyperoxia has been associated with decrease in body temperature of rats (10), rectal temperature was monitored in all phases of Predictive Studies V, both as a potential indicator of CNS O_2 toxicity and for its potential effects as a modifier of O_2-related ventilatory change.

PROCEDURES AND METHODS

Oxygen Pressure-Time Exposures

The Predictive Studies V Program includes continuous hyperoxic exposures at pressures of 1.5, 2.0, 2.5, and 3.0 ATA, with normoxic controls at 1.0 ATA (Fig. 1). This summary report does not include information obtained at 2.0 ATA because that series is at present incomplete. Respiratory reactivity to CO_2 and hypoxic ventilatory response were measured at 1.0 ATA both before and after the exposures to hyperoxia at 1.5 and 2.5 ATA.



Respiratory-metabolic measurements during quiet period of EEG recording:

Ventilation	End-tidal P_{CO_2}
Tidal volume	Rate of CO_2 elimination
Frequency	

Fig. 1. Pressure-time "profiles" for Predictive Studies V investigations at 3.0, 2.5, 2.0, 1.5, and 0.2 ATA oxygen pressure. Symbols (X) indicate approximate times at which ventilatory measurements were made during quiet periods of EEG recording.

Procedures for Measurement of Ventilatory Parameters During Hyperoxic Exposures

Inspired respiratory flow, end-tidal P_{CO_2}, body temperature, and inspired oxygen concentration were monitored continuously. Expired gas was accumulated in a bag over a 3-min collection period for measurement of volume and analysis of gas composition. These collection periods were scheduled during predetermined "quiet" intervals when the subject was at rest for recording the EEG. These intervals were a component part of integrated "measurement modules," during which sequences of measurements were made related to a variety of physiologic systems in these investigations of specific organ oxygen tolerance (4). The pressure-time profiles indicating times at which resting ventilatory parameters were measured at each exposure pressure are shown in Fig. 1.

Apparatus for Measurement of Resting Ventilatory Parameters During Hyperoxic Exposures

Inspiratory flow for each breath was measured by pneumotachograph, recorded onto a magnetic tape recorder, and displayed on an oscilloscope for visual monitoring of flow pattern. Expired gas was collected into a meteorological balloon which was emptied promptly through the chamber hull to a dry gasometer for measurement of collected volume. A portion of this gas was bypassed through electronic gas analyzers to determine mixed expired CO_2 and O_2 concentrations. A sample of expired gas from each expiration was diverted from the space just distal to the expiratory check valve of the subject's lightweight oronasal mask (approx. 100 cc dead space) through the chamber hull to a rapid response CO_2 analyzer for measurement of end-tidal P_{CO_2}. The end-tidal values were recorded for all breaths as the peaks of the end-expiratory "washout" curves. Mean values of end-tidal P_{CO_2} for each expired-gas collection period were obtained from the largest peak values, corresponding to the largest tidal volumes in the period. This procedure minimized the effect of mask dead space and provided close agreement between end-tidal and arterial P_{CO_2} values. A sample of gas was taken from a fitting on the mask to a rapid response oxygen analyzer outside the chamber for measurement of inspired O_2 level. Any leak which might dilute the oxygen within the mask could thereby be detected rapidly. Chamber ambient and subject rectal temperatures were monitored with thermistor sensors.

Procedures and Apparatus for Pre- and Postexposure Measurement of Ventilatory Responses to Hypercapnia and to Hypoxia at 1.0 ATA

Preexposure ventilatory responses to progressive hypercapnia and to progressive hypoxia of supine subjects were determined in duplicate, in a laboratory room at 1.0 ATA by rebreathing methods (11, 12), an hour or more following a light breakfast. They preceded the 1.5 ATA exposures by about 6 h and were 10 h before exposure at 2.5 ATA. Postexposure measurements were obtained 2 or 3 h after exposure at 1.5 or 2.5 ATA, respectively. Little or no

sleep was possible in the intervening period, and sustenance was primarily a 5% dextrose solution by continuous intravenous infusion, supplemented by light carbohydrate snacks.

RESULTS

Focus in this summary report is on comparison of mean values at the different hyperoxic pressures investigated. Results are presented in Fig. 2 for ventilation, end-tidal P_{CO_2}, and rate of CO_2 elimination; in Fig. 3 for ventilatory responses to CO_2; in Fig. 4 for ventilatory responses to hypoxia; and in Fig. 5 for body temperature changes.

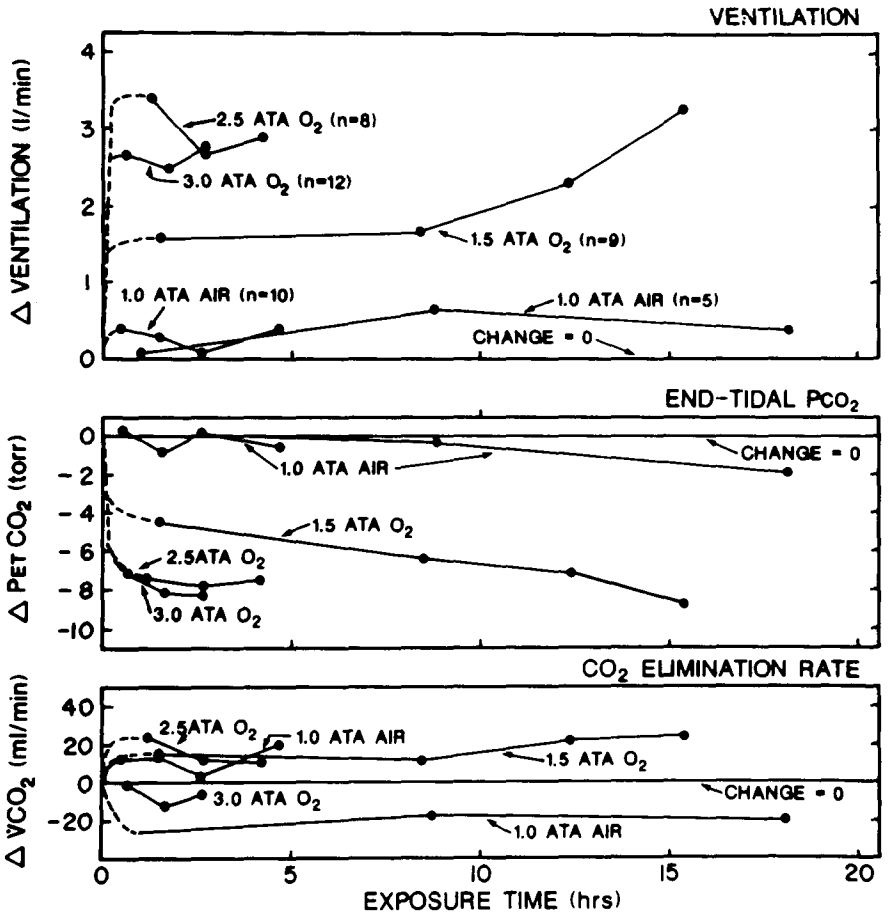


Fig. 2. Effects of oxygen at 0.2, 1.5, 2.5, and 3.0 ATA on ventilation, end-tidal P_{CO_2}, and rate of CO_2 elimination. Changes are from preexposure reference levels in air at 1.0 ATA.

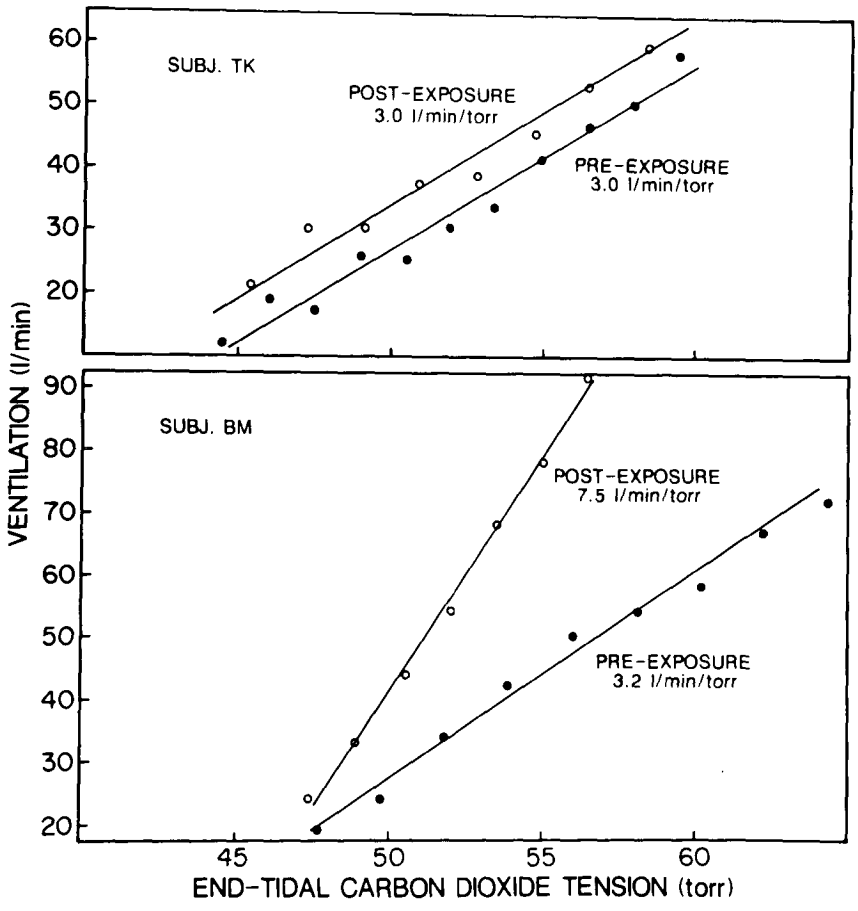


Fig. 3. Effects of hyperoxia on ventilatory response to CO_2 in 2 subjects following exposure at 2.5 ATA O_2. See text for explanation.

Ventilation, End-Tidal P_{CO_2}, and CO_2 Elimination Rate

Increments in mean values of ventilation and decrements in mean values of end-tidal P_{CO_2} occurred during hyperoxia at 1.5, 2.5, and 3.0 ATA (Fig. 2). The increased ventilation was greater in magnitude early in exposure at 2.5 and 3.0 ATA than it was early in the 1.5 ATA exposures. Correspondingly, the decrements in end-tidal P_{CO_2} were greater at 2.5 and 3.0 ATA than they were early in exposure at 1.5 ATA.

These changes were of the same magnitude and did not progress with time at the 2.5 and 3.0 ATA pressures. In contrast, although the changes in ventilation and end-tidal P_{CO_2} at 1.5 ATA were smaller in magnitude than were those at 2.5 and 3.0 ATA early in exposure, these changes did become greater over time. By the end of the 1.5 ATA exposures, the increment in ventilation and decrement in P_{CO_2} were as great as those at the higher

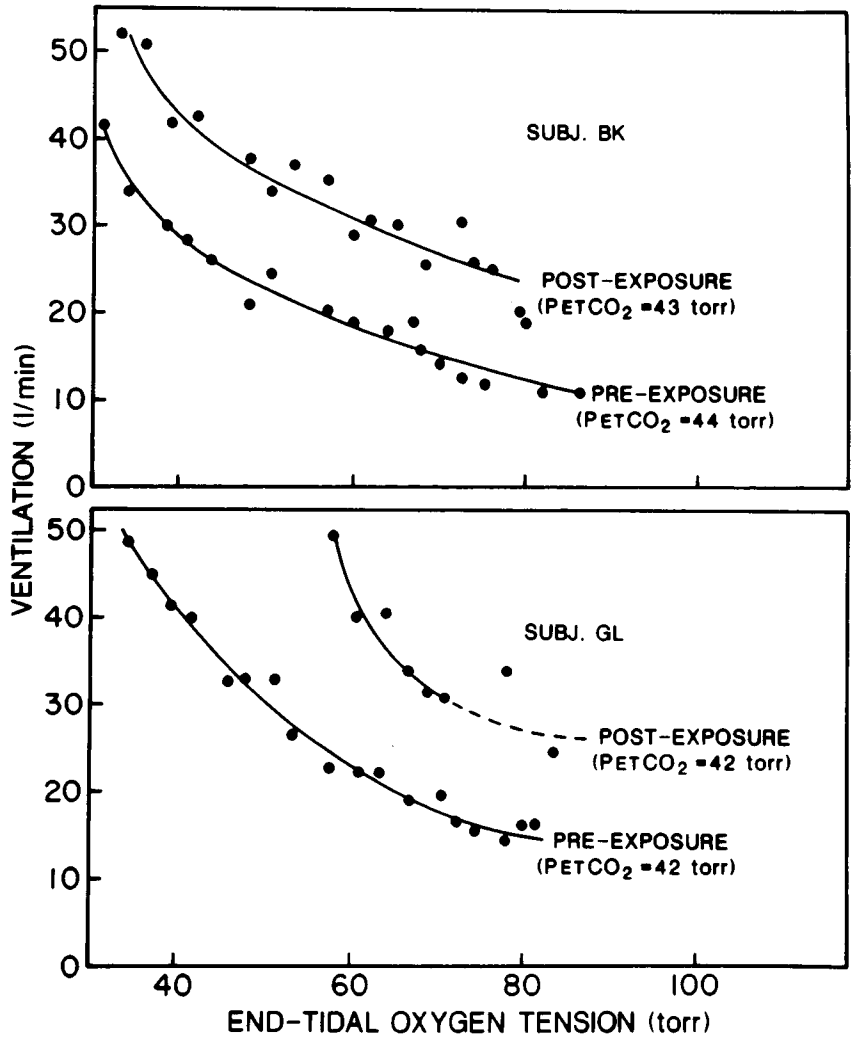


Fig. 4. Effects of hyperoxia on hypoxic ventilatory response in 2 subjects following exposure at 1.5 ATA O_2. See text for explanation.

pressures. There were relatively small increments in mean values of ventilation and correspondingly small changes in mean values of end-tidal P_{CO_2} during normoxic control "exposures."

Changes in rate of CO_2 elimination were small and not progressive at any of the exposure conditions. This parameter was sufficiently stable to not influence the effects of hyperoxia on ventilatory or thermal homeostasis.

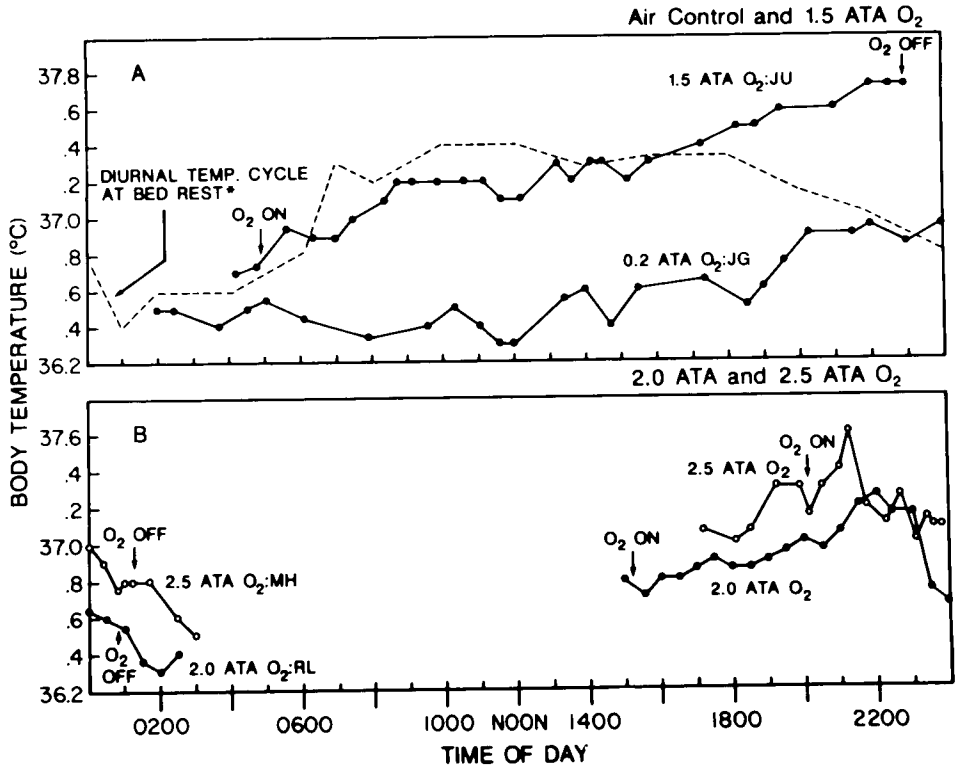


Fig. 5. Body temperatures of individual subjects during exposures. Overall patterns of change are typical for the subjects at each oxygen pressure. See text for explanation.

Respiratory CO_2 Reactivity

Following exposures to oxygen at 0.2, 1.5, and 2.5 ATA, mean values of respiratory CO_2 reactivity were increased above preexposure values for the 1.5 and 2.5 ATA exposure groups. Mean pre- and postexposure reactivities were 3.3 and 4.9 liter•min^{-1}•Torr^{-1} P_{CO_2}, respectively, for the 1.5 ATA exposure group. Corresponding values for the 2.5 ATA group were 3.1 and 4.8 liter•min^{-1}•Torr^{-1} P_{CO_2}. Individual results for 2 of the subjects from this group are shown in Fig. 3 to illustrate the range of responses among subjects. For subject TK (*top*) there was no change in reactivity, whereas for subject BM (*bottom*), postexposure reactivity was more than doubled. Reactivity was increased by 100% or more in 4 of the 1.5 ATA and in 4 of the 2.5 ATA O_2 subjects, whereas in the other subjects changes were small. All the individual postexposure respiratory CO_2 reactivities observed fall into the range of responses of healthy subjects (13). Respiratory CO_2 reactivities measured on subsequent days in the 2.5 ATA O_2 group were back at the preexposure control level.

Ventilatory Responses to Progressive Hypoxia

In no subject was the hypoxic ventilatory response abolished. The postexposure hypoxic ventilatory responses were usually displaced above the preexposure curves after exposure at 1.5 ATA O_2, but not after the 2.5 ATA exposures. There was no increase in degree of curvature in some subjects (Fig. 4, *top*). Other subjects had an increase in degree of curvature (Fig. 4, *bottom*). Quantitative analyses of the hypoxic ventilatory responses, which were obtained before and after the exposures to 0.2, 1.5, and 2.5 ATA O_2, are not currently available. By inspection, the degree of curvature (related to hypoxic respiratory sensitivity) may be increased somewhat in 4 of 9 subjects following exposure to 1.5 ATA O_2, and in 3 of 8 subjects following exposure to 2.5 ATA O_2. Of these 7 subjects with possibly increased hypoxic sensitivity, 6 also had increased respiratory CO_2 reactivity.

Thermal Homeostasis

With a single exception, patterns of change in body temperature were similar for individual subjects of the 1.0 ATA air exposure series and the 1.5 ATA O_2 exposure series. Individual patterns of temperature change are shown in Fig. 5; *A* shows temperature "profiles" for single subjects at 1.0 ATA air and at 1.5 ATA O_2, along with diurnal temperature change of normal individuals on bed rest (14), with nocturnal sleep. The natural diurnal cycle is absent for both the subject at 1.0 ATA air and at 1.5 ATA O_2. The patterns of change at these two pressures are virtually identical. In contrast to the increments in body temperature at 1.0 ATA air and 1.5 ATA O_2, body temperatures decreased following the initiation of hyperoxia at 2.0 and 2.5 ATA (Fig. 5 *B*).

DISCUSSION

Altered respiratory homeostasis during prolonged hyperoxia was evident in the subjects at each of the pressures investigated, in the form of increased ventilation and decreased $P_{ET_{CO_2}}$. The initial changes, which persisted, were presumably physiologic in origin (1). Within the preplanned pressure time limits selected for these exposures, the observed effects were not of sufficient magnitude to impair function. However, continued hyperoxic exposure beyond the limits of these investigations could result in progressive changes of functional significance.

Persistent mild increment in ventilation and decrement in end-tidal P_{CO_2} during hyperoxia at 3.0 and 2.5 ATA were similar in magnitude to those seen in man during shorter exposures to oxygen at 3.5 ATA (15). Although these changes did not progress over the hyperoxic exposures in the majority of subjects at 2.5 and 3.0 ATA (Fig. 3), functionally significant changes in respiratory parameters did develop progressively over the 30 min preceding onset of seizure in the single subject who experienced an O_2-related convulsion at 3 h of exposure at 3.0 ATA (4, 6, 16).

The smaller early exposure increment in ventilation during hyperoxia at 1.5 ATA most likely reflects lesser intensity of the same effects that caused ventilation to increase at 2.5 and 3.0 ATA. The progressive increase in ventilation later in the 1.5 ATA exposure reflects either progressive development of toxic effects of hyperoxia directly on mechanisms concerned with the regulation of respiration, or indirect effects related to progressive increase in severity of pulmonary symptoms (5). It is predictable that the difference in gas density between air at 1.0 ATA (reference level) and oxygen even at 3.0 ATA had only a negligible effect on ventilation (17).

The decrease in end-tidal P_{CO_2} observed at 1.5, 2.5, and 3.0 ATA (Fig. 2, *center*) were entirely a consequence of increased ventilation, since the rates of CO_2 elimination (Fig. 2, *bottom*), and most likely the metabolic rates as well, did not change significantly during hyperoxia as compared to preexposure levels. This current observation for prolonged hyperoxia confirms an earlier observation for a shorter exposure to oxygen at 3.5 ATA (15).

Respiratory reactivity to carbon dioxide is reduced below air-breathing levels when it is measured *during* brief exposures to hyperoxia (3), due to suppression by hyperoxia of peripheral chemoreceptor activity (2) and possibly due to reduction of central respiratory chemoreceptor reactivity as well (18). For several reasons, respiratory CO_2 reactivity could not be measured *during* the oxygen exposures of Predictive Studies V; therefore the effect of hyperoxia on respiratory CO_2 reactivity during prolonged oxygen breathing at 1.5 and 2.5 ATA cannot now be assessed.

Sleep deprivation has been reported to reduce hypercapnic ventilatory response to CO_2; 24 h of sleeplessness reduced reactivity by 24% in 13 healthy men (19). This was not confirmed in the 5 subjects of the 1.0 ATA air × 20 h experiment series of Predictive Studies V. Nevertheless the potential effect of sleep deprivation must be considered in investigations involving prolonged periods of wakefulness.

It is important to note that ventilatory responses to progressive hypoxia were not abolished in any of the subjects of Predictive Studies V. Abolition of carotid body reactivity to hypoxia as well as its associated ventilatory response has been reported for cats exposed to oxygen at 1.0 ATA for 65 h (20). Rather, the enhanced hypoxic responses (Fig. 4) observed for some of the subjects of the 2.5 and 1.5 ATA O_2 exposure series could be related to increased input from peripheral receptors or enhanced central responsiveness to peripheral stimuli.

Altered thermal homeostasis manifested as progressive drop in body core temperature with rate of fall proportional to degree of hyperoxia has been reported for rats (10). A similar effect was expected to be more difficult to detect in man due to immensely greater body mass. Initially, body temperature was recorded only intermittently during the 3.0 ATA O_2 exposures which initiated Predictive Studies V, because it was felt that 3.5-h exposure was too brief to allow any derangement of thermal homeostasis to be expressed. Observed temperature changes were unremarkable until a distinct fall in body

temperature was observed in the single subject who experienced an oxygen-related seizure in the periods preceding and following the convulsion (4, 16). Continuous recordings of body temperature were made in all subsequent exposures. Preliminary analysis indicates that thermal homeostasis may be altered in man by hyperoxia at oxygen pressures greater than 1.5 ATA (Fig. 5). Since rate of CO_2 elimination did not change over the exposure periods (Fig. 2), the observed decreased temperatures seem unrelated to metabolic changes. The observed small decrements are not physiologically important, and are of no significance to use of hypoxia in therapy or in decompression for increased rate of inert gas elimination.

SUMMARY

Altered respiratory homeostasis was evident during exposures at 2.5 and 3.0 ATA O_2 as nonprogressive increment in ventilation and reciprocal decrement in $P_{ET}CO_2$. During exposure at 1.5 ATA O_2 these changes were progressive. Mean values of respiratory reactivity to CO_2 were somewhat increased following prolonged hyperoxia at 1.5 and 2.5 ATA, as compared to preexposure mean values. Hypoxic ventilatory response was unchanged or enhanced after oxygen exposures at 1.5 and 2.5 ATA. Observed respiratory and body temperature changes were not of sufficient magnitude to impair function.

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HUMAN CIRCULATORY RESPONSES TO PROLONGED HYPERBARIC HYPEROXIA IN PREDICTIVE STUDIES V

J. B. Pisarello, J. M. Clark, C. J. Lambertsen, and R. Gelfand

In its various applications, oxygen breathing in man is limited by toxic effects that become more severe as inspired oxygen pressure and exposure duration are increased. Although all tissues, organs, and systems are potentially susceptible to oxygen effects, only pulmonary and CNS manifestations of oxygen poisoning have been studied extensively during long exposures. With respect to cardiocirculatory phenomena during oxygen breathing in man, most previous studies have focused on acute responses (1, 2), whereas those investigations that contained prolonged exposures have not included detailed evaluation of circulatory parameters (3, 4). Consequently, relatively little is known about physiologic and toxic effects of prolonged hyperbaric oxygen exposure on the cardiocirculatory system in man.

This summary report presents selected results of cardiocirculatory measurements in healthy volunteers who breathed 100% O_2 continuously at 3.0 ATA for up to 3.5 h, at 2.5 ATA for up to 6.0 h, at 2.0 ATA for up to 11.9 h, and at 1.5 ATA for up to 19.0 h. These data were collected as part of a broad program of investigation (Predictive Studies V) designed to measure effects of hyperbaric hyperoxia on human organ systems and functions (5-7).

METHODS

Each experiment included air breathing, sea-level control measurements before measurements during oxygen breathing at increased pressure. During each exposure to hyperoxia, cardiovascular data were obtained repeatedly: early in the oxygen exposure to investigate acute responses and later in the oxygen breathing period to evaluate effects of prolonged exposure (5). All measurements reported here (unless noted otherwise) were performed at rest in the

supine position, after a period of stabilization.

Cardiovascular Measurements

Cardiac electrical activity was continuously monitored during each exposure and stored on magnetic tape for later computer analysis. Electrical impedance cardiography (8) was utilized to follow changes in cardiac output throughout the exposure. Arterial blood pressure was measured intermittently by the cuff method during the 3.0 ATA series and by indwelling catheter in the 2.5 ATA, 2.0 ATA, and 1.5 ATA series. Systemic vascular resistance was calculated from cardiac output and mean blood pressure data, assuming constant right atrial pressure. Values are expressed as percent changes from preexposure values.

Responses to Active Standing

Blood pressure and heart rate responses to active standing (9) were measured before and during the oxygen exposures at 2.5, 2.0, and 1.5 ATA to investigate the effect of hyperbaric O_2 breathing on autonomic modulation of orthostatic reflexes. To perform this measurement, ECG and arterial blood pressure were continuously recorded while the subject actively rose from the supine to the upright position in less than 5 s. Instantaneous heart rates while supine and during the initial 60 to 90 s after active standing were obtained from an ECG-activated cardiotachometer. The pattern of this response has been shown previously to be a reproducible phenomenon in the evaluation of baroreflex autonomic function (9).

Liver Blood Flow

To study possible oxygen effects on regional hepatic circulation, liver blood flow was measured as the rate of indocyanine (ICG) clearance (10) at sea level and during early and late oxygen exposure at 2.0 and 1.5 ATA. For each flow measurement, 0.5 mg/kg of ICG was injected i.v. while the subject was resting in a supine position. Blood sampling was performed at 0, 1, 2, 3, 6, 9, 12, 15, 18, and 21 min from the indwelling arterial catheter. Plasma concentration of ICG was determined spectrophotometrically, using the subjects preinjection arterial sample as a blank to construct a standard calibration curve.

RESULTS

Cardiovascular Oxygen Tolerance Limits at 3.0, 2.5, 2.0, and 1.5 ATA

In the 3.0 ATA series of exposures, all but 2 were continued to the predetermined maximum duration of 3.5 h. One of the 2 exceptions was terminated at 2.5 h because of excessive bradycardia, and the other was stopped at 3.0 h upon the onset of an oxygen convulsion. Two exposures at 2.5 ATA and 1 at 1.5 ATA were stopped at 5.5, 5.8, and 17.7 h, respectively, when the subjects developed asymptomatic, unifocal, ventricular ectopic activity of

increasing frequency. All remaining exposures at 2.5, 2.0, and 1.5 ATA that were terminated before predetermined maximum durations were stopped on the basis of pulmonary symptoms and/or changes in lung volumes and flow rates.

Effects on Resting Heart Rate and Cardiac Rhythm

As observed by others during short hyperbaric oxygen exposures (1, 2), initial responses in the 3.0, 2.5, and 2.0 ATA series were characterized by average reductions in sinus node frequency discharge of approximately 6 to 12 beats/min. An initial average reduction of 3 beats/min was observed in the 1.5 ATA series (Table 1). Sinus arrhythmia, sinus pauses, and occasional periods of nodal control occurred early in the exposures at 3.0, 2.5, and 2.0 ATA (Fig. 1). These phenomena were usually transient and were not associated with symptoms in any subject except the 1 at 3.0 ATA who developed progressive, severe bradycardia. Immediately after termination of oxygen breathing at 2.5 h, this subject had a sinus pause of 13 s in association with a 20-s period of syncope. Sinus rhythm returned promptly at the rate of 60/min and, within 20 s, he became mentally alert. No further ECG abnormalities occurred, and postexposure measurements were completed.

Table 1

Resting Heart Rate During Oxygen Breathing in Man, Beats/Min, Mean ± SE

Pressure	Preexposure	Early Exposure	Late Exposure
3.0 ATA ($n = 12$)	60.1 ± 4	54.2 ± 3	53.0 ± 2
2.5 ATA ($n = 8$)	57.3 ± 2	45.0 ± 2*	47.1 ± 2*
2.0 ATA ($n = 7$)	59.0 ± 4	50.0 ± 4	63.5 ± 2**
1.5 ATA ($n = 7$)	59.1 ± 5	56.2 ± 3	64.2 ± 5

* $P < 0.05$ compared to preexposure. ** $P < 0.05$ compared to early exposure.

In contrast to the sustained decreases in resting heart rate observed at 3.0 and 2.5 ATA, initial decrease in sinus frequency discharge at 2.0 and 1.5 ATA was followed after about 3 h of exposure by progressive acceleration. At 9.8 h of exposure in the 2.0 ATA series, pulse rate was significantly greater than the initial oxygen exposure value (Table 1). Along with increased frequency of sinus discharge at 2.0 and 1.5 ATA, sinus pauses disappeared and periods of nodal control occurred. In some cases, atrial and ventricular premature depolarizations were observed during the later part of the 2.5, 2.0, and 1.5 ATA exposures (Fig. 2). This ectopic activity was infrequent and not associated with symptoms during the 2.0 ATA exposure series. However, in 1 subject at 1.5 ATA, and in 2 others at 2.5 ATA, exposures were terminated because of increasing numbers of unifocal, premature ventricular depolarizations. None of the 3 subjects had associated symptoms, and the continuous monitoring demonstrated complete disappearance of ectopic phenomena within 3 h of exposure termination.

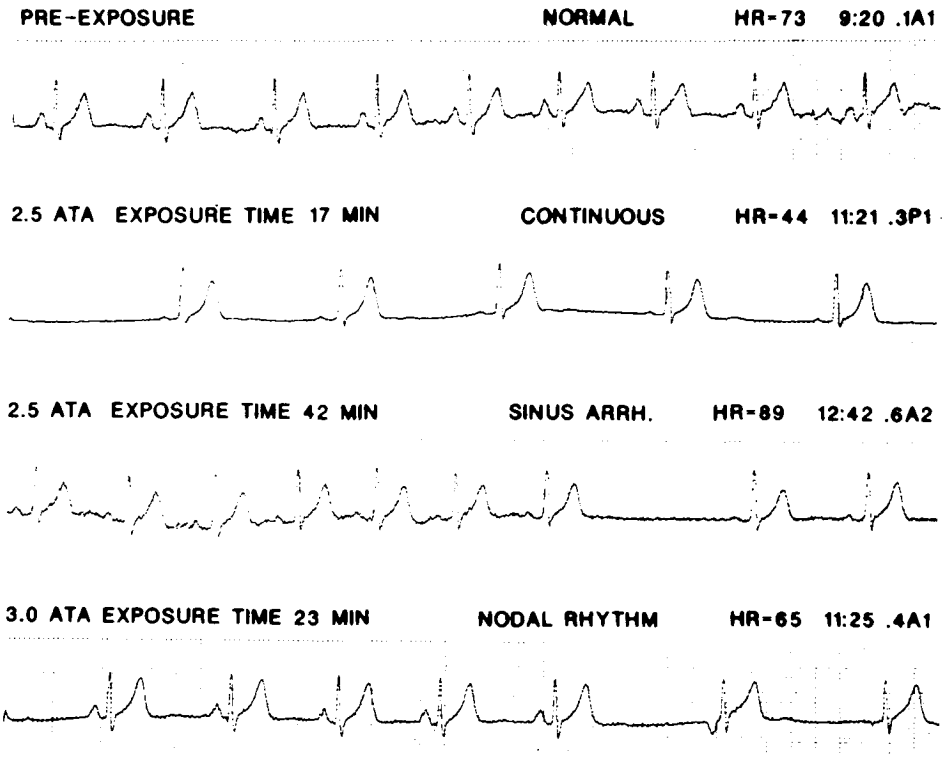

Fig. 1. Electrocardiographic features during the 1st h of oxygen exposure in man at 2.5 and 3.0 ATA. Top to bottom: normal sea-level tracing, sinus bradycardia, sinus arrhythmia, and nodal escape (last two beats).

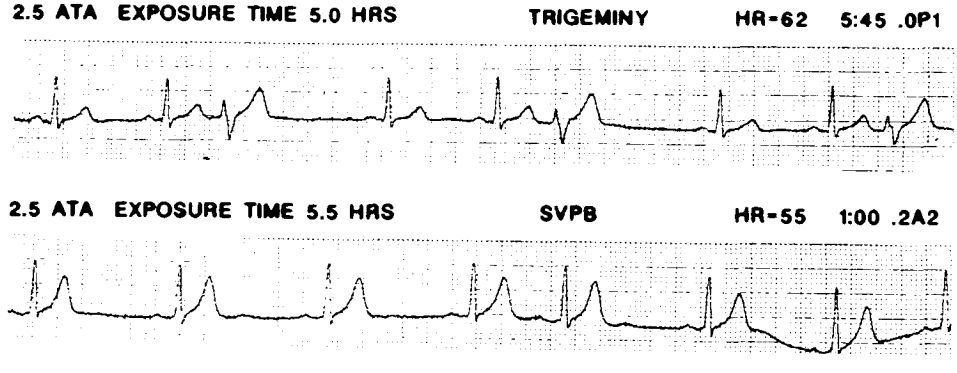

Fig. 2. Electrocardiogram of a subject exposed to oxygen at 2.5 ATA. Ectopic premature beats of ventricular and supraventricular origin are demonstrated in the top and bottom tracings, respectively.

Effects on Cardiac Output and Regional Hepatic Circulation

Resting cardiac output variations during oxygen breathing in general paralleled variations in heart rate (Table 2). Initial decrements were observed at all four pressures, with progressive late increments during the 2.0 and 1.5 ATA exposures, in association with accelerations of pulse rate. These changes were of small magnitude and not statistically significant.

Table 2
Cardiac Output During Oxygen Breathing in Man, % Variation

Pressure	Early Exposure, %	Late Exposure, %
3.0 ATA ($n = 12$)	- 8	- 9
2.5 ATA ($n = 7$)	- 15	- 10
2.0 ATA ($n = 6$)	- 13	- 6
1.5 ATA ($n = 7$)	- 16	- 7

Liver blood flow determinations by the green-dye clearance technique at 1.5 and 2.0 ATA demonstrated no changes in this parameter during early or late oxygen exposure. Results of the 1.5 ATA exposures are shown in Fig. 3.

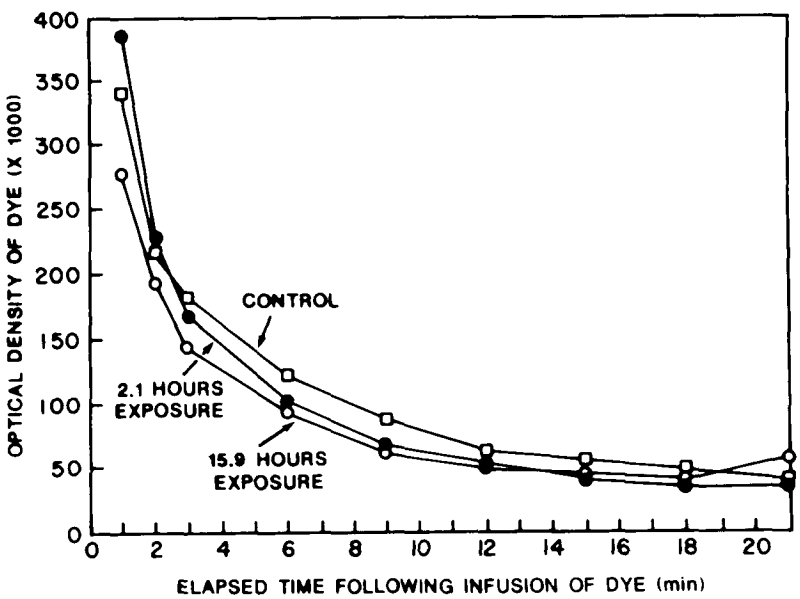

Fig. 3. Indocyanine dye clearance curves reflecting hepatic blood flow at sea level and after 2.1 and 15.0 h of oxygen breathing at 1.5 ATA in man. There are no statistical differences among curves. Average data for 7 subjects are shown.

Effects on Arterial Blood Pressure and Systemic Vascular Resistance

Average changes in resting arterial blood pressure during early oxygen exposure at 3.0, 2.5, and 1.5 ATA indicated decreases of 0.5, 6.2, and 7.7 mmHg, respectively, and an increase of 12.2 mmHg at 2.0 ATA (Table 3). Only the decrement at 2.5 ATA was statistically significant. During late oxygen exposure at 3.0, 2.5, and 2.0 ATA, average values of mean arterial pressure exceeded preexposure control values by 4.4, 1.0, and 3.4 mmHg, respectively (Table 3). The late exposure value at 1.5 ATA was 4.6 mmHg less than control. None of these late exposure changes was statistically significant.

Table 3
Mean Arterial Blood Pressure During Oxygen Breathing in Man, mmHg, Mean ± SE

Pressure	Preexposure	Early Exposure	Late Exposure
3.0 ATA ($n = 12$)	90.0 ± 4	89.5 ± 3	94.4 ± 4
2.5 ATA ($n = 7$)	94.9 ± 4	88.7 ± 3*	95.9 ± 2
2.0 ATA ($n = 6$)	84.4 ± 3	96.6 ± 4	87.8 ± 8
1.5 ATA ($n = 7$)	97.3 ± 4	89.6 ± 3	92.7 ± 4

* $P < 0.05$ compared to preexposure value.

Average calculated values of systemic vascular resistance during early oxygen exposure at 3.0, 2.5, 2.0, and 1.5 ATA were increased by 8, 9, 31, and 6%, respectively (Table 4). During late oxygen exposure, values of systemic vascular resistance increased further to 16 and 11% above control values at 3.0 and 2.5 ATA, respectively, and decreased at 2.0 ATA to 10% with respect to control values. None of these changes was statistically significant.

Table 4
Systemic Vascular Resistance During Oxygen Breathing in Man, % Variation

Pressure	Early Exposure, %	Late Exposure, %
3.0 ATA ($n = 12$)	+ 8	+ 16
2.5 ATA ($n = 7$)	+ 9	+ 11
2.0 ATA ($n = 6$)	+ 31	+ 10
1.5 ATA ($n = 7$)	+ 6	+ 1

Hemodynamic Responses to Active Standing

Normal acceleration of heart rate in response to an abrupt transition from supine to standing positions occurred during both early and late oxygen exposure in all 9 subjects studied at 1.5 ATA and in 6 of the 7 subjects exposed to oxygen at 2.0 ATA. However, in 1 of the 2.0 ATA subjects, complete blunting of the normal tachycardic response to active standing was observed in follow-up measurements 2 h after termination of an 8-h exposure

(Fig. 4). This event was not associated with symptoms, and arterial blood pressure did not fall.

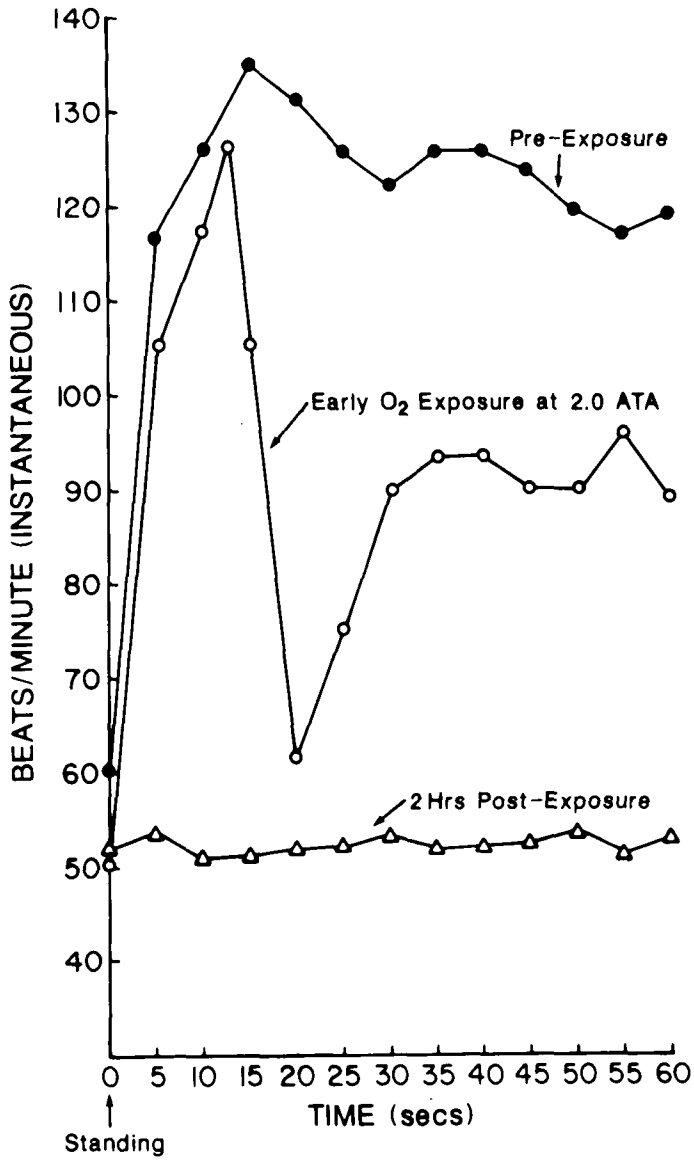

Fig. 4. Immediate heart rate responses to standing in 1 subject before, during, and after 8 h of oxygen breathing at 2.0 ATA. Note the complete blunting of this response in the postexposure curve. Despite failure of pulse rate acceleration, blood pressure in the standing position was maintained and the subject experienced no symptoms.

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THE SUZANNE KRONHEIM MEMORIAL LECTURE, 1986

9th International Symposium on Underwater and Hyperbaric Physiology
Undersea and Hyperbaric Medical Society, Bethesda, MD, 1987

WALLACE FENN, THE MAP MAKER

H. Rahn

It was my great pleasure to review with you an imaginary book entitled "An Atlas of Gas Exchange" (Fig. 1). It is a book that might have been edited by Wallace O. Fenn, Distinguished University Professor of Physiology at The University of Rochester, New York, were he alive today. The chapters and illustrations are by his many disciples. As the contents indicate, it will take us on many journeys into strange lands, using the map and the roads which he first discovered and which his disciples all over the world have followed and embellished to understand basic rules of gas exchange.

Fenn always saw the big picture, the whole world. So let us start with a global view of gas exchange (Fig. 2). What you see beyond the Earth is not a satellite. It represents all the free gas on our planet gathered into a volume at 1 atmosphere and has a diameter of 1200 miles (1). The internal sphere has a diameter of 740 miles and represents oxygen. Upon liquefaction these diameters shrink to 130 and 80 miles, respectively (1). If you now spread out this world of gas in two dimensions (Fig. 3), devoted entirely to the metabolic gases, you have a map in which longitude is expressed in units of O_2 tensions and latitude in units of CO_2 tensions. Along the periphery of this map you see the not-too-well-defined borders of the Forbidden Zones, CO_2 Narcosis, O_2 Toxicity, Acapnia, and Hypoxia. The circle represents our home, the normal lung composition of warm-blooded animals including man. Such a map beckons the adventurer to explore all directions of the compass.

How do you travel when you leave home, what are the laws that dictate the routes of travel, what rules must you observe, where on this map is your performance impaired, and how far can you penetrate into the forbidden zones and still return? Many of these questions were answered in the form of equations. But Fenn showed us how to graph them on the O_2-CO_2 diagram, which helped others to easily understand them, use them, and make their own

An Atlas of Gas Exchange
Editor: Wallace O. Fenn
Illustrators: His Disciples

Content

Journey to Mt. Everest
Journey into the Deep
Journey into the Lung
Journey into the Egg
Journey from Water to Land
Journey from Land to Water

Fig. 1.

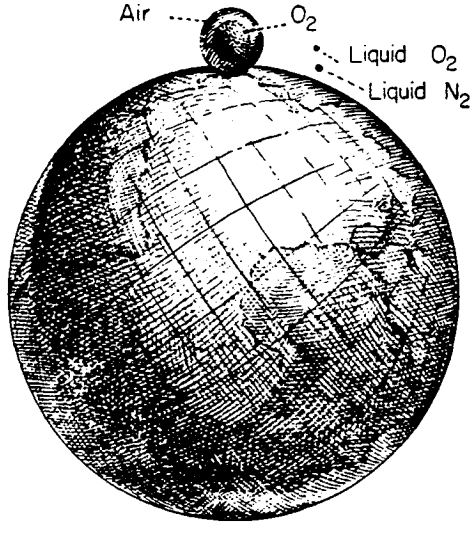

Fig. 2.

explorations and predictions. Today I want to briefly review some of these explorations that his disciples have made.

The O_2-CO_2 diagram was introduced by Fenn to respiration physiologists exactly 40 yr ago in a paper entitled "A theoretical study of the composition of the alveolar air at altitude" (2). It had a most unusual introduction, stating that the "oxygen-carbon dioxide diagram [is] a very great aid in accurate thinking. It has been useful in so many different problems that we consider it worthwhile to describe it in this paper so that it can be made available to others." This was a most unusual and in retrospect a rather prophetic statement from an author publishing his very first paper in the area of respiration physiology, at the age of 53.

But the circumstances were also unusual because by the time Wallace Fenn (Fig. 4) began his studies in respiration he had just been elected to the National Academy of Sciences for his contributions in muscle and electrolyte physiology. He was the first to demonstrate that muscle shortening is an active process, now known as the Fenn Effect, and ushered in the era of electrolyte physiology, demonstrating that potassium is not locked up in muscle during contraction but is lost in exchange with sodium and that excitation is coupled to changes in membrane permeability, a powerful and far-reaching pronouncement 50 yr ago. Today these are accepted concepts that students in medicine learn during their first year.

During the mid 1950s, after putting his indelible stamp on pulmonary gas exchange and lung mechanics [for review *see* Otis and Rahn (3)], Fenn became intrigued with two new frontiers—man's exploration in space and in ocean depths. For Fenn, every problem of applied human physiology was "considered basic if the investigator put some basic thinking into it." His last benchwork was on the partial molar volume concepts for determining the volume O_2 occupies in hemoglobin when subjected to high pressures. His last theoretical study predicted the changes in partial pressures of oxygen dissolved at great depth. This was shortly before he died at the age of 78.

His long and active career brought him many honors and recognitions. The one that probably gave this shy and modest man his greatest reward was his election as President of the International Union of Physiological Sciences to bring together for peaceful discussions thousands of physiologists from all countries of the world. [For further details *see* Biographical Memoirs of W. O. Fenn (4)].

RULES OF THE ROAD

Before we journey forward we must first understand the "rules of the road" (5). One way to start is to first locate the variables that determine our resting alveolar gas values, our home (Fig. 5). Why is our home at a P_{CO_2} of 40 and a P_{O_2} of 100? Given a mixed venous point, $\bar{v}$, on the one hand and an inspired point, P_I, on the other as well as an RQ of 0.85, one can plot only two RQ lines, a gas line and a blood line. Where they intersect at P_A is

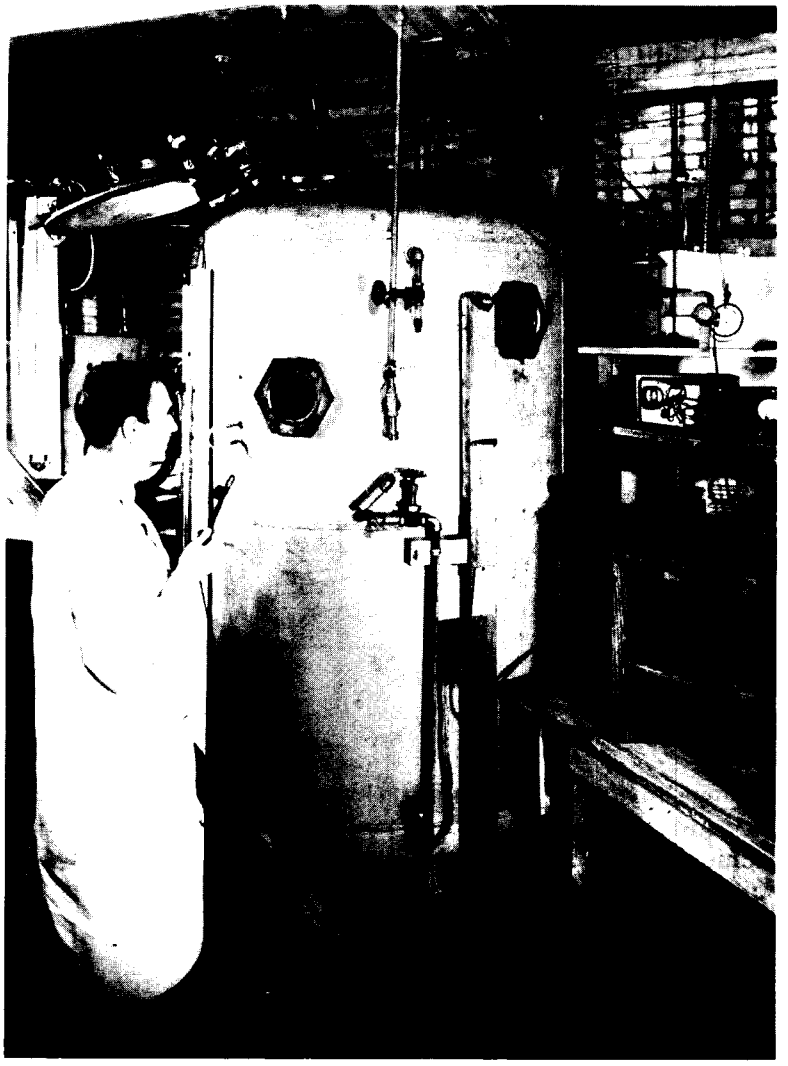

Fig. 6.

But below you see another line. These points are from mountain climbers after acclimation. These could go much higher. They obviously ventilated more, had lower P_{CO_2} and higher P_{O_2} at any altitude. The last two points are from 23,000 ft. But look at the P_{CO_2}—15 Torr. Could this be right? At that time many people doubted it. It meant that they were breathing at rest 2.5 times more than at sea level. And what about people on Everest? Extrapolating, one might predict a P_{CO_2} of about 10 Torr to survive—but was this possible? A resting ventilation 4 times normal?

For the answer we had to wait 30 yr. Here you see the circle in John

West's slide (Fig. 8). Yes—it was about 8 Torr (7). It meant that they were ventilating 5 times normal. Remember that, for I will bring it up later.

JOURNEY INTO THE DEEP

Let us now go in the opposite direction—holding our breaths under water. Here you see 2 diving patterns of the Ama of Japan and Korea (8) harvesting the ocean floor (Fig. 9). In Fig. 10 you see Dr. Hong instructing an Ama how to deliver her alveolar gas sample into this special device while she is at the bottom. I can assure you that that was not easy. Here you see the alveolar pathway for a 10- and a 20-m dive (Fig. 11). During descent the lung volume is compressed, all gas tensions increase, and all gases pass from the lung to the blood. Once on the bottom, O_2 values fall. However, during ascent from 10 m the lung volume must double and triple when ascending from 20 m, suddenly reducing the P_{O_2}, in these two examples to less than 30 Torr. Such a value is most likely less than the mixed venous blood and temporarily causes a reversal of the O_2 transport. This is the dangerous period, when ascent blackout occurs, and is responsible for many accidents among breath-hold divers (9). How small the margin is between safe and impaired performance at the end of a dive is shown in Fig. 12 which depicts regions of performance based on psychomotor tests. This invaluable diagram was mapped by Wallace Fenn (10). The point on the right is the average alveolar value upon return from dives between 10 and 27 m (11). The arterial saturation is 80% and the performance level is adequate. On the left you see the average value of 11 dives where the alveolar P_{O_2} was 27 Torr and the arterial saturation 55%. Obviously these divers were very close to blackout and unconsciousness.

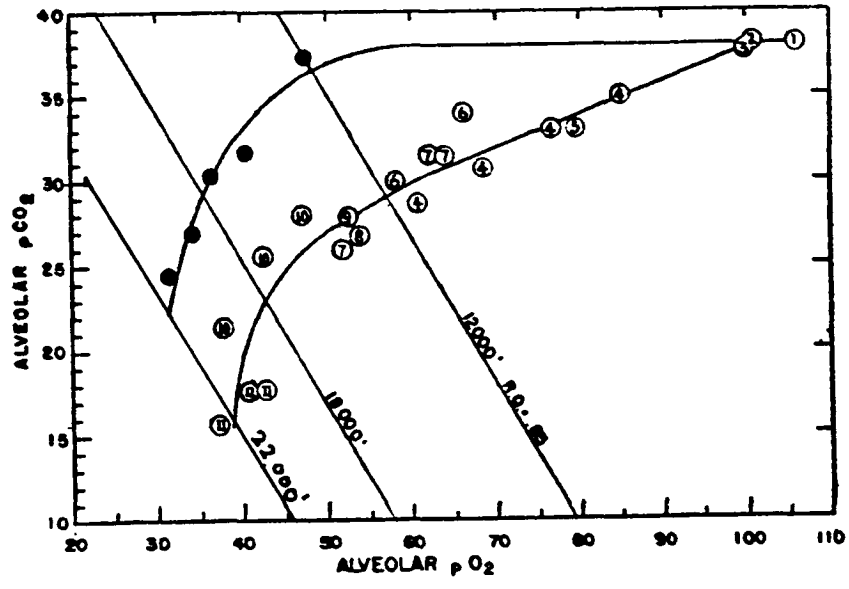

Fig. 7.

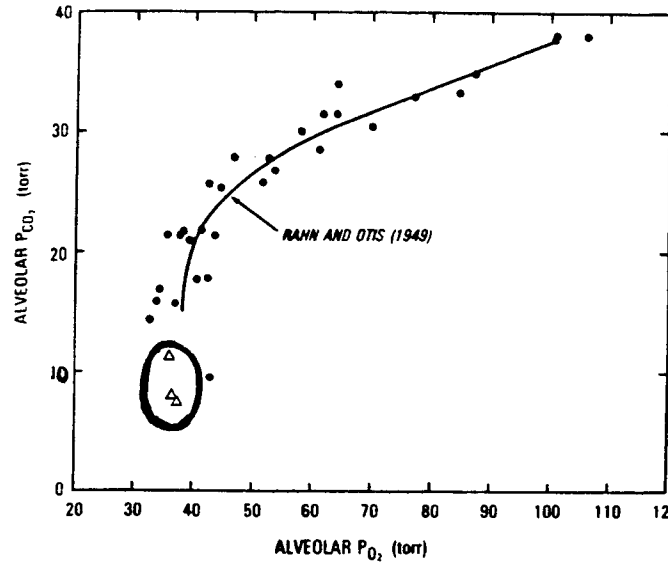
ALVEOLAR P_{CO_2} (torr)
ALVEOLAR P_{O_2} (torr)
RAHN AND OTIS (1949)
0
10
20
30
40
20
30
40
50
60
70
80
90
100
110
120

Fig. 8.

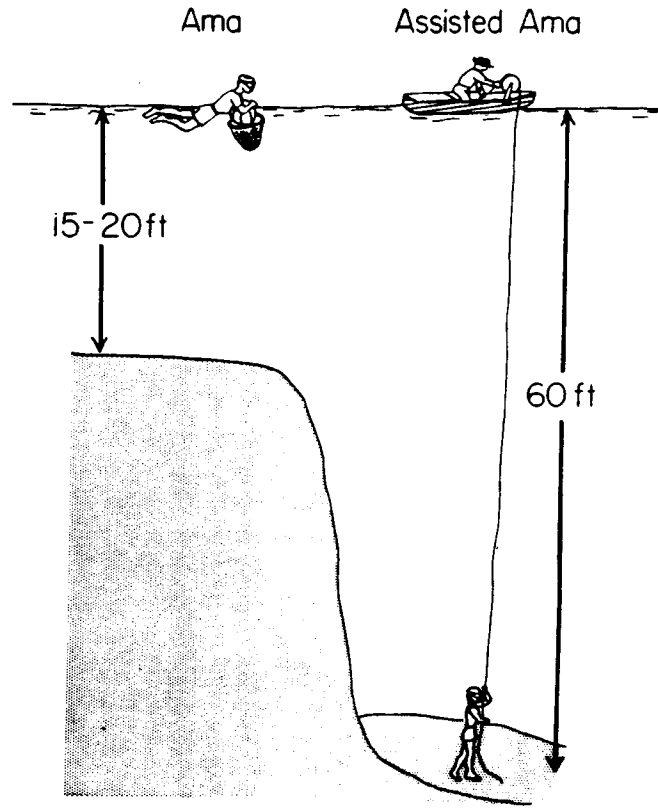
Ama
Assisted Ama
15-20ft
60ft

Fig. 9.

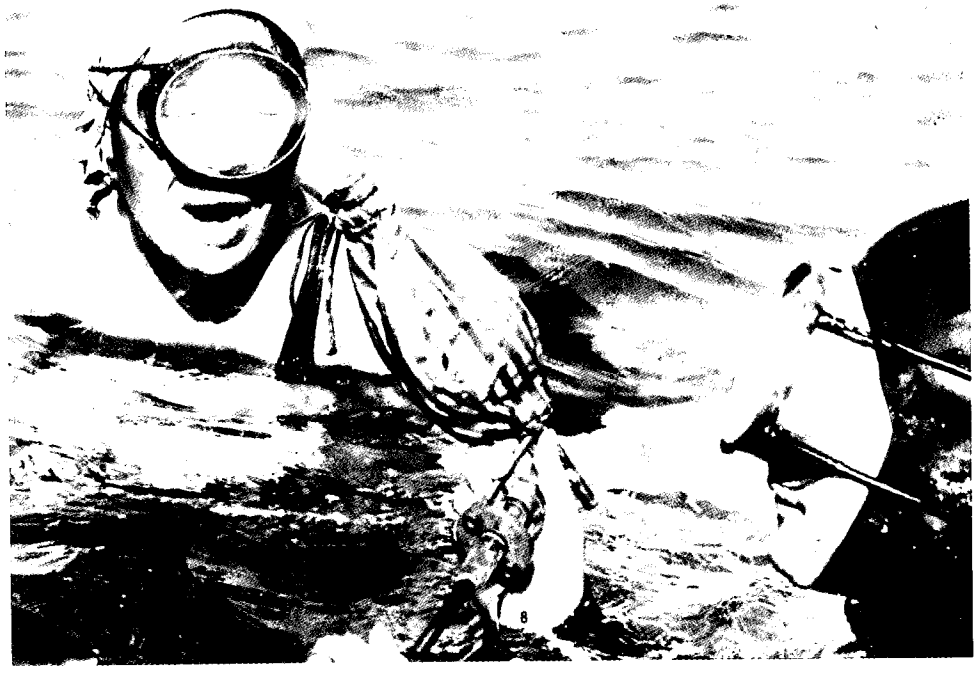

Fig. 10.

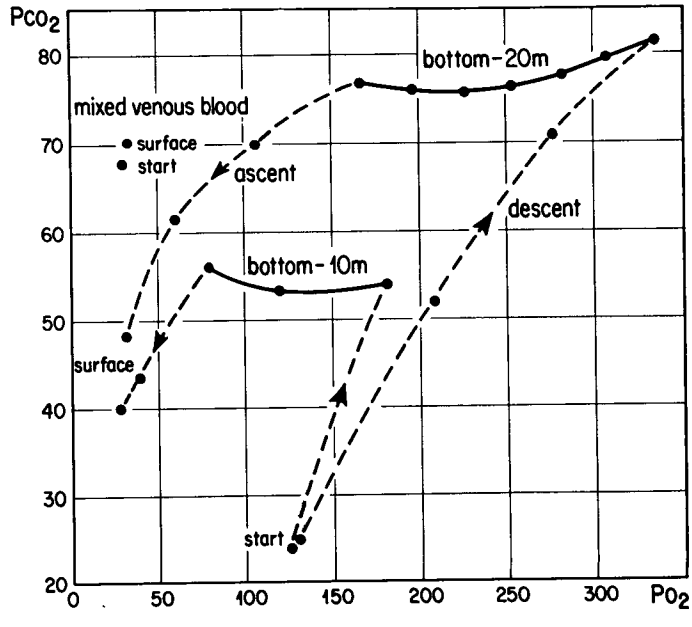
Pco2
80
70
60
50
40
30
20
0
50
100
150
200
250
300
Po2
bottom-20m
mixed venous blood
surface
start
ascent
descent
bottom-10m
surface
start

Fig. 11.

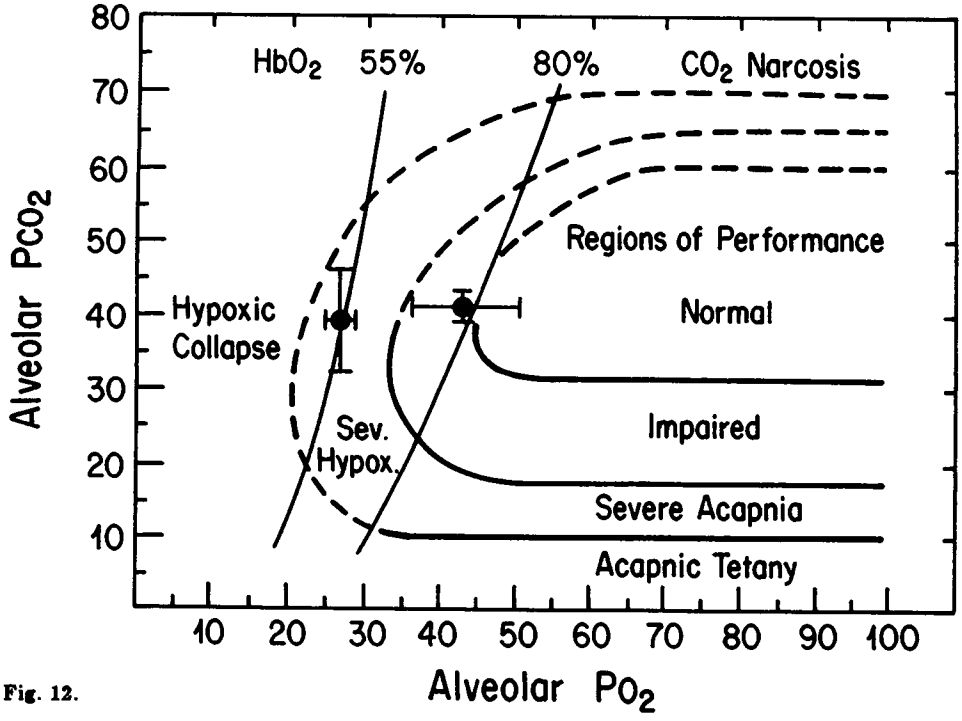

Fig. 12.

Has the deepest breath-hold dive been achieved in the competition between Mayol of France and Maiorca of Italy? Mayol recorded a depth of 105 m and Maiorca is not far behind. In contrast to Mt. Everest, the alveolar O_2 at these depths must be the highest that man has experienced without resorting to exposures in a hyperbaric chamber or to breathing oxygen. These changes in alveolar O_2 during a 100-m dive have recently been modeled (12). In Fig. 13 you see change in alveolar P_{O_2} arriving at 100 m after 110 s. You will notice that all O_2 values, regardless of the O_2 consumption, reach a value above 1 atm on the bottom, in contrast to the values depicted below by the *heavy line* which represents the average value of a similar breath-hold for 9 subjects at the surface (13). What happens upon ascent when the lung volume expands? This is shown for the last 60 s in Fig. 14. At the *arrow*, alveolar P_{O_2} falls below the mixed venous level and reversal of O_2 flux is initiated.

JOURNEYS INTO THE LUNG

How does the gas composition in our lung vary among the 200 million alveoli, which most likely are not equally ventilated or perfused. The O_2-CO_2 diagram became an important road map in the solution of this question because the answer, at that time, could not be solved by equations but only graphically. Here you see a graphic solution (Fig. 15). Using the roads that Fenn had charted and locating the mixed venous and inspired air point, there was only

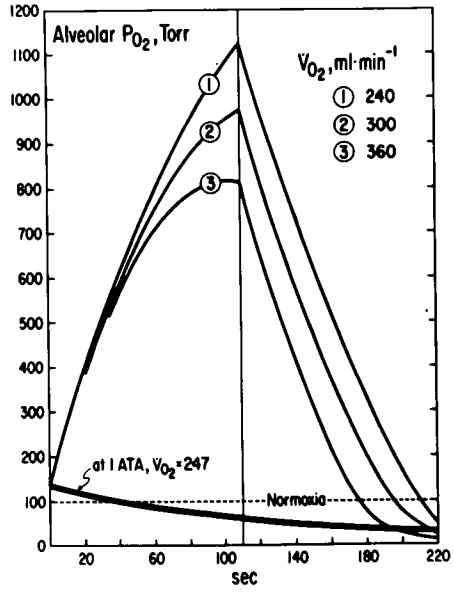

Fig. 13.

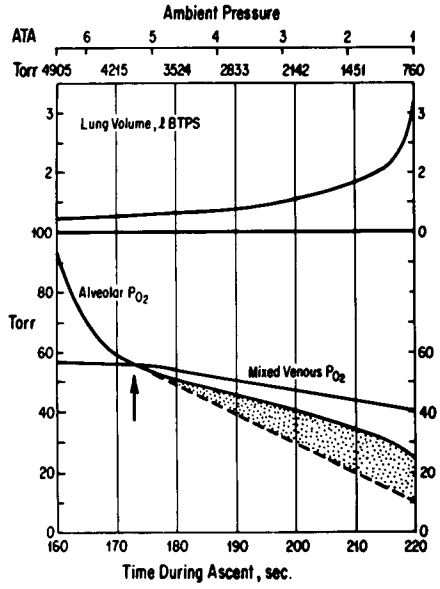

Fig. 14.

one line depicting all the possible combinations of O_2 and CO_2 which could possibly exist. Furthermore, it showed us that any point on this line was the consequence of a given ventilation:perfusion ratio which, theoretically, ranged from 0 at the mixed venous point to infinity at the inspired point (5).

This was the diagram 37 yr ago, and in 1961 it attracted John West who in London had measured the regional differences of V_A/Q in man as shown in the *upper part* of Fig. 16. I invited him to come to Buffalo to translate his observations with the help of the O_2/CO_2 diagram. In Fig. 16 you see his classic analysis, with which you are all familiar (14).

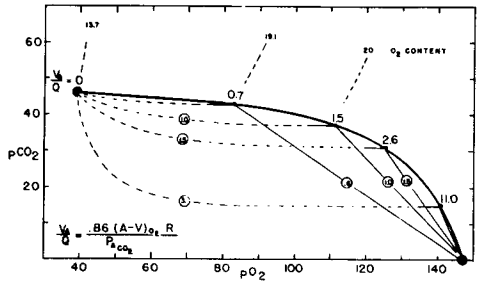

Fig. 15.

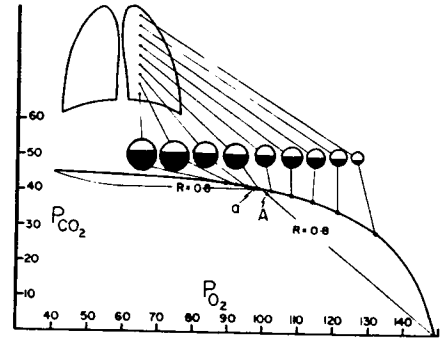

Fig. 16.

JOURNEY INTO THE CHICKEN EGG

The VA/Q approach to analyzing lung function has now become a cornerstone in pulmonary physiology. However, during the last 10 yr it has also become clear that alveolar bulk flow may not penetrate into the peripheral areas of our alveoli. Rather, it looks as if gas-phase diffusion may be the ultimate transport in our alveoli. For example, in Fig. 17 you see the time it takes for an O_2 molecule to travel from one bronchiole to its successive daughter branches. As you approach the alveoli, the volume of conducting airways increases and the time for convective transport takes longer and longer. On the other hand, as the daughter branches become shorter, the time for diffusive transport becomes shorter. It would appear that diffusive transport dominates near the alveolar level.

However, to study the role of gas-phase diffusion in the lung is difficult. So we went to the chicken egg where all transport is by diffusion through pores. These I have visualized for you (Fig. 18) by pressurizing under water the gas space of an egg. You see how many pores it has—10,000—each with a radius equal to that of a red blood cell. Sotherland et al. (15) developed a method that showed that the local gas tensions between the shell and the capillaries varied along the axis of the egg (Fig. 19). This space is analogous to our alveolar space. When these values are transferred to the O_2-CO_2 diagram (Fig. 20), you see a diagram similar to that of the lung (Fig. 16). The solid curve represents all the possible gas tensions that can theoretically exist. However, the curve is not a VA/Q line but rather it is the first gas-phase diffusion-perfusion line. If gas-phase perfusion is the ultimate transport in our alveoli, maybe these parameters dictate in part the regional gas tensions in our lung.

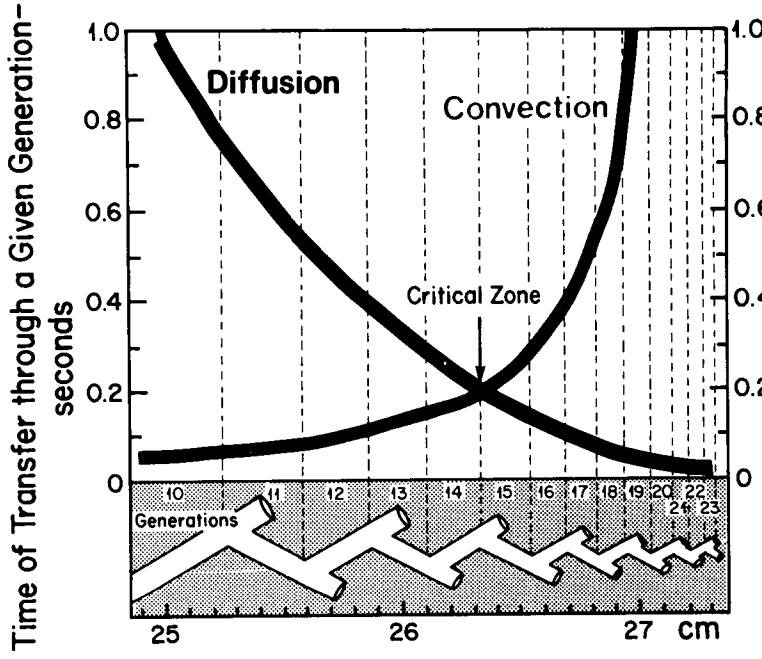
Time of Transfer through a Given Generation-seconds
1.0
0.8
0.6
0.4
0.2
0
Diffusion
Convection
Critical Zone
10
11
12
13
14
15
16
17
18
19
20
22
24
23
Generations
25
26
27 cm

Fig. 17.

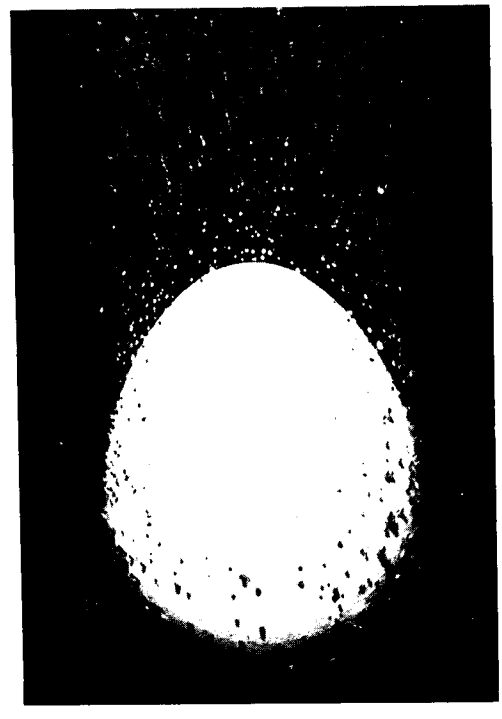

Fig. 18.

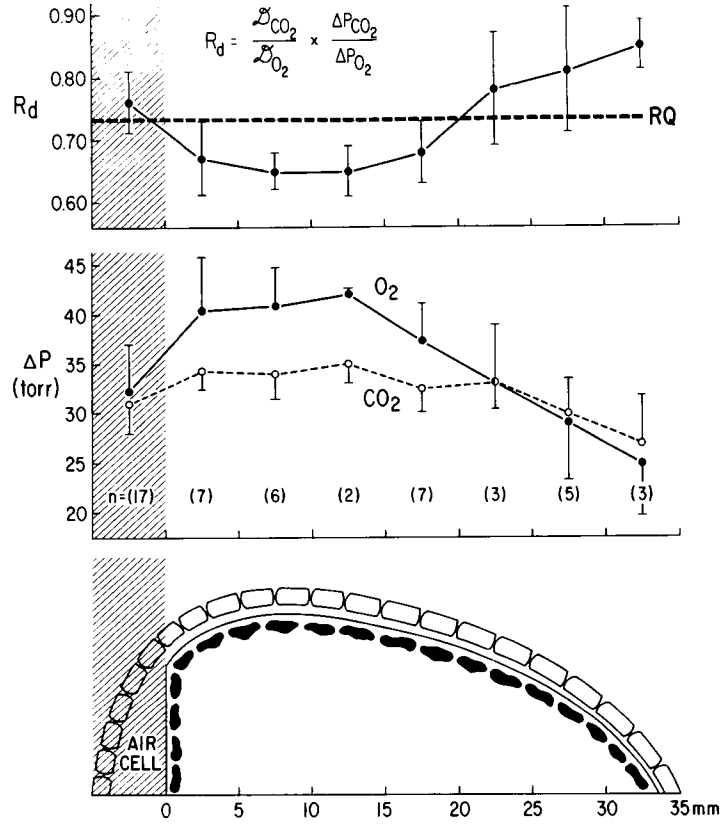
0.90
0.80
0.70
0.60
Rd
$R_d = \frac{\dot{V}_{CO_2}}{\dot{V}_{O_2}} \times \frac{\Delta P_{CO_2}}{\Delta P_{O_2}}$
RQ
45
40
35
30
25
20
ΔP (torr)
O2
CO2
n=(17)
(7)
(6)
(2)
(7)
(3)
(5)
(3)
AIR CELL
0
5
10
15
20
25
30
35mm

Fig. 19.

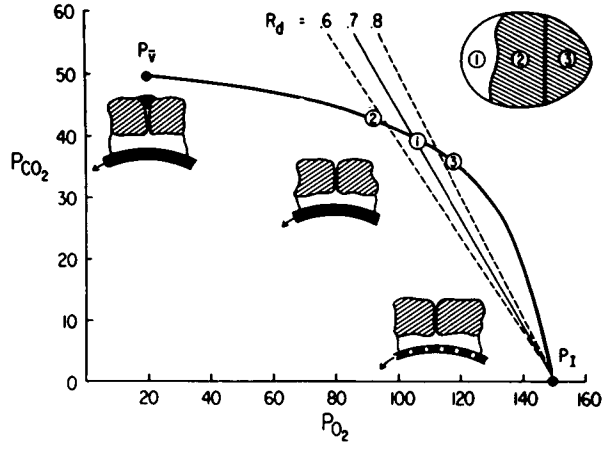
60
50
40
30
20
10
0
P_{CO_2}
R_d = .6 .7 .8
$P_{\bar{v}}$
P_I
20
40
60
80
100
120
140
160
P_{O_2}

Fig. 20.

JOURNEY FROM WATER TO LAND

What about our ancestors, the water-breathing fish? What were the respiratory problems as they made their transition from water to land, from "fish to philosopher." For orientation we must first establish water breathers on the O_2-CO_2 diagram (16). In Fig. 21 you see for the same RQ the O_2 and CO_2 tensions for air and water breathers. The latter are confined to the *lower curve* because in a water medium O_2 solubility is some 20 times less than CO_2 (in air O_2 and CO_2 solubilities are the same). Thus the typical CO_2 tension in fish must always be less than 5 Torr. Furthermore, fish must ventilate about 10 times more than air breathers to extract the same amount of oxygen.

Let me return briefly to our man on Mt. Everest who is breathing 5 times more than at sea level, with a P_{CO_2} of 8 Torr (Fig. 22). He has almost become a fish which breathes 10 times more with a P_{CO_2} = 4 Torr. The ventilatory response on Mt. Everest is due to the low O_2 tension, but in water is due to the low O_2 solubility.

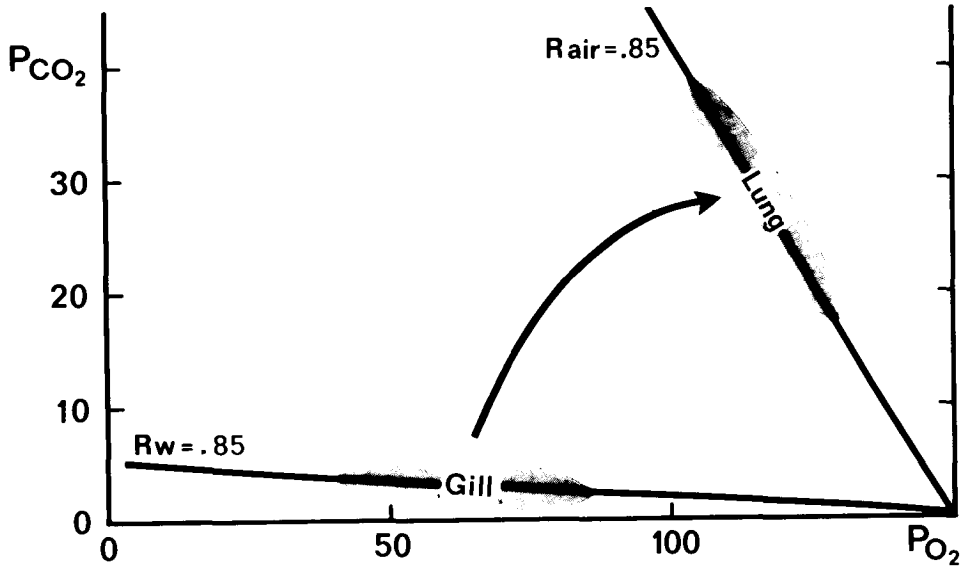

Fig. 21.

	P_{CO_2}	Ventilation Ratio
Man on Everest	8	5
Fish	4	10

Fig. 22.

Here you see (Fig. 23) an example of how our ancestors tried to bridge the gap between water and air breathing. This is the garfish taking in a breath of fresh air. In winter he behaves like a fish under the Buffalo ice, with a low P_{CO_2}, but in summer he becomes a facultative air breather and his P_{CO_2} increases (17). In Fig. 24 you see an overview of the evolutionary transition from water to air breathing (18). My evolutionary tree stands with the fish in water. Some of these became air breathers and were caught forever at the air-water interface where we still find them today. Others, however, made the transition to land, and the reptiles were the first to develop the modern lung which allowed them to leave the water, armor their skin to prevent water loss, and become truly terrestrial dwellers.

As you see on the *right*, the problem in this transition was not the O_2, but the increased CO_2 tension dictated by physicochemical properties of gases in air. But this also required enormous changes in the acid-base balance, as can be appreciated in the diagram to the *left* of the tree. The two lines represent the blood buffer curves for a water breather, the trout, with a P_{CO_2} value of 2 and the air breathing turtle with a P_{CO_2} of 25 Torr.

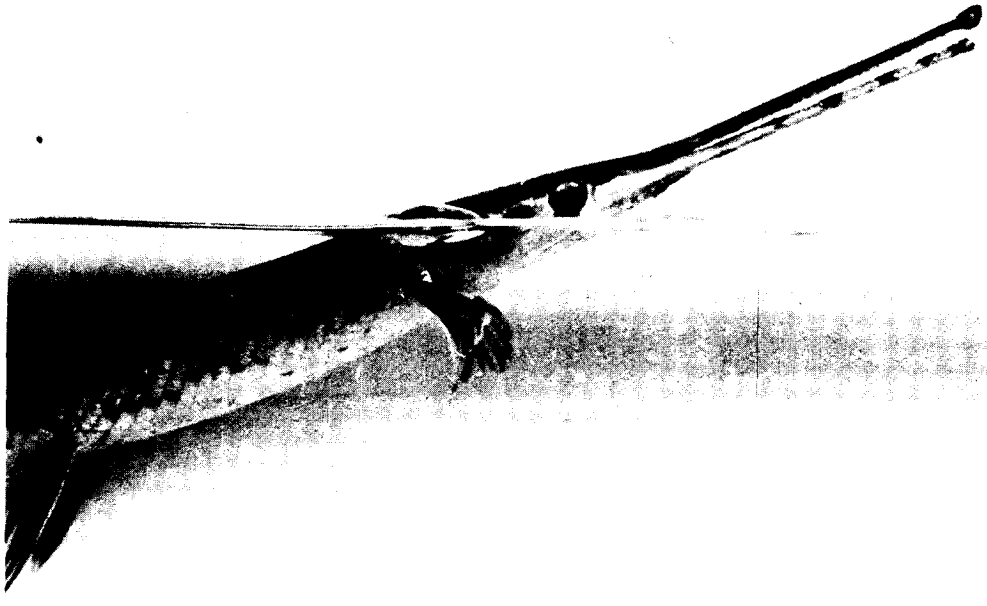

Fig. 23.

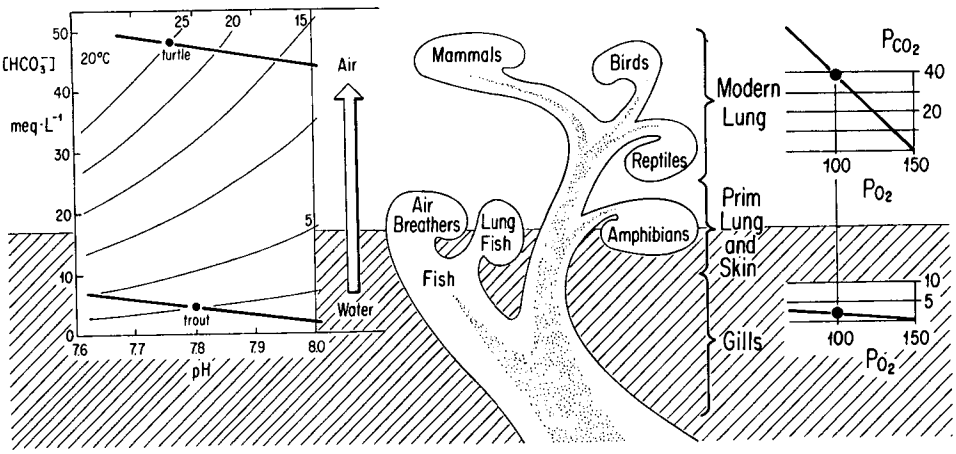

Fig. 24.

JOURNEY FROM LAND TO WATER

Water-Breathing Man

Could man breathe water like a fish (Fig. 25)? Could a dog become a dog fish? This was a question pursued by J. Kylstra. He started in Holland, experimenting with mice, and later came to Buffalo to continue with dogs. Here you see the experimental set-up designed by E. Lanphier (Fig. 26). Saline was equilibrated with O_2 at 4 ATA in a conventional glass-lined, home water heater located on the floor above the pressure chamber and used to ventilate the lungs of dogs in a pressure chamber at 4 ATA (19). Here you see 3 happy survivors (Fig. 27) after 1 h of water breathing, with J. Kylstra at the *right*, at the *left* Orr Reynolds, who came to present each dog with the Dog Hero Award of the Year from the National Society for Medical Research.

The advantages of liquid breathing are obvious. Replacing lung N_2 or He with water theoretically allows man to dive to any depth and ascend without fear of decompression sickness. Imagination shows another approach to water breathing, a technique for replacing the lung by a fish gill. When the infant is born his placenta is exchanged for an artificial gill, which he later learns to move in water to exchange O_2 and CO_2 (Fig. 28). Such a man would become cold blooded and his arterial P_{CO_2} and bicarbonate reduced to levels of a fish. Obviously, this is not a realistic approach even though it provides a unique challenge to our surgeons.

DIVING MAN VS. DIVING INSECTS

Thus man has turned to other devices to conquer the depths while maintaining a normal body temperature and normal acid-base balance. Facing the 21st Century with a world population of 6 billion people, the demand for

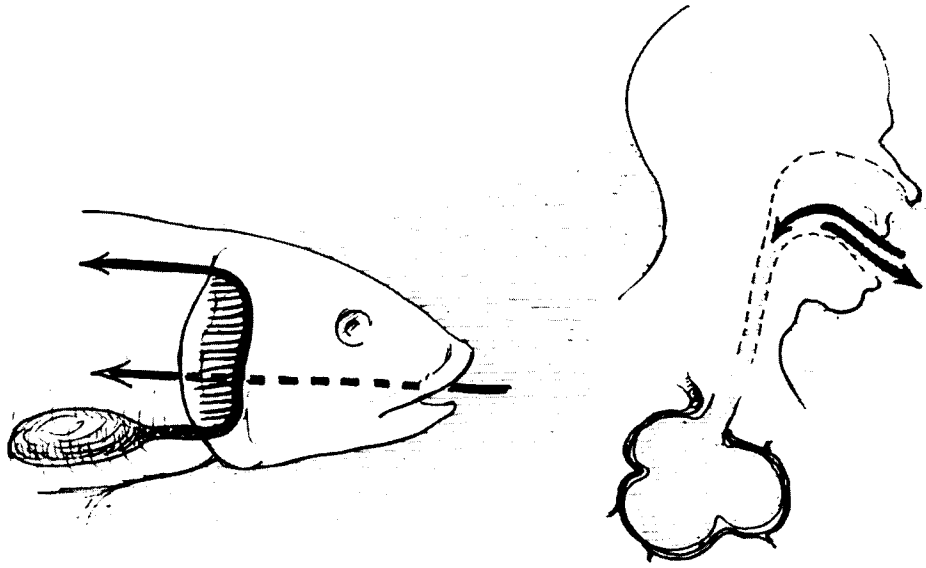

Fig. 25.

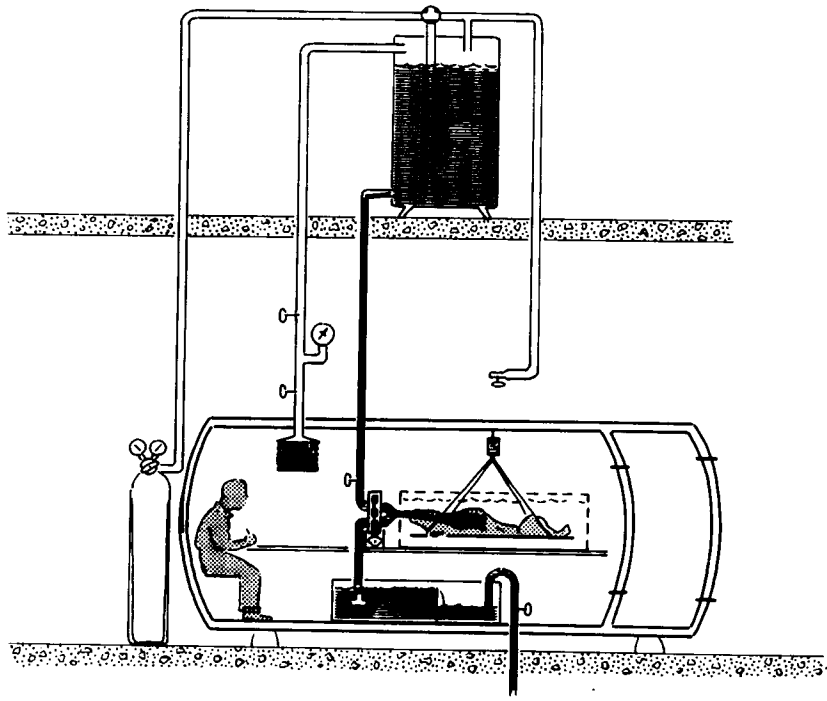

Fig. 26.

Fig. 27.

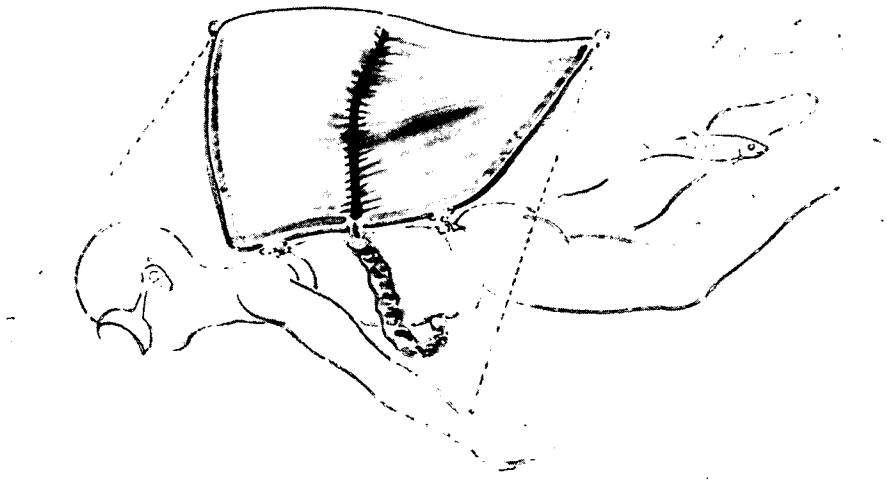

Fig. 28.

new frontiers increases the exploitation for underwater mineral sources, oil, and gas and for marine farming of plants and animals. The unexploited continental shelf (up to a depth of 200 m) is enormous. Its surface is equal to the continent of Africa and larger than the surface of the moon (Fig. 29).

Although man prides himself on his inventiveness to conquer the depths, it is only an imitation of what the insects accomplished more than 200 million yr ago. They were driven by their own population pressure as they conquered the world by invading every conceivable ecologic niche except the ocean.

They were the first to use snorkels, such as a crane-fly larva (Fig. 30). On the *right* you see man—as depicted in the ancient literature. This was obviously imaginary. As Fenn pointed out with the pressure volume diagram of the chest and lungs, which he was the first to introduce (20), a snorkeler could submerse only a few centimeters (Fig. 31). Figure 32 compares surface-supplied gas techniques. Certain insect larvae learned to tap the oxygen-conducting vessels in plant roots, which here are likened to a hose diver. Underwater houses were pioneered by water spiders, which spin a web and fill it with air obtained at the surface. In Fig. 33 you see the similarity between the spider house and the Cousteau-Bond underwater dwellings.

THE INCOMPRESSIBLE GAS GILL

Insects also learned to pick up air at the surface, stow it under their wing covers, and use it as a gas gill. This technique extends the O_2 content about 8 times while the gill gas volume slowly collapses because of the escape of N_2 (21). This is shown in Fig. 34. As the O_2 fraction of the gas gill is reduced, the N_2 fraction increases and thereby raises the N_2 tension above that in the water, which at any depth is 0.79 ATA. Thus N_2 gas diffuses into the water and causes the gill to collapse. When the insect returns to the surface for another "refill" it is to replenish the gas gill with N_2 as much as with O_2. This obviously was not thought about when a patent was awarded in 1966 for a human gas gill (as I visualize this device in Fig. 35). Obviously it would slowly collapse because of the escape of N_2 and the deeper the dive the faster the collapse. Thus, compressible gas gills are only temporarily useful, extending their initial O_2 supply about eight-fold.

However, as Thorpe and Crisp (22) were first to discover, some insects solved even this problem by inventing the remarkable plastron gas gill which up to a depth of 30 to 40 m is incompressible, a feat man has not yet achieved but may in the future. The secret of these insects was to cover the plastron over each spiracle with tiny hairs, 2 million/mm^2. These are shown in the *upper part* of Fig. 36, where you see the meniscus between the gas gill space and the water. The dimensions are such that the surface tension will withstand a hydrostatic pressure differential of up to 4 ATA and thereby render the gas gill incompressible. Thus, O_2 can freely diffuse into this space and CO_2 escape, while the N_2 partial pressure is in equilibrium with that of the water. These animals never had to surface.

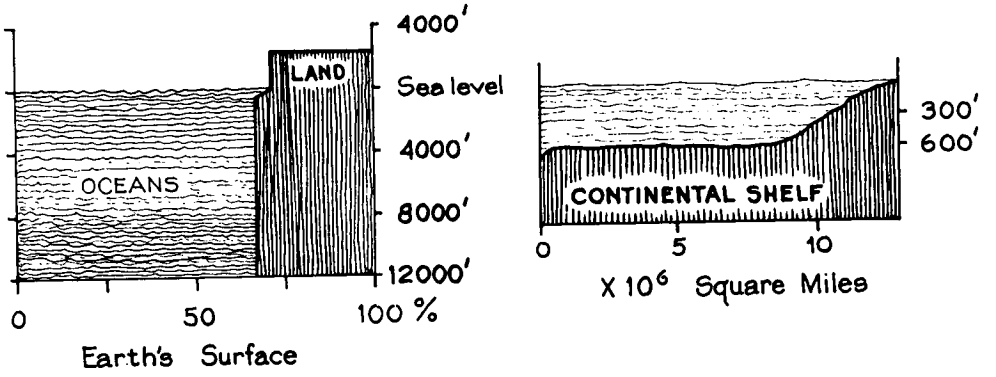
4000′
LAND
Sea level
4000′
OCEANS
8000′
12000′
0
50
100 %
Earth's Surface
300′
600′
CONTINENTAL SHELF
0
5
10
X 10⁶ Square Miles

Fig. 29.

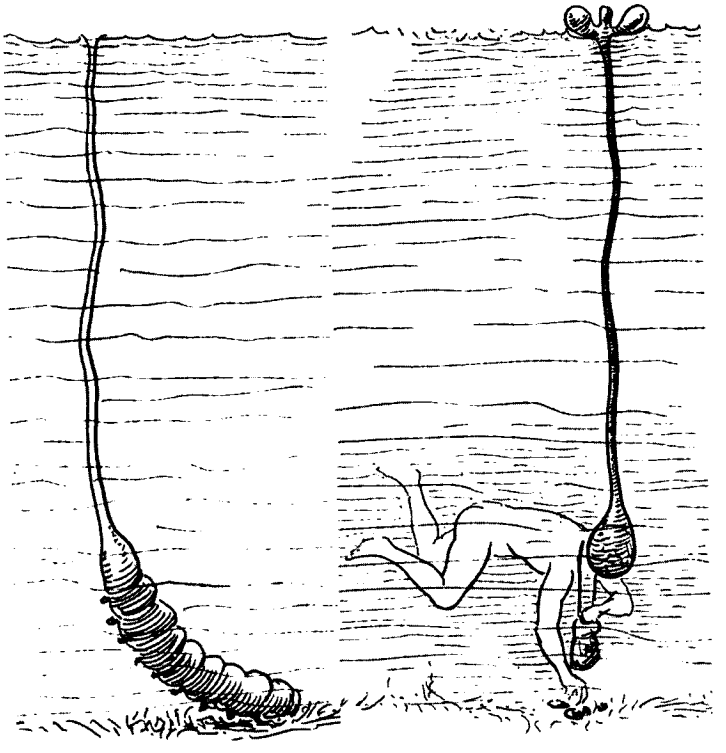

Fig. 30.

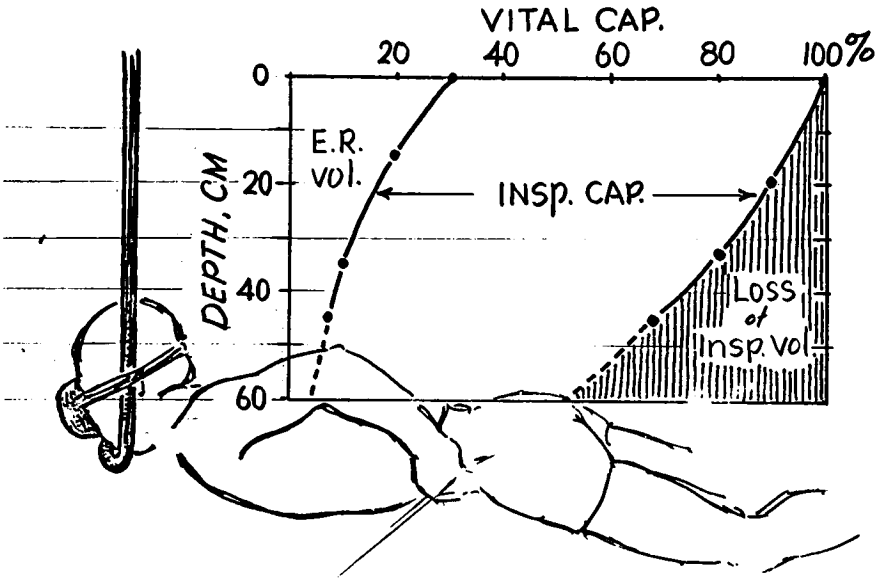
VITAL CAP.
20
40
60
80
100%
0
20
40
60
DEPTH, CM
E.R.
vol.
INSP. CAP.
Loss
of
Insp. vol

Fig. 31.

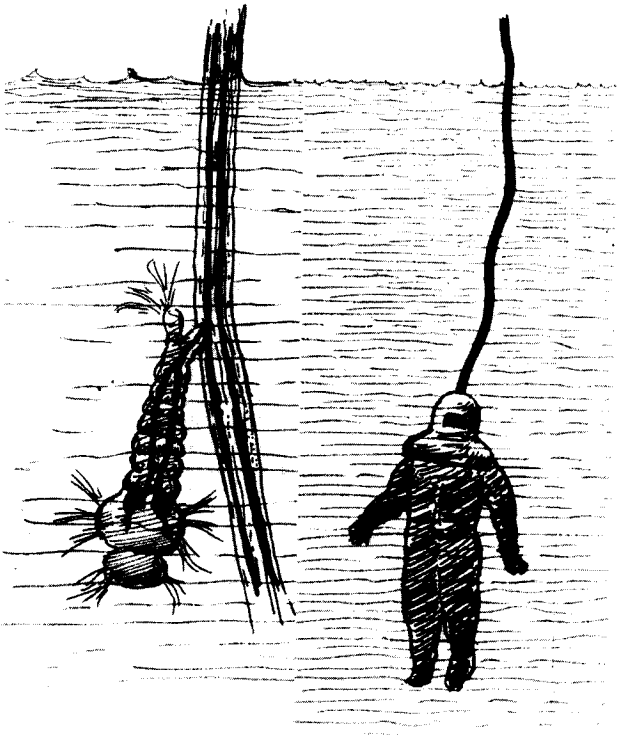

Fig. 32.

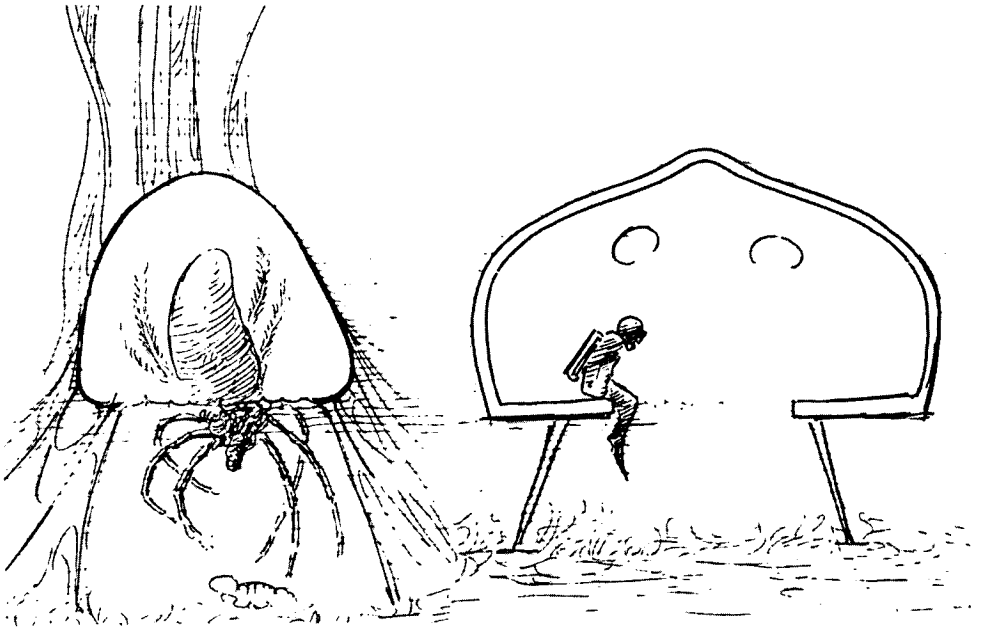

Fig. 33.

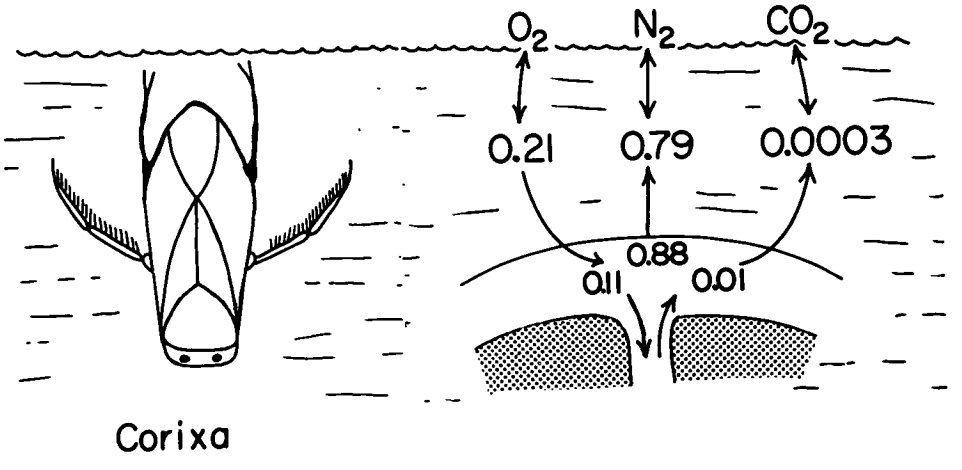

Fig. 34.

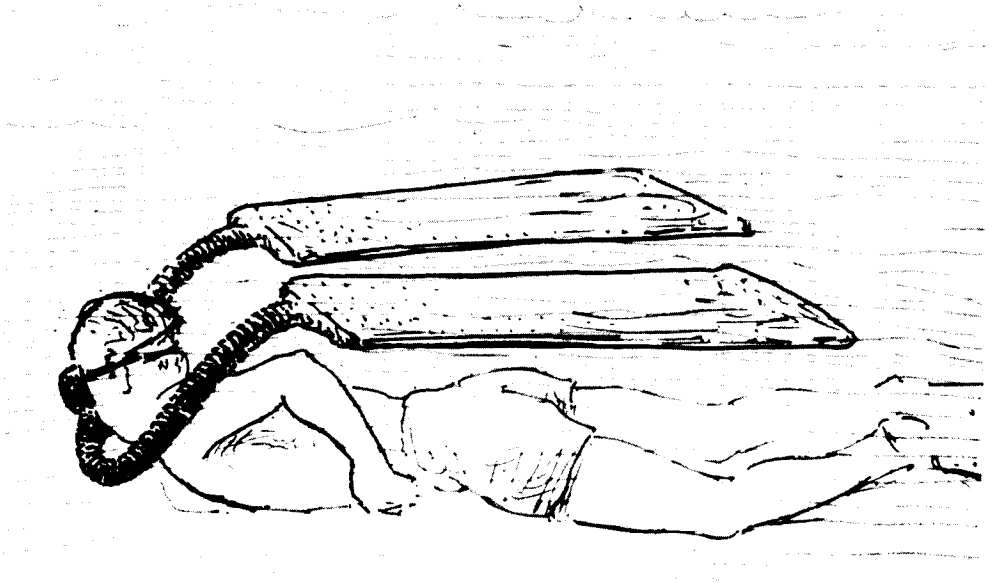

Fig. 35.

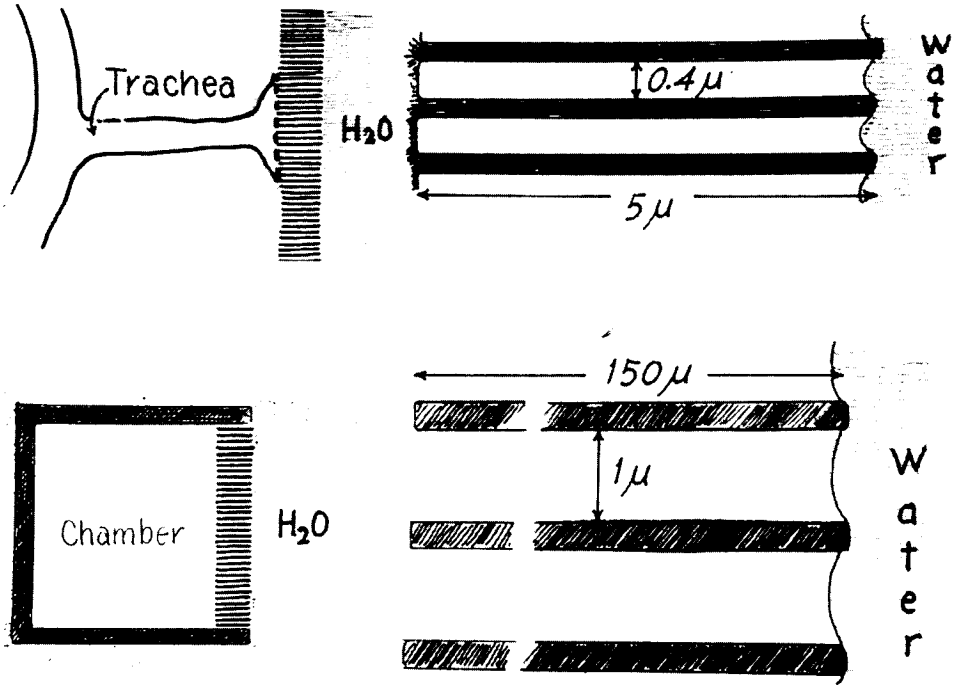

Fig. 36.

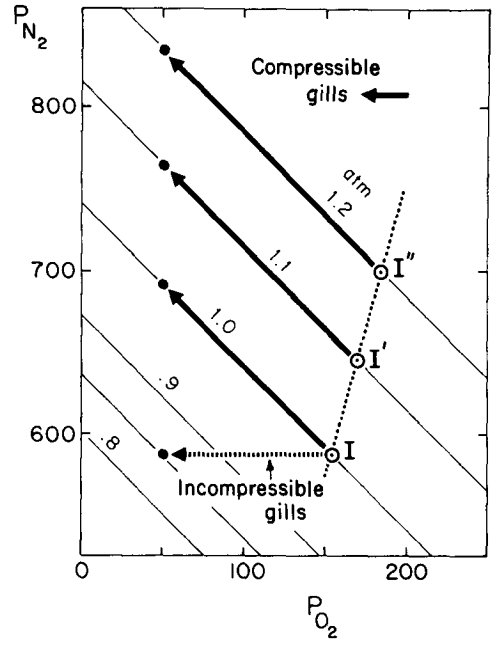
P_{N_2}
800
700
600
Compressible gills
atm
1.2
1.1
1.0
.9
.8
I''
I'
I
Incompressible gills
0
100
200
P_{O_2}

Fig. 37.

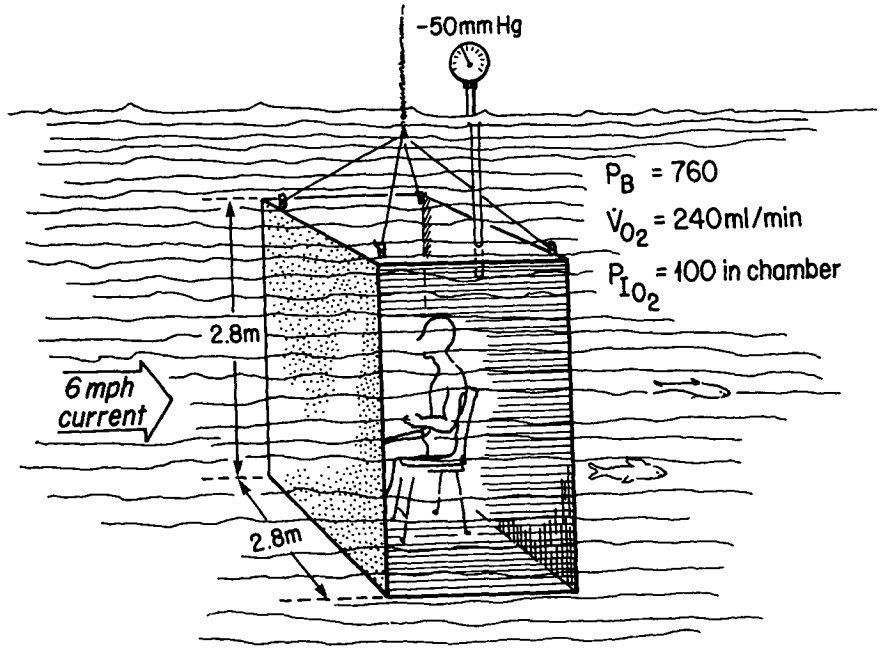
-50mm Hg
P_B = 760
$\dot{V}_{O_2}$ = 240ml/min
$P_{I_{O_2}}$ = 100 in chamber
2.8m
6 mph current
2.8m

Fig. 38.

The difference in behavior of a compressible and an incompressible gas gill is shown in Fig. 37, which depicts the events not on an O_2-CO_2 map but on an O_2-N_2 diagram where the concentration of CO_2 in the underwater gas gill system becomes negligible (21). *Point I* is air composition of a gas gill at the surface. When O_2 is removed, the *diagonal arrow* shows the increases in N_2 tension in a compressible gill. In the incompressible plastron gill, the removal of O_2 does not change the N_2 tension, only the total gas pressure. Returning to Fig. 36, in the *lower part* you see Paganelli's imitation of a plastron gill by covering a chamber with hydrophobic millipore paper having hole dimensions comparable to those of the insect. Into this incompressible gas chamber he placed small animals, which survived just like the insects (23).

With this in mind I show the last Fig. 38, which depicts a man sitting behind a sheet of millipore paper. The total gas pressure will be slightly less than 1 ATA at any depth, and the flow of the Niagara River is such as to reduce the boundary layer over the millipore surface, insuring an inspired O_2 tension of 100 Torr (23). Insects have been doing this for millions of years.

EPILOGUE

I have led you through various selected vignettes from "An Atlas of Gas Exchange." These are only a few examples illustrating how gas exchange can be understood. You will have noticed that I did not use any equations because Wallace Fenn showed us 40 yr ago how to graph them. This was the birth of the O_2-CO_2 diagram which told you not only what went on in the lung but also in the blood. Most importantly, it allowed you to predict events.

Today we take all this for granted and build upon the roads that he so clearly charted. When he plowed a field in virgin soil, he made his furrows deep and straight so that those who followed would not lose their way.

If today you and I can see farther than he, let us remember it is because we are standing on his shoulders.

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Acknowledgment

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SESSION 13: HYPERBARIC OXYGEN THERAPY I

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GAS GANGRENE AND HYPERBARIC OXYGEN: RESULTS AND EXPERIENCES FROM 1960 TO 1985

D. J. Bakker

Gas gangrene is an acute, rapidly progressive, nonpyogenic gas-forming and necrotizing infection of muscles, skin, and subcutaneous tissues. The infection is caused by anaerobic spore-forming bacteria of the genus Clostridium, primarily *Clostridium welchii* or *Clostridium perfringens.*

Diagnosis of gas gangrene should be exclusively reserved for invasive anaerobic clostridial infections of the muscles, characterized by profound toxemia, extensive edema, massive death of tissue, and a variable degree of gas production. Great differential-diagnostic problems can arise with infected vascular gangrene which does not always follow the described chronic and benign course.

Clostridial spores are responsible for contamination and the source of these spores is either exogenous or endogenous. Clostridia and clostridial spores are particularly ubiquitous in nature (soil, sand, street dust), and therefore nearly all exogenous infections occur in patients with compound and complicated fractures with soft tissue injuries after street accidents. A minority is seen after operations, injections, infusions, criminal abortion, etc. Clostridial myositis and myonecrosis as endogenous infections are caused by contamination from a clostridial focus in the body, e.g., gas gangrene of the abdominal wall after gallbladder or urinary tract operations in patients with clostridial contamination of bile or urine.

Clostridial myonecrosis is a highly fulminating infectious disease which is often fatal. Before hyperbaric oxygen became known as a therapeutic modality, mortality ranged from 30 to 50% or even up to 100% (1-9).

Treatment of gas gangrene was almost entirely limited to surgical intervention to remove all dead and diseased tissue from the infected area. Therefore it was necessary to amputate or exarticulate as soon as possible and

as generously as possible to remove all diseased tissue from healthy tissue. Tissue-saving procedures were discarded in favor of life-saving operations. Mortality remained between 20 and 50%, and well over 10% even at a very early stage of the disease. Moreover, patients whose lives were saved were often seriously disabled and subjected to long physical and psychologic rehabilitation programs.

These major operative interventions, already difficult in cases of gas gangrene of the extremities, can be virtually impossible when the infection is localized on the chest, abdominal wall, or even intrathoracic or intraperitoneally. The borderline between infected and healthy tissue is not well defined, and although sparse excision results in further spread, wide excision ensures serious disability. Finally, these very ill patients are not good candidates for major surgery.

This situation changed completely when hyperbaric oxygen was found to be beneficial in gas gangrene (10-13) in our clinic in 1960. Hyperbaric oxygen therapy is first directed to save the patients life and to cure the gas gangrene; next to save limbs and/or other tissues as much as possible; and finally to stimulate demarcation and allow a more rapid distinction between dead and viable tissue, generally within 24 h after the first treatment.

CLINICAL PICTURE

The clinical picture of gas gangrene is that of a rapidly spreading wound infection a few hours to a few days (range 1 h to 41 d) (13) after an accidental injury, an operation, or after minor trauma. The earliest sign is extreme pain in the wound area. Body temperature rises quickly. The patient becomes seriously ill, somnolent, and comatose, and without treatment is soon in septic shock. Kidney function is impaired, blood pressure is lowered, and jaundice gives warning of hemolysis caused by circulating toxins. Gas bubbles escape from the wound and are palpable in the tissues. The color of the skin around the wound is copper or bronze and the wound has a sickly sweet odor. In later stages, bullae and vesicles filled with a reddish fluid are seen.

Clostridial myositis gives a special x-ray picture of featherlike air figures in muscle tissue. Spread of the infection can be from 10 to 15 cm/h. Diagnosis, differential diagnosis, etiology, pathophysiology, bacteriology, and clinical presentation have been extensively described in our monograph (13) on hyperbaric oxygen and infectious diseases.

RATIONALE FOR HBO IN GAS GANGRENE

Clostridium perfringens is not a strict anaerobe because it does not form spores in tissues; for this to occur anaerobic conditions are necessary. More than 20 different exotoxins produced by Clostridium have been identified, the most dangerous being the oxygen-stable lecithinase-C, alpha-toxin, which is hemolytic, tissue necrotizing, and lethal.

For the onset of gas gangrene, two conditions must exist: a) the presence of clostridial spores and b) an area of lowered oxidation-reduction potential, e.g., by extensive soft tissue damage and necrotic muscle tissue—an area with such a low Po_2 that clostridial spores can flourish into the vegetative forms. Clostridial bacteria surround themselves with produced toxins; local host defense mechanisms are abolished when toxin concentration is sufficiently high, followed by ever increasing tissue destruction and further clostridial growth.

Action of hyperbaric oxygen on Clostridia is based on the forming of H_2O_2 in the absence of catalase, a normal aerobic defense mechanism against the affect of oxygen radicals. Thus, hyperbaric oxygen seems to be bacteriostatic and bactericidal for *C. perfringens* and other Clostridia.

As soon as the patient is breathing 100% O_2 by mask in a multiplace or freely in a monoplace chamber at 2.5 to 3.0 bar, tissue Po_2 around and even inside the infected area rises to over 250 mmHg so that the production of alpha-toxin stops completely (14-17); within 30 min the circulating toxin is fixed to the living cells and the growth of Clostridia is limited. After a short interval, the hemolytic tissue necrotizing and lethal activity of the Clostridia ceases. Between two hyperbaric oxygen sessions, when the patient is at sea-level pressure, alpha-toxin production starts again, but before the dangerous level is reached the next session stops production once more. Intermittent periods without alpha-toxin production and rapid destruction of circulating alpha-toxin enables the body to build up its own defense mechanisms.

The temporary arrest of alpha-toxin production may lead to a change in the environment of Clostridia which consequently no longer meets the requirements for optimum function of Clostridium. The transiently increased pH, the arrest of the activity of proteolytic enzymes in the tissue, and the consequent arrest of the release of amino acids in the lesion may result in a condition in the surrounding tissues that is not ideal for function and multiplication of anaerobic microorganisms.

In the infected area, the circulation is improved by diminished edema and compression of gas bubbles. Necrotic tissue is already lost at this point, but the quantity of tissue lost is always far less than that expected before hyperbaric treatment.

MATERIAL AND METHODS

From 1960 to 1985, 547 patients (100%) were admitted on suspicion of gas gangrene. In 409 cases (72.9%) the diagnosis was clinically and bacteriologically proven. Of these 409, 309 were men (75.5%) and 100, women (24.5%).

The patients were divided into 3 groups: posttraumatic gas gangrene (n = 257), postoperative (n = 124), and gas gangrene from miscellaneous causes (n = 28).

In the *trauma group*, 185 patients acquired gas gangrene after a traffic accident.

In the *operation group*, 61 patients acquired gas gangrene after amputation for arteriosclerotic and/or diabetic gangrene of the lower extremities, 9, after colon surgery, and 9, after cholecystectomy. Practically every operation, even the most "sterile," can be followed by gas gangrene, as our patients demonstrate.

In the *miscellaneous group*, gas gangrene occurred after intramuscular injection (n = 8), criminal abortion (n = 3), knee-joint puncture (n = 3), intravenous infusion (n = 3), insect sting (n = 2), bladder catheterization (n = 1), ulcus cruris (n = 1), cowhorn (n = 1), acute pancreatitis (n = 1), and in 5 patients with generalized gas gangrene no port of entry could be found. These cases are sometimes referred to as "spontaneous" gas gangrene. Obduction can reveal large bowel pathology (carcinoma or diverticulitis).

One hundred ten of the patients were between the ages of 15 and 25, all traffic-accident victims. Saving tissue or limbs in these patients is even more important than in the higher age groups. In the age group 50 to 70 yr, 130 patients were mainly amputations and postcolon surgery (a minority of 45 patients were posttraumatic). Gas gangrene occurs at every age; however, in our patient population the ages 15 to 25 and 60 to 70 were predominant.

WOUND AND BLOOD CULTURES

All wound cultures were positive for one or more of the pathogenic Clostridia; a prerequisite to be included in this series. *Clostridium perfringens* alone was cultured in 357 patients (87.3%); *Clostridium perfringens* alone or in combination with another Clostridium was found in 392 patients (95.8%). Only 1 type of Clostridium was found in 373 patients (91.2%); 2 different types in 34 patients (8.3%); 3 different types in 1 patient, and 4 different types in 1 patient.

Blood cultures for one or more of the pathogenic Clostridia were positive in 103 out of 409 patients (25.2%). (However, no blood culture could be found for ± 15% of the patients treated between 1960 and 1970.) In the *trauma group*, 56/257 blood cultures were positive (21.8%), *postoperative*, 36/124 (29.0%), and *miscellaneous*, 11/28 (39.3%). In these groups *Clostridium perfringens* was found in 92.9, 97.2, and 90.9%, respectively.

MORTALITY

When discussing mortality it is important to distinguish between those patients who died because of manifest gas gangrene, whose wound and/or blood cultures were still positive at the moment of death, and those patients who died later when the gas gangrene was considered cured. The cause of death in the latter cases was myocardial infarction, pulmonary emboli, metastases of a large bowel carcinoma, or sepsis due to other microorganisms.

The 50 patients who died with manifest gas gangrene did so within 24 h after treatment began, before the fourth hyperbaric session (5 patients died

shortly after admission, too late for any therapy, 21 died between the first and second sessions, 14 patients died between the second and the third sessions, and 10 died after the third session but before the fourth session began, i.e., within 24 h).

Mortality in the *trauma group* was 18/257 (7.0%). In the group with a positive blood culture it was 9/56 (16.1%). Another 11 patients (4.3%) died after full treatment when the gas gangrene was cured. In the *postoperative group* mortality was 22/124 (17.7%); with a positive blood culture 12/36 (33.3%). Another 17 patients (13.7%) died later from other causes. The *miscellaneous group* is the most serious; 8/28 (28.6%) patients died with manifest gas gangrene; later, another 8 (28.6%) died from other causes. The total mortality with manifest gas gangrene in the whole group was 48/409 (11.7%).

LIMB-SAVING OR TISSUE-SAVING CAPACITY OF HBO

The limb-saving or tissue-saving capacity of hyperbaric oxygen treatment can be shown in relation to the number of necessary amputations. After hyperbaric oxygen therapy, 40 amputations were necessary out of 313 patients with gas gangrene of the extremities [another 55 patients were amputated because of arteriosclerotic and/or diabetic gangrene, 13 patients underwent amputation before hyperbaric oxygen therapy because of gas gangrene in the referring hospital (mainly before 1965), 13 reamputations had to be performed in the already amputated group, and in 7 patients the trauma preceding gas gangrene was traumatic leg or arm amputation]. Of the 40 amputations, 21 extremities could be considered as already lost at admission. These amputations, better called necrotomies, were delayed until hyperbaric oxygen therapy was finished. Amputation rate was 19/313 (6.1%); thus, with the 13 reamputations in the operation group, the total was 32/313 (10.0%).

In the last 5 yr, relatively less gas gangrene was seen in the trauma group and more in the operation group, mainly after vascular surgery.

TREATMENT SCHEME

During the first 24 h after admission, gas gangrene patients are treated 3 times with hyperbaric oxygen, 90 min at 3 bar, 100% O_2 in a multiplace chamber. Very serious cases are treated 4 times, but this is seldom necessary. In the second and third 24-h periods, patients are treated 4 more times.

Antibiotic therapy consists of 6×10^6 U penicillin i.v. over a 7-d period, and most patients are treated in the intensive care unit.

DISCUSSION

Hyperbaric oxygen is now widely accepted as a primary mode of treatment for gas gangrene, although antibiotic and surgical treatment continue to play important roles. The timing of the surgery is a question that arises frequently.

Our approach has always been conservative with delayed surgery, for which we have been criticized. The problem in gas gangrene, however, is not the necrotic nor the healthy tissue, but the quickly advancing phlegmon in between. In treating gas gangrene, alpha-toxin production must be halted as soon as possible, and to do this patients must be treated adequately with hyperbaric oxygen.

The obvious effect of stimulation of the demarcation prevents the loss of much tissue that can be saved with immediate hyperbaric oxygen treatment.

Time is of the utmost importance. Suspicion of gas gangrene is sufficient reason to refer a patient for HBO treatment. It is not necessary to discuss surgery until after the 3rd, 4th, or 7th session. Our results show that delayed surgery does not add to morbidity and mortality; mortality and amputation figures in The Netherlands are among the lowest in the world.

Higher mortality among the *miscellaneous group* is because no one suspected gas gangrene after such a trivial "trauma" and referrals come too late. We do see less gas gangrene after trauma today because more external fixating devices and fewer internal osteosynthesis are used in complicated fractures. A second factor in this group may be that less experienced surgeons in smaller hospitals are performing more vascular surgery; below knee amputation in poorly vascularized tissue is another reason.

To prevent the onset of gas gangrene it is important to recognize that the condition of the wound is a more critical factor than the presence of Clostridial spores.

In summary, proper wound management is the only way to prevent gas gangrene, but if infection does occur, hyperbaric oxygen should be the primary treatment to reduce morbidity and mortality.

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TREATMENT OF GAS GANGRENE BY HYPERBARIC OXYGEN

H. Tamura, K. Takao, K. Okafuji, K. Uchida, and M. Kawashima

In Japan, gas gangrene is uncommon, but some cases do occur. If gas gangrene is diagnosed, adequate treatment is necessary to save life or affected limbs. Brummelkamp et al. (4) reported the treatment of clostridial infection with hyperbaric oxygen (HBO) drenching in 26 cases. Since then, considerable data have been published on this subject and many authors have proved the effectiveness of HBO therapy. Between 1973 and 1985, we treated 22 patients with gas gangrene and confirmed the effectiveness of HBO therapy.

MATERIALS AND METHODS

Patients

Of the 22 patients, 21 were transferred from other hospitals and one was admitted directly. They were referred for HBO therapy with suspected clostridial gas gangrene.

The 22 patients consisted of 21 men and 1 woman with mean age of 42 yr (range 18-70). Fourteen patients were infected in a lower limb, 4 in the upper arm, 3 in the abdominal wall, and 1 in the hip area. Seventeen infections occurred following inadequate treatment after trauma, 3 infections in the abdominal wall followed operative procedures, 1 arose spontaneously following diabetic ulcer of the leg, and 1 occurred following an abscess of the anal region. Only 1 patient suffered from coexistent diabetes (Tables 1, 2).

Bacteriology

Smear and culture from wounds and blood were performed routinely. Both aerobic and anaerobic techniques for isolating organisms were used. Causative organisms, genus clostridium, were found in 12 patients, and nonclostridial organisms in 10 patients. Among the 12 clostridial with gas gangrene, *Clostridium perfringens* was found in 11 patients (91.2%) and *Clostridium*

Table 1
Sex, Age, Site and Organism of Clostridial Gas Gangrene

Case No.	Sex	Age	Site of Gas Gangrene	Organism
1	M	46	left lower leg	*C. perfringens*
2	M	31	right upper leg	*C. perfringens*
3	M	26	right lower leg	*C. perfringens*
4	M	33	abdominal wall	*C. perfringens*
5	M	43	left leg	*C. perfringens*
6	M	69	right lower arm	*C. perfringens*
7	M	19	right foot	*C. perfringens*
8	M	34	left lower leg	*C. perfringens*
9	M	18	right upper leg	*C. perfringens*
10	M	52	abdominal wall	*C. septicum*
11	M	49	right leg	*C. perfringens*
12	M	22	right lower leg	*C. perfringens*

Table 2
Sex, Age, Site, and Causative Organism
of Nonclostridial Gas Gangrene

Case No.	Sex	Age	Site of Gas Gangrene	Causative Organism
13	M	70	abdominal wall	*E. coli*
14	M	29	left lower leg right lower leg	*E. coli*
15	M	46	right lower arm	*Aeromonas hydrophila*
16	M	34	left lower leg	unknown
17	M	67	right lower arm	Streptococcus
18	M	48	right elbow	Streptococcus
19	M	33	left upper arm	unknown
20	M	22	left leg	*E. coli*
21	F	64	left foot	Streptococcus
22	M	70	hip	*E. coli*

septicum was found in 1 patient (8.8%). Of the 10 nonclostridial gas gangrene patients, *Escherichia coli* was found in 4 patients, *Streptococcus pyogenes* in 3, *Aeromonas hydrophila* in 1, and unknown in 2.

RESULTS

All 22 patients were treated with HBO. The average period from onset to first HBO treatment in all cases was 5.8 d (range 2-16 d); 4.9 d (range 2-10) in clostridial cases, 6.9 d (range 2-16) in nonclostridial cases.

Twenty patients out of 22 survived. The causative organisms in 2 patients who died were *C. septicum* and *E. coli*. Mortality was 9.1% (2:22); 8.3% in clostridial gas gangrene, 10% in nonclostridial gas gangrene. Rate of limb salvage without amputation was 50% (9:18), 50% in clostridial gas gangrene, 50% in nonclostridial gas gangrene (Tables 3, 4).

DISCUSSION

Age, Sex, and Site Distribution in Japan

In Japan, 171 cases with gas gangrene were reported up to 1985, including our 22 cases. The peak age was the 20s (25%), followed by the 30s (21.4%), 40s (17.1%), and teens (15.7%). The main etiology of gas gangrene in Japan is trauma, so young and middle generations have a greater chance of being infected (Fig. 1).

The hundred and seventy-one patients with proved gas gangrene consisted of 133 men (77.8%) and 32 women (18.7%); 6 cases were unknown. This is because in Japan men are more often involved in traffic or labor accidents than women (Fig. 2). Gas gangrene occurred most frequently in the lower limb (54.4%), followed by upper limb (14.6%), the abdominal wall (10.1%), and the anal region (8.7%) (Table 5).

Clinical and Radiologic Features Between Clostridial and Nonclostridial Gas Gangrene

In typical clostridial gas gangrene, one of the first signs is extreme pain in the wound area, accompanied by severe swelling and high fever. The patient becomes severely ill. As the disease progresses, a bronze discoloration appears on the affected skin. Discoloration advances rapidly, accompanied by discharge from the wound, with a characteristic foul odor. Crepitus may be palpable in the affected area. In our series, all patients were admitted to our hospital in this advanced stage. If we do not treat the patients in this stage, the phlegmon will progress aggressively and severe toxemia will develop. Finally, the patients die because of high toxicity.

In nonclostridial gas gangrene, clinical features were almost the same, but some differences were noted between clostridial and nonclostridial gas gangrene. In our experience, nonclostridial gas gangrene progresses slower than clostridial gas gangrene. The average period from onset to first HBO treatment in nonclostridial gas gangrene was 6.9 d vs. 4.9 d in clostridial gas gangrene. The affected area was localized, except in well-advanced cases. Discoloration of the skin was not so remarkable. We had the impression that in nonclostridial gas gangrene, phlegmon did not spread rapidly and damage to muscle

Table 3
Outcome of HBO Therapy for Clostridial Gas Gangrene

Case No.	Period from Onset to First HBO, d	HBO Treatment, times	Outcome
1	3	4	left upper leg amputation
2	10	6	right upper leg amputation
3	2	3	full recovery
4	10	2	full recovery
5	5	5	left upper leg amputation
6	7	6	right upper arm amputation
7	5	8	left lower leg amputation
8	3	4	full recovery
9	5	6	full recovery
10	2	5	death
11	5	5	full recovery
12	2	4	full recovery

Table 4
Outcome of HBO Therapy for Nonclostridial Gas Gangrene

Case No.	Period from Onset to First HBO, d	HBO Treatment, times	Outcome
13	2	4	death
14	7	5	left knee amputation right upper arm amputation
15	13	7	right upper arm amputation
16	6	6	left upper arm amputation
17	4	3	full recovery
18	16	4	full recovery
19	3	1	full recovery
20	12	4	full recovery
21	3	2	left lower leg amputation
22	3	8	full recovery

was not as severe as in clostridial gas gangrene; the damage seen in subcutaneous tissue was severe.

Generally speaking, many nonclostridial patients suffered from diabetes with orthopedic vascular problems, but in our series only 1 patient suffered

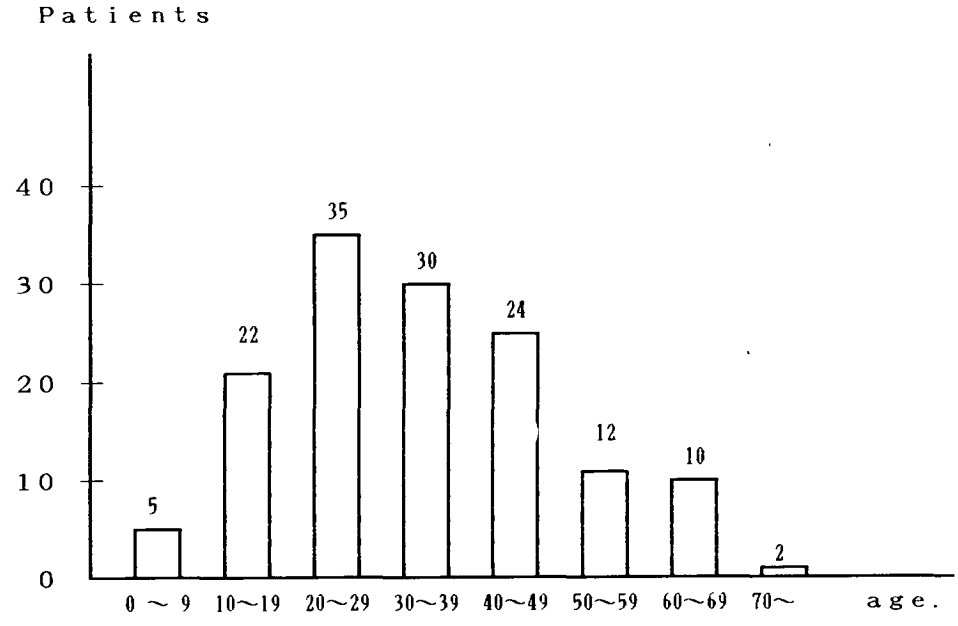

Fig. 1. Age distribution of 171 patients in Japan.

from coexisting diabetes.

Radiologic proof of gas formation is very important. A radiologic feature of clostridial gas gangrene is seen as gas formation, featherlike in appearance in muscle tissue. In our series the radiologic feature of nonclostridial gas gangrene was seen as streaked gas formation in the subcutaneous tissue, not featherlike, although such gas formation was sometimes seen in early stages of clostridial gas gangrene. The quantity of gas in nonclostridial gas gangrene seemed to be less than in clostridial gas gangrene.

Prophylaxis

In Japan, gas gangrene generally occurs after trauma and was seen in 77.3% of our cases. We think that the condition of the wound is far more important from the onset of gas gangrene than the presence of clostridial or nonclostridial organisms. Proper wound management is very important in the prevention of gas gangrene: meticulous debridement, irrigation, adequate drainage, loose suture of wounds, and use of broad spectrum antibiotics, especially penicillin. We have treated many patients with traumatic wounds (500-1500 in a year) but we have no experience with gas gangrene.

We have not used gas gangrene antitoxin recently because its effects are doubtful in the treatment of advancing clostridial gas gangrene, and precaution is necessary to avoid anaphylactic shock.

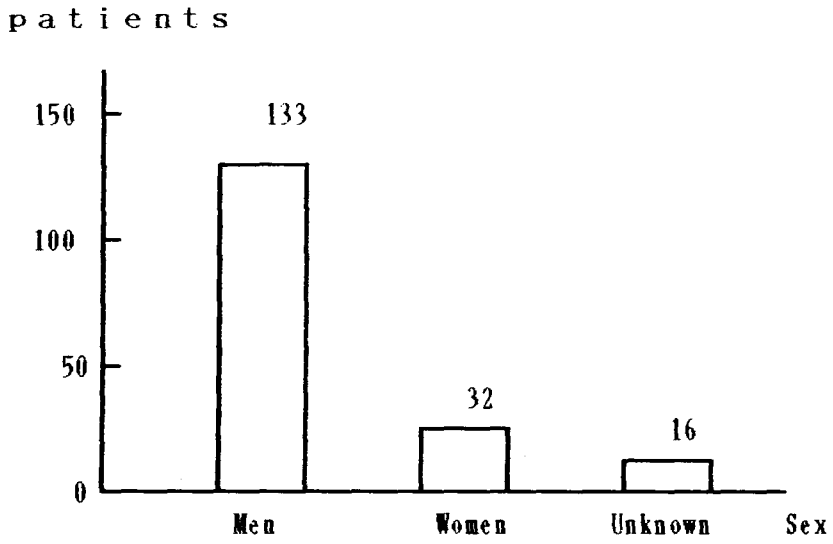

Fig. 2 Sex distribution of 171 patients in Japan.

Fig. 2. Sex distribution of 171 patients in Japan.

Table 5
Site of Gas Gangrene in 171 Patients in Japan

Site	Patients
Lower limb	93
Upper limb	25
Abdominal wall	18
Anal region	15
Widely spreading	15
Face or head	5

Treatment

Antibiotic therapy, surgical debridement, and HBO therapy are necessary in established gas gangrene. In our hospital, penicillin in doses of 5 to 10 million units were administered routinely and a broad spectrum of antibiotics was often administered because of the existence of mixed infections.

Before HBO therapy became available, extensive surgical debridement and radical amputation including viable tissue, was necessary to save life. Thanks to the effects of HBO, such a surgical approach was unnecessary. When a patient with gas gangrene is admitted to our hospital, we perform minimal

debridement and incisions into the phlegmonous area in cases with massive necrosis. HBO therapy is started as quickly as possible. After several HBO treatments the general condition is often improved dramatically and the wound well demarcated. At that time we excise necrotic tissue and perform amputation, if necessary.

With HBO therapy, the prognosis of clostridial gas gangrene is completely altered. Mortality and frequency of amputation can be drastically reduced. Before HBO therapy became available, mortality in patients with serious gas gangrene ranged from 30 to 50%. After application of HBO therapy became available, mortality ranged from 10 to 20%. In our 12 patients with clostridial gas gangrene, mortality was 8.3%; only 1 patient infected with *C. septicums* died, and all 11 patients infected with *C. perfringens* survived. We usually note immediate clinical improvement during or after the first HBO therapy with those patients having clostridial gas gangrene.

The effects of HBO are improvement in oxygen supply to poorly vascularized tissue and inhibition of the production of the virulent alpha toxin which causes hemolysis, muscle necrosis, skin necrosis, serious systemic effects such as cardiotoxicity, and brain dysfunction. Once toxin production is halted, the disease cycle is broken and the residual toxin is fixed rapidly. Rapid clinical improvement follows.

Generally, the value of HBO therapy in nonclostridial gas gangrene is questionable. We think that HBO therapy for nonclostridial gas gangrene is useful as an adjunctive treatment. It is very difficult and dangerous to decide quickly whether a gas-forming infection is clostridial or nonclostridial. The presumptive diagnosis is based on the clinical appearance of the patient and demonstration of gram-positive rods. If the patient with gas gangrene has received antibiotic therapy before admittance, gram-positive rods may not be demonstrated on smear. Cultures are taken, but treatment is initiated immediately before bacteriologic results are known, because of the possibility of rapid progression of the illness. In gas-forming infections, HBO therapy should be started immediately until the diagnosis is demonstrated bacteriologically.

Figure 3 shows our HBO treatment schedule. Patients with gas gangrene are placed in the large hyperbaric chamber with our medical staff, air is compressed to 2.8 ATA, and the patient begins to breath pure oxygen through a mask at 2.8 ATA. Oxygen inhalation is continued for 1 h, after which decompression is according to schedule. We continue the table every day until the general condition is improved and gas formation disappears. Average number of HBO treatments is 4.6, range 1 to 8.

Generally, oxygen toxicity is rare under 3 ATA; none of our patients gave any evidence of oxygen toxicity.

CONCLUSION

We treated 22 patients with clostridial and nonclostridial gas gangrene. The mortality was 9.1% 5(2:22) and the rate of limb salvage was 50% (9:18).

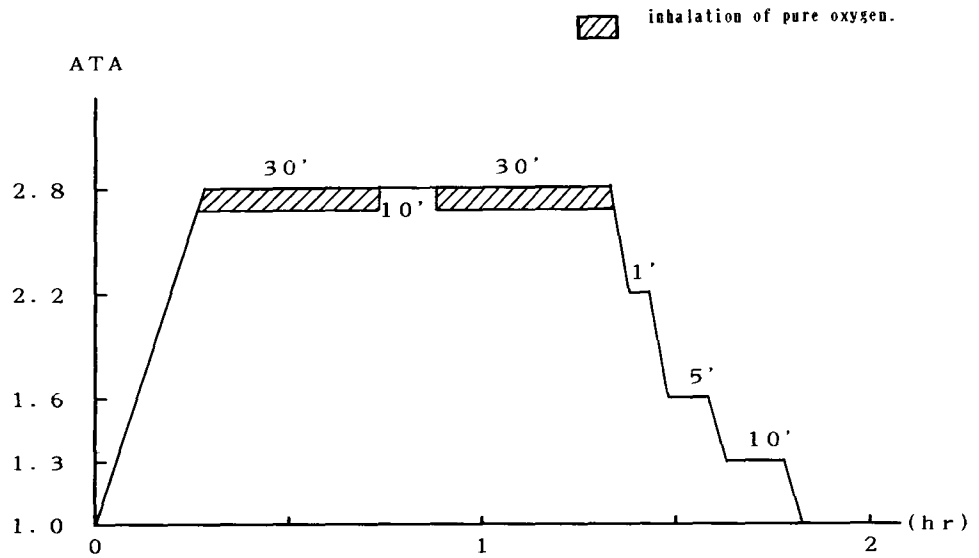

Fig. 3. Treatment schedule of HBO for clostridial gas gangrene.

Antibiotics, surgical debridement, and HBO therapy were important components in treatment of patients with gas gangrene. HBO therapy was especially effective in saving lives and affected limbs. We also noted a difference in clinical and radiologic features between clostridial and nonclostridial gas gangrene.

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HYPERBARIC OXYGEN THERAPY OF ISCHEMIC SKIN FLAPS: CLINICAL AND EXPERIMENTAL STUDY

M. Ueda, T. Kaneda, H. Takahashi, and K. Sakakibara

Rapid strides in the field of plastic surgery have brought about development of many kinds of flap surgery in recent years. Flaps are now used frequently for head and neck reconstruction. With appropriate design, most of the transplanted flaps survive without difficulty, but occasionally and unintentionally clinical evidence may develop indicating disturbed circulation of the flap. When the trouble exists in arterial supply to a skin flap cyanosis or the so-called white flap develops. When the trouble involves the venous system of the flap, congestion develops in the flap, producing a so-called blue flap (1). If these changes develop further into necrosis of the flap, the results may not be limited to the area of reconstructive surgery, but may lead to increased mortality (2). Consequently, it is necessary not only that the cause of this type of flap condition be removed, but that some method to improve circulation be found (3-5).

Chemotherapy (6-11) with vasodilators or tissue metabolism activators have been used to prevent flap necrosis. However, such agents will never restore a compromised flap to full function. Although surgical methods to revive the impaired circulation of a compromised flap have been pursued extensively, we noted marked blood flow improvement in ischemic flaps using hyperbaric oxygen (HBO). This report covers our experience with ischemic flaps and studies to determine the optimum conditions for use of HBO. We will also demonstrate experimentally the mechanism of HBO improvement of impaired flap circulation.

CLINICAL STUDY

Clinical Cases

Twenty-six patients from the Department of Oral Surgery, Nagoya

University Hospital, in whom ischemic conditions developed, either during or immediately after flap surgery, were studied for 8 yr, from 1977 to 1985. Sixteen were males and 10 were females, and their ages ranged from 14 to 66 yr. Common signs were blue color, edema, and the absence of bleeding at the edges of the flaps. Their primary diseases and the size of flaps are shown in Table 1.

Table 1
Clinical Cases Treated with HBO

case	sex	age	primary disease	size of flap (mm)
1	F	22	deformity of upper lip (cleft lip)	12 × 10
2	F	14		20 × 16
3	M	15		15 × 15
4	M	18		15 × 13
5	F	16		10 × 10
6	F	18		15 × 13
7	M	21		20 × 18
8	F	16		15 × 13.5
9	F	17		10 × 8
10	M	18		10 × 7
11	M	16		12 × 5
12	M	15		14 × 13
13	F	43	lower lip cancer	30×20
14	M	50		65×20, 65×20
15	M	66		75×15
16	M	60	mouth floor cancer	200×100
17	F	58		230×100
18	M	58		60×40
19	M	24	tongue cancer	100×80
20	M	50		70×50
21	F	61	mandibular cancer	60×40, 80×60
22	M	65		25×20
23	M	49	buccal mucosal cancer	300×80
24	M	29	cleft palate	15×30, 15×30
25	F	15	microstomia	25×15, 25×15
26	M	20	Palatal fistula	20×40

Twelve patients had transplanting of Abbe flaps to correct lip deformities after cleft lip surgery, and 3 used transplantation of deltopectoral (D-P) flaps for immediate reconstruction after removal of mouth floor or tongue cancers.

Nasolabial flaps were used in 2 cases of lower lip cancer, and pectoralis major myocutaneous (PM-MC) flaps were used in 2 cases for reconstructive surgery of tongue cancers. Sternocleidomastoid myocutaneous (STM-MC) flaps, Estlander flaps, cervical flaps, and forehead flaps were used individually in reconstruction after malignant tumors were removed. In addition, minor flaps were used in 3 cases for various types of oral surgery (Table 2).

Table 2
Types of Flaps Treated with HBO

Flaps	Cases
Abbe flap	12
D-P flap	3
Nasolabial flap	2
PM-MC flap	2
STM-MC flap	1
Estlander flap	1
Cervical flap	1
Forehead flap	1
Tongue flap	1
Palatal flap	1
Mucosal flap	1
Total	26

D-P flap = deltopectoral flap; PM-MC flap = pectoralis major myocutaneous flap; STM-MC flap = sternocleidomastoid myocutaneous flap.

The sizes of the transplanted flaps ranged from 230 × 100 mm maximum to 10 × 7 mm minimum. Twenty-three of the 26 flaps were axial pattern flaps with distinct feeding arteries, but the remaining 3 were random-pattern flaps, nourished by the subdermal plexus only.

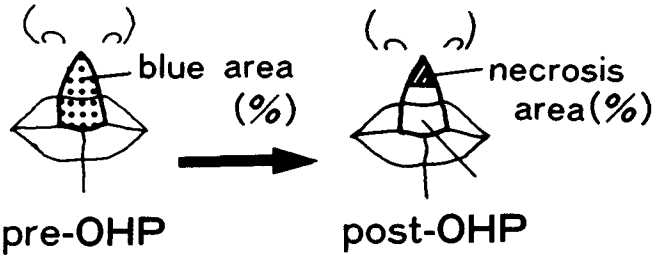
Methods of Administering HBO

Hyperbaric oxygen was administered in the Department of Hyperbaric Medicine, Nagoya University Hospital. The chamber has capacity for 10 patients in a supine position. The maximum pressure was 3 ATA. The chamber was compressed with pressurized air, and each patient was given pure oxygen through an oxygen mask. Carbon dioxide concentration and humidity within the chamber were maintained at constant levels.

Patients with comparatively mild ischemic conditions were given HBO at 2 ATA once a day for 60 min, 6 d/wk. Patients with severe symptoms were given HBO at 2 ATA for 60 min and at 3 ATA for 60 min twice a day. Electrocardiogram, chest x-ray, and eustachian tubes were checked before HBO was given.

Methods of Judging Clinical Results

Emphasis was placed on the relationship between the conditions of HBO and the degree of circulatory improvement of flaps in this study. The criterion for evaluating flap circulation was the degree of recovery (%), calculated as the difference between the necrotic area of a flap (%) after HBO and the blue area (%) before HBO (Fig. 1). In addition, change in the color of the flaps after HBO was observed, and the time when no further improvement was noted was recorded as the date of flap stabilization. The number of HBO treatments, the starting date of HBO, and other factors were also studied. Finally, the relationship between the start of HBO and the degree of circulatory improvement in the 12 cases in which Abbe flaps were transplanted, where the surgical methods and the recipient site were virtually the same, were subjected to statistical analysis to determine the optimum schedule of HBO.



volume of recovery(%)

=blue area(%) − necrosis area(%)

Fig. 1. Method of judging effect of HBO degree of recovery is blue area of flap minus necrotic area of flap after HBO.

Clinical Results

Twenty-six patients received HBO treatments 3 to 46 times. The average number of treatments was 19.4. For 2 patients, HBO was discontinued because they developed severe otalgia during therapy. Five patients started HBO on the same day that surgery was performed, 7 started the day after surgery, 5 started 3 d after surgery, and 4 started 4 d after surgery. Thus, the majority of patients started HBO by the 4th d after surgery. Three patients started HBO on the 5th, 8th, and 14th postoperative d, respectively, when the patients were sufficiently stable. The patient who received HBO on the 14th postoperative d was 1 of the patients who had received flap transplantation to cover a palatal splint. The long delay was due to failure to recognize disturbed circulation of the flap. The average time between surgery and the start of HBO was 2.6 d (Table 3).

Table 3
Therapy Condition of HBO on Ischemic Flaps

Case	Start of HBO, POD*	Pressure, ATA	Treatment Period, min	No of Times
1	3	2	60	11
2	3	2	60	18
3	4	3	60	14
4	1	2	60	17
5	1	2	60	19
6	1	2	60	19
7	0	2	60	5
8	4	2	60	9
9	1	2, 3	60, 60	25
10	0	2, 3	60, 60	35
11	1	2, 3	60, 60	21
12	0	3	60	19
13	3	2	60	16
14	2	2, 3	60, 60	46
15	2	2	60	8
16	4	2	60	8
17	8	2	60	3
18	1	2, 3	60, 60	28
19	4	2	60	—
20	1	2, 3	60, 60	36
21	5	2, 3	60, 60	36
22	0	2	60	10
23	2	2, 3	60, 60	10
24	14	2, 3	60, 60	18
25	3	2	60	—
26	0	2, 3	60, 60	34
Mean	2.6			19.4

* POD = postoperative day.

Circulatory improvement in flaps was observed in all cases after HBO. The flaps in all patients were discolored before HBO therapy, but changed to a fresh pink color just after the initial HBO, and the viable area increased according to the increase of HBO. An average of 2.7 d was required before the condition stabilized and the flap color showed no further change.

Eleven patients given HBO achieved 100% recovery of the degree of circulation, and another 5 patients achieved 95% recovery. Partial necrosis of 15 to 20% was observed in the remaining 10 patients, resulting in an average of 92.1% improvement rate overall. Even when there was partial necrosis, it was limited to the superficial layer at the extreme edges of the flap (Table 4).

Table 4
Effects of HBO on Ischemic Flaps*

Case	Blue Area of Flap, %	Necrotic Area of Flap, %	Degree of Recovery, %	Date of Symptom Fixed, POD**
1	100	5	95	4
2	100	15	85	3
3	100	15	85	2
4	100	0	100	1
5	100	0	100	1
6	100	0	100	2
7	100	0	100	2
8	100	5	95	7
9	100	0	100	2
10	100	0	100	1
11	100	15	85	7
12	100	0	100	1
13	100	5	95	3
14	100	20	80	7
15	100	20	80	3
16	100	0	100	1
17	100	0	100	1
18	100	0	100	4
19	100	20	80	stop
20	100	5	85	1
21	100	20	80	8
22	100	5	95	1
23	100	0	100	1
24	100	5	95	1
25	100	20	80	stop
26	100	20	80	0

* 100% Recovery of the Circulation was Observed in 11 Flaps; 95% Recovery in 5 Flaps; and 80 to 85% Recovery in 10. Average Recovery Rate was 92.1%. ** POD = Postoperative day.

Following is a description of some of the representative cases.

Case 11: 16-yr-old male, with an Abbe flap transplantation to correct lip deformity after bilateral cleft lip and palate surgery. The entire flap became dark pink immediately after surgery (Fig. 2, *top*). Since a high degree of circulatory disturbance was suspected, HBO at 2 ATA for 60 min and 3 ATA for 60 min was administered on consecutive days. On the 5th postoperative d, 85% of the area of the distal portion recovered, where there was superficial necrosis (Fig. 2, *bottom*).

Fig. 2. Case 11, 16-yr-old male. Top, upper lip deformity and Abbe flap transfer. Immediately after the operation the entire flap became dark pink and revealed severe edema. Bottom, 7 d after HBO; 85% of the flap survived.

Case 21: 50-yr-old male, with a sternocleidomastoid flap transplanted after resection of tongue and mouth floor cancer. The patient was given chemotherapy with peplomycin before surgery, then underwent a partial resection of the mandible and hemiglossectomy, followed by a sternocleidomastoid myocutaneous flap with a 60 × 40 mm skin paddle transplanted for reconstruction of the tongue and mouth floor (Fig. 3). During surgery, the flap began to turn blue and there was no bleeding from the edges of the flap. Next day, the skin paddle became extremely edematous, with total necrosis of the flap due to circulatory disturbances (Fig. 4, *top*). The same HBO therapy was administered as that in case 11. HBO was continued until 28 treatments were administered. The color of the flap improved immediately, with complete recovery after the 8th HBO treatment. Therapy resulted in 100% recovery (Fig. 4, *bottom*).

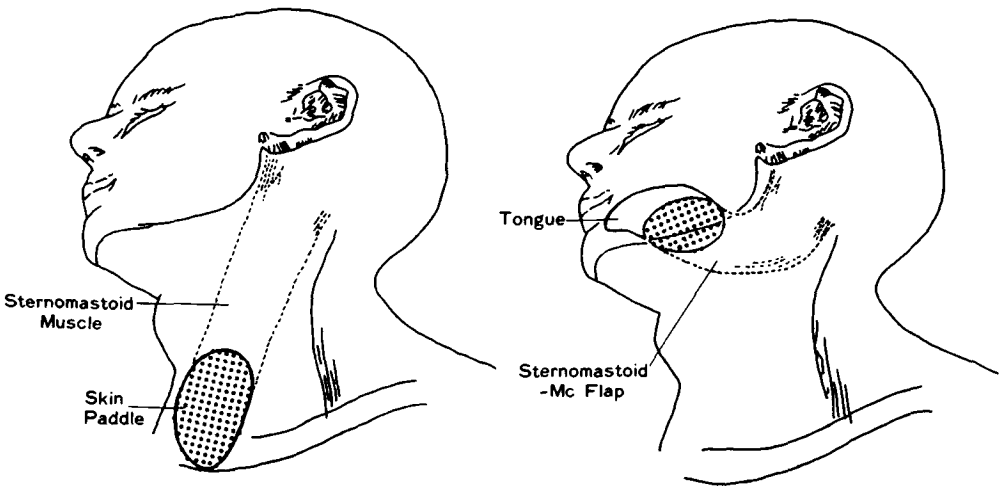


Fig. 3. The method of reconstruction in case 21. The tongue and mouth floor was reconstructed with sternocleidomastoid myocutaneous flap.

Finally, the relationship between the start of HBO and the degree of circulatory improvement of 12 cases is shown in Fig. 5. All were transplanted Abbe flaps and the surgical procedures were virtually the same. The degree of improvement is shown on the vertical axis, and the starting point of HBO is shown on the horizontal axis. In calculating a regression line, $Y = 100.1 - 2.8X$ was obtained and the correlation coefficient was -0.806. This suggests that an early start of HBO results in a higher degree of circulatory improvement.

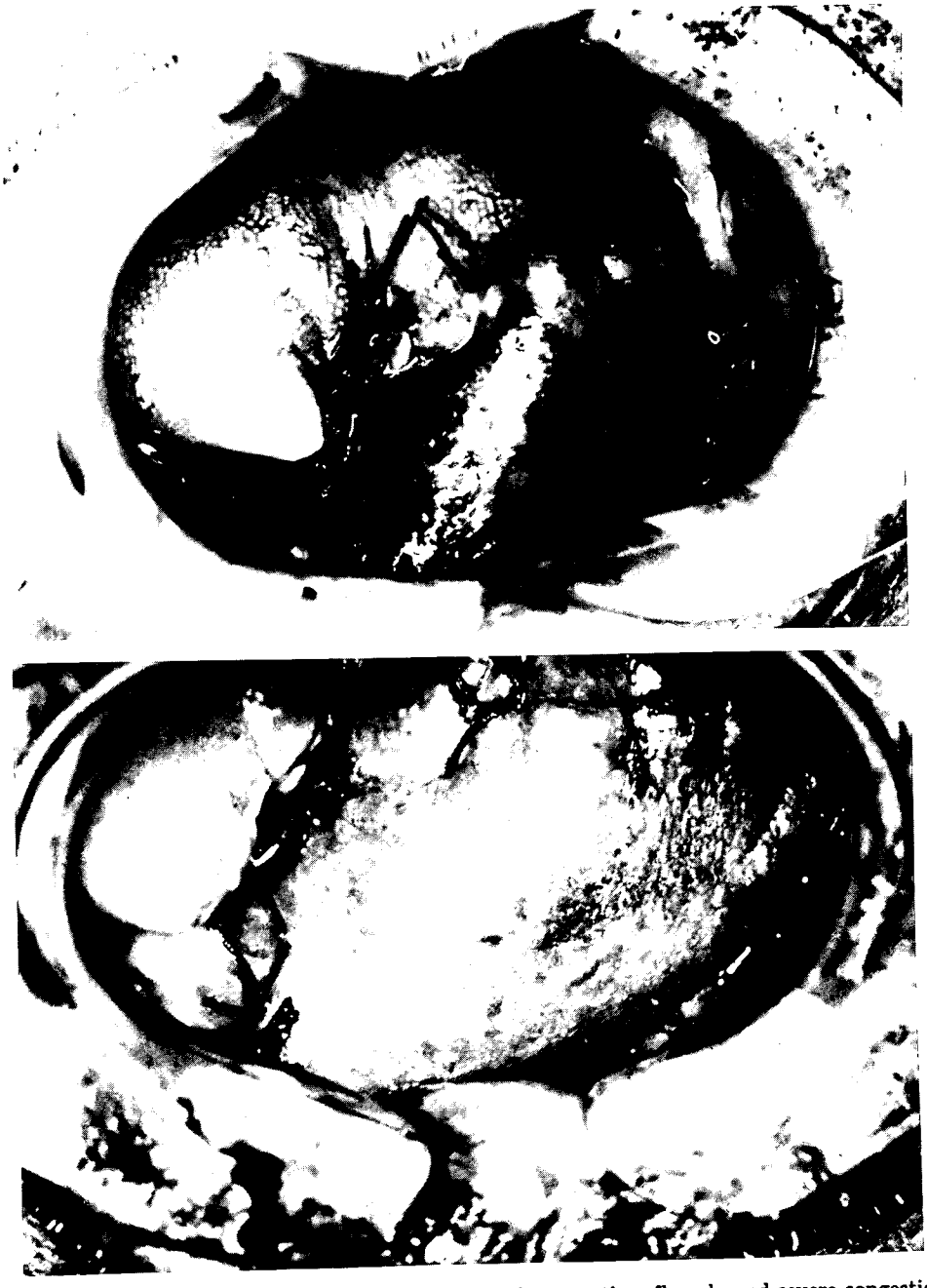


Fig. 4. Case 21, 50-yr-old male. Top, 1st d after the operation, flap showed severe congestion and edema; no bleeding was observed from the skin paddle. Bottom, 4th d after the operation (treated with HBO 8 times); flap color was improved remarkably; skin paddle improved to normal skin color; flap survived completely.

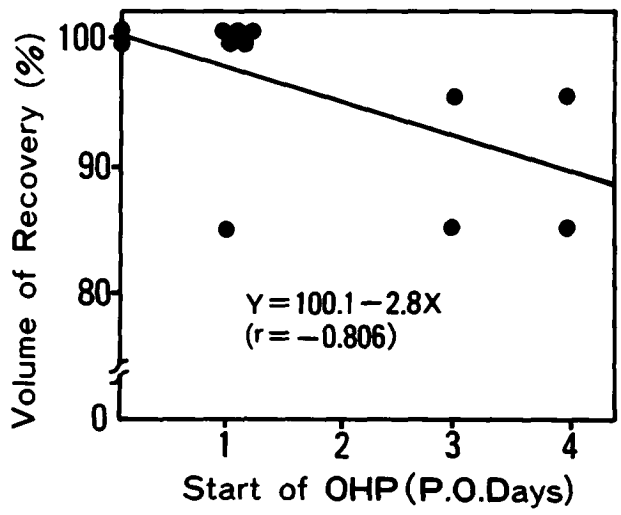


Fig. 5. Relationship between the start of HBO and the degree of circulatory improvement in Abbe flap. Graph shows that the earlier HBO is started, the higher the degree of circulatory improvement.

EXPERIMENTAL STUDY

The following experiments were conducted to demonstrate the mechanism of HBO in treating ischemic flaps.

Materials and Methods

The experiment was conducted according to the method used by Tan et al. (12). Experimental animals were 30 Wistar rats with body weights of 250 g. Sodium pentobarbital (50 mg/kg) was administered i.p. An island flap was designed on the lower abdomen using the superficial epigastric artery as a feeding artery to the lower abdomen (Fig. 6). To induce ischemic conditions in the flaps, the flap blood supply was cut off by clamping the feeding artery for 60 min. The clamp was then removed and blood flow restored. HBO was administered immediately to the experimental group of rats at 2 ATA for 60 min. Another group of rats used as control did not receive HBO treatment. Blood flow was measured before and after HBO using a laser Doppler flow meter (13, 14) (Periflux®, Perimed Co. Ltd., Sweden). Blood flow was measured in the center and at 4 points around the perimeter of the flap, then an average measurement was calculated.

RESULTS

In the control group, blood flow was reduced to 12 to 13% of unclamped

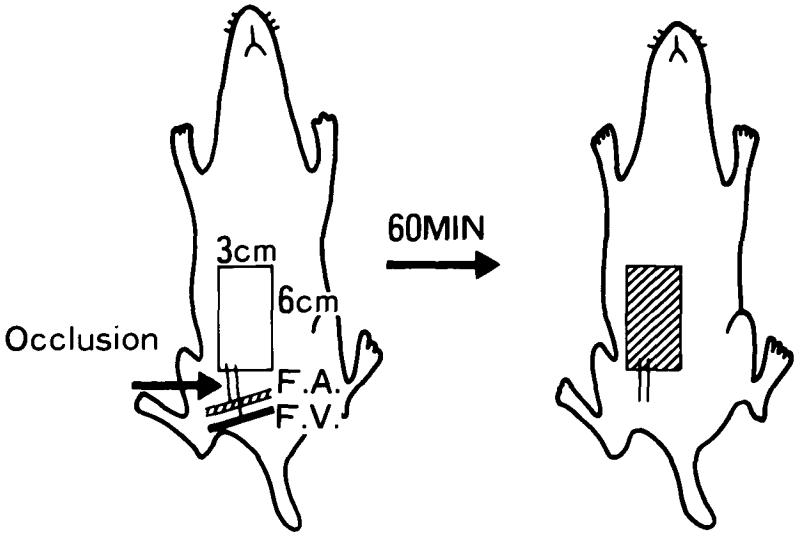


Fig. 6. Experimental methods. An island flap was elevated on the lower abdomen of the rat. The feeding vessels were clamped for 60 min. The rat was treated with HBO at 2 ATA, 100% O_2, 60 min. Blood flow of the flap was measured at pre- and post-HBO with laser Doppler flow meter.

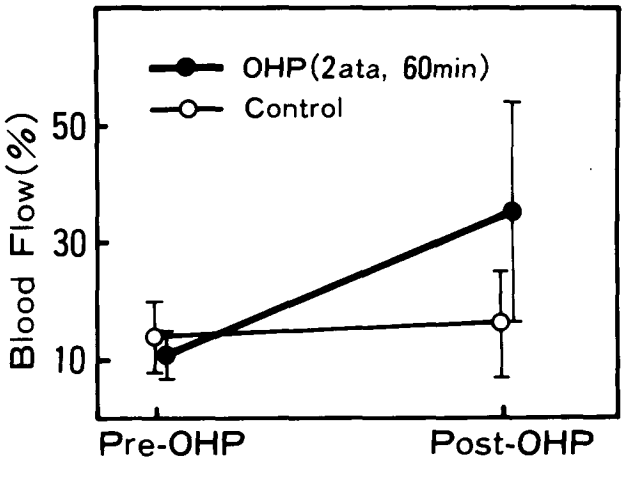


Fig. 7. Blood flow of the ischemic island flap and normal flap. In the ischemic flap the blood flow is increased remarkably after HBO, but in normal skin flap no change of blood flow was observed.

valves for 60 min, with virtually no change observed. In the experimental group, blood flow in the flap was 10% before HBO and 32% after, which is a significant increase in the blood flow when compared to the control group (Fig. 7).

It was confirmed experimentally that blood flow is increased significantly by HBO in ischemic flaps. Based on the mechanism of flap recovery, we suggest that HBO for compromised skin flaps be continued for 2 wk after surgery.

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HYPERBARIC OXYGENATION TO ISCHEMIC ULCERS IN COMBINATION WITH SYMPATHETIC DENERVATION AND PGE_1 INFUSION

H. Urayama, Y. Watanabe, and T. Iwa

Arterial reconstruction is the first choice for pain, ulcer, and/or necrosis of extremities in obstructive arterial diseases. But in cases where arterial obstruction is too distal, the run-off of the distal artery is poor, and the organ functions deteriorated with age or complications, such as myocardial infarction, conservative therapy is preferred. Hyperbaric oxygenation (HBO) is now considered a useful conservative therapy. In this report, the effect of HBO in combination with sympathetic denervation and prostaglandin E_1 (PGE_1) infusion on ischemic ulcers is evaluated.

MATERIALS AND METHODS

Thirty-one patients with ischemic ulcer were treated with conservative therapy from 1978 to 1984; 28 men and 3 women, ranging in age from 28 to 79 yr (mean: 48.5). Distribution of diseases was 11 cases of arteriosclerosis obliterans, 17 of thromboangiitis obliterans, 2 of systemic scleroderma, and 1 case of arterial injury (Table 1). Ten patients were treated with HBO in combination with sympathetic denervation and PGE_1 infusion (group I) (Table 2); 21 cases were treated by sympathetic denervation and PGE_1 infusion without HBO (group II).

Hyperbaric oxygen treatment was carried out in a monoplace chamber at 3 atmospheres absolute pressure (3 ATA), breathing 100% oxygen. The number of HBO treatments was determined by change in the ulcer and complications such as barotrauma. Plethysmogram, skin temperature, and transcutaneous oxygen pressure (t_c Po_2) were monitored during HBO treatment in 5 patients, no. 6 to 10. The plethysmogram was taken at the toe or finger of the diseased extrem-

Table 1
Distribution of Diagnosis and Therapy in Group I (Treated with HBO) and Group II (Treated without HBO)

	Diagnosis			PGE_1 Infusion			
Group	ASO	TAO	Others	i.v.	i.a.	Sympathetic Resection	Denervation Block
I	4	6	0	7	3	6	4
II	7	11	3	16	5	14	7

ASO = arteriosclerosis obliterans; TAO = thromboangiitis obliterans; i.v. = intravenous; i.a. = intraarterial.

ity by photoelectric plethysmograph of the reflected light type. The skin temperature was monitored at the dorsum pedis or the dorsum manus of the diseased extremity by the thermistor sensor. The t_c Po_2 was monitored at the border of the ulcer by the electrode of the transcutaneous blood gas analyzer.

Table 2
Patients with HBO Treatment (Group I)

Patient No.	Diagnosis	Age	Sex	Sympathetic Denervation	PGE_1 Infusion	HBO Times	Response of Ischemic Ulcer
1	TAO	39	M	Resection	i.v.	30	PH
2	TAO	37	M	Block	i.v.	8	PH
3	TAO	38	M	Resection	i.v.	46	PH
4	TAO	53	M	Resection	i.v.	7	PR
5	TAO	43	M	Resection	i.a.	11	CH
6	ASO	67	M	Block	i.a.	8	CH
7	ASO	68	F	Block	i.v.	10	CH
8	TAO	46	M	Resection	i.a.	10	PR
9	ASO	70	M	Block	i.v.	20	PH
10	ASO	74	F	Resection	i.v.	20	CH

TAO = thromboangiitis obliterans; ASO = arteriosclerosis obliterans; i.v. = intravenous; i.a. = intraarterial; CH = complete healing; PH = partial healing; PR = pain relief without healing.

Sympathetic denervation was performed by operative resection or phenol block of sympathetic ganglia and nerves of Th I, II, and III in thoracic sympathetic denervation, and L II, III, and IV in lumbar sympathetic denervation. PGE_1 infusion was performed by continuous intraarterial infusion of the dose of 0.05 to 0.2 $ng \cdot kg^{-1} \cdot min^{-1}$, or continuous intravenous infusion of the dose of 5 to 10 $ng \cdot kg^{-1} \cdot min^{-1}$. Responses to conservative therapy were

divided into four categories: complete healing of the ulcer (CH), partial healing of the ulcer (PH), pain relief without healing of the ulcer (PR), and no change (NC).

RESULTS

In patient 6, the plethysmogram showed increased amplitude of the pulse wave by sympathetic denervation and PGE_1 infusion, and during HBO the amplitude decreased before but did not change after sympathetic denervation and PGE_1 infusion (Fig. 1). In patient 7, t_c Po_2 in room air breathing was increased threefold after sympathetic denervation and PGE_1 infusion than before, and t_c Po_2 during HBO treatment was increased fivefold after sympathetic denervation and PGE_1 infusion than before (Fig. 2). The skin temperature of 5 patients, 6 to 10, rose by 1.0°C in mean value at compression and fell by 1.0°C at decompression, while the room temperature rose by 0.6°C at compression and fell 1.6°C during decompression (Fig. 3). The amplitude of pulse wave of the plethysmogram in 5 patients, 6 to 10, was decreased by 0.6 mm in mean value during HBO treatment, but it was negligible (Fig. 4). In 5 patients, 6 to 10, t_c Po_2 before HBO was 39 + 22 mmHg (mean ± SD), and during HBO it increased to 283 ± 139 mmHg (Fig. 5). All patients in group I (treated with HBO) became pain-free, compared to group II (treated without HBO) in which 2 patients remained unchanged. Complete or partial healing of the ulcer was accomplished in 8 of 10 patients (80%) in group I, and 11 of 21 patients (52%) in group II (Table 3).

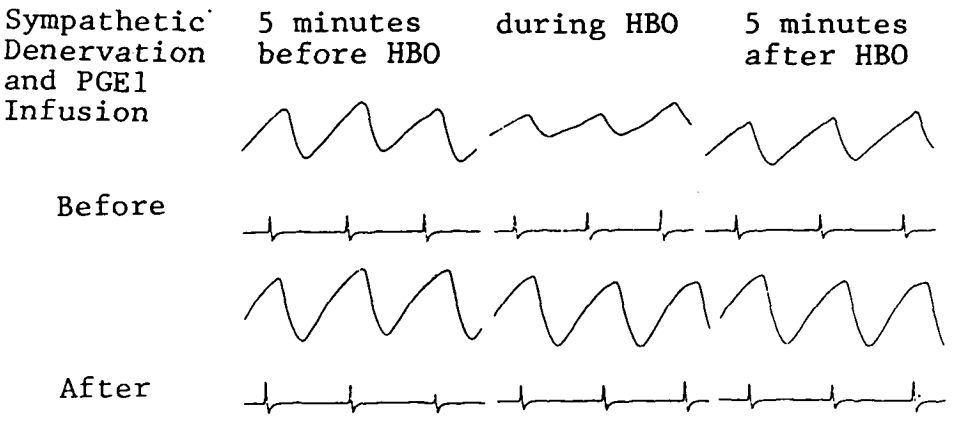

Fig. 1. Wave forms of the plethysmogram before and after sympathetic denervation and PGE_1 infusion in patient 6.

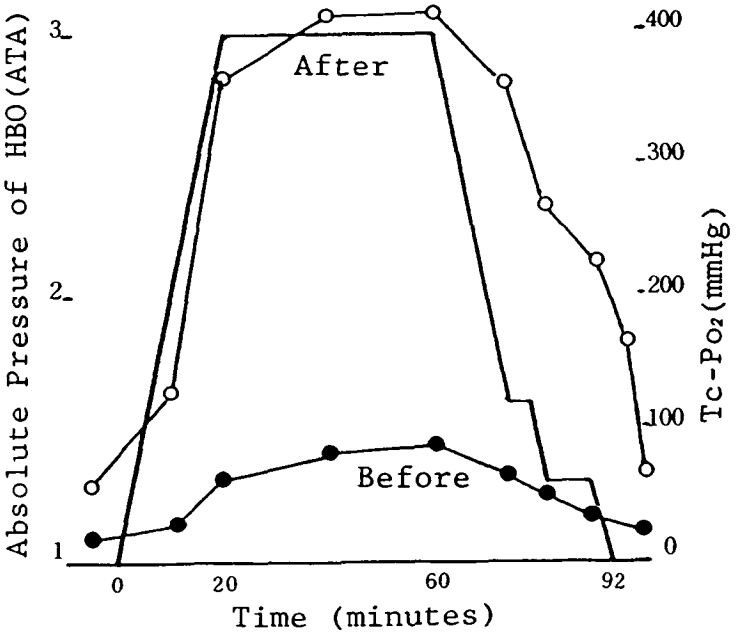

Fig. 2. Transcutaneous oxygen pressure ($t_c Po_2$) during HBO treatment before and after sympathetic denervation and PGE_1 infusion in patient 7.

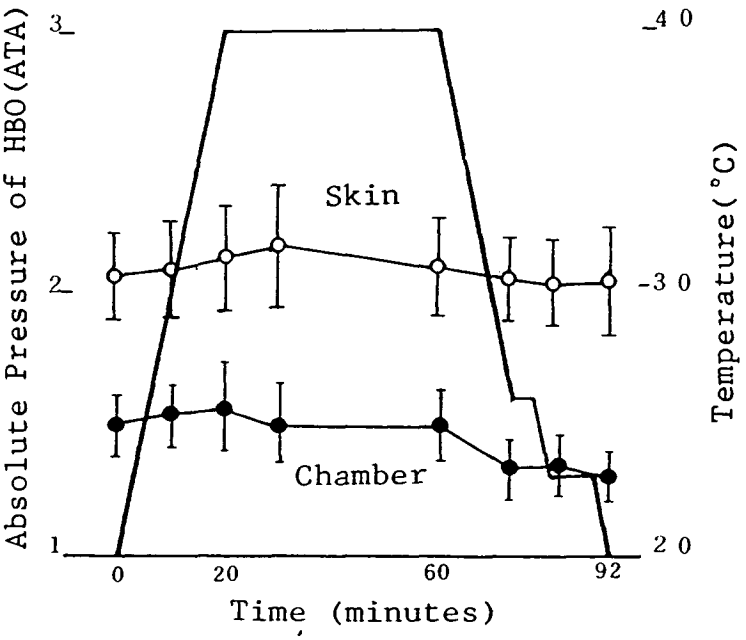

Fig. 3. Skin and chamber temperatures during HBO treatment in 5 patients, 6 to 10. Values are mean ± SD.

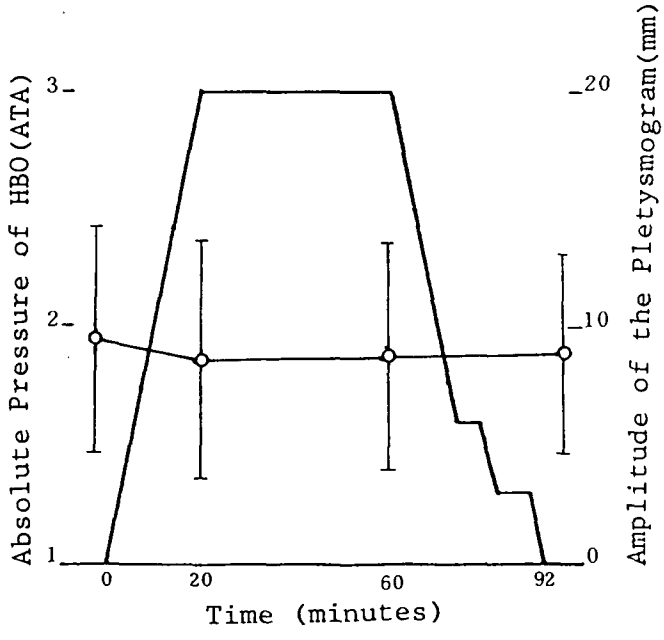

Fig. 4. Amplitude of the plethysmogram during HBO treatment in 5 patients, 6 to 10. Values are mean ± SD.

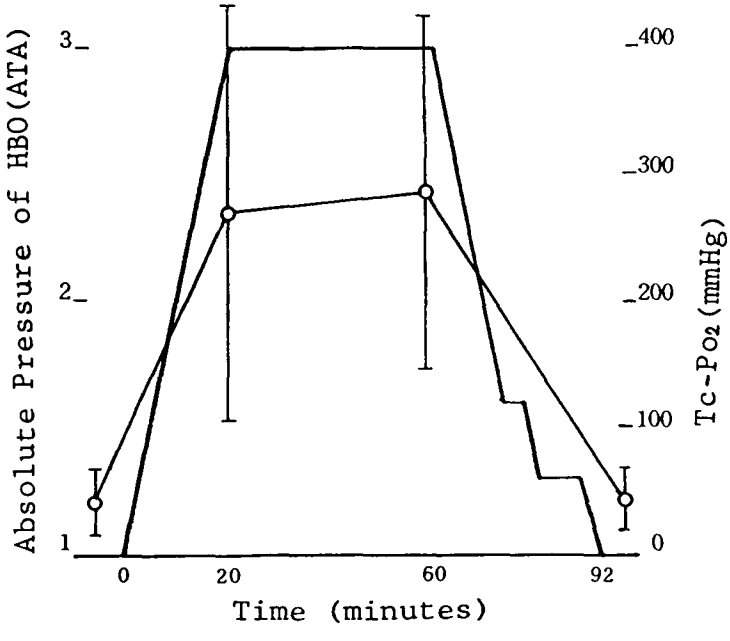

Fig. 5. Transcutaneous oxygen pressure during HBO treatment in 5 patients, 6 to 10. Values are mean ± SD.

Table 3
Response of Ischemic Ulcers in Group I (Treated with HBO) and Group II (Treated without HBO)

Group	No. of Cases	Response				CH + PH, %
		CH	PH	PR	NC	
I	10	4	4	2	0	80
II	21	2	9	8	2	52

CH = complete healing; PH = partial healing; PR = pain relief without healing; NC = no change.

DISCUSSION

Ischemic ulcer was induced by impaired arterial blood supply which carries nutrition and oxygen to the tissues. HBO increases the oxygen supply to the tissues, but in HBO breathing 100% oxygen, the rich, oxygenated blood cannot reach the tissues if the blood supply is compromised severely. Sympathetic denervation dilates the vessels by relaxing nerve-controlled vasoconstriction (1). PGE_1 dilates the vessels directly, relaxing smooth muscles of the wall (2), and increases the peripheral perfusion by correcting erythrocyte deformability (3). As the plethysmogram showed increased amplitude in patient 6, sympathetic denervation and PGE_1 infusion increase the blood flow to the extremity of the ischemic ulcer. HBO reduces the cardiac output and regional blood flow (4, 5). Sympathetic denervation and PGE_1 infusion block the vasocontractor effects of HBO, as shown by the plethysmogram during HBO. The skin temperature changed in proportion to the room temperature of the hyperbaric chamber. The room temperature is held constant by ventilation and air conditioning, but it rises on compressing and falls on decompressing by Boyle-Charles law. The maximum difference of the skin temperature of 1°C should not affect the amplitude of the plethysmogram (6).

The skin temperature and the plethysmogram were taken on normal skin of the diseased extremity, whereas the $t_c\ Po_2$ was taken at the border of the ischemic ulcer. Healing depends on the perfusion to the edge of the ulcer where it takes place. As in case 7, sympathetic denervation and PGE_1 infusion not only increased the $t_c\ Po_2$ at the border of the ischemic ulcer at one atmosphere but also had a synergistic effect with HBO to the $t_c\ Po_2$ (7). The $t_c\ Po_2$ increased 5 to 10 times by the combination therapy and that was enough for healing of the ischemic ulcer. However, in 2 patients, 4 and 8, no healing of the ulcer occurred. In is possible that the peripheral vessels were already dilated maximally before treatment, and that the limiting factor was not oxygen. Although the oxygen cannot promote the healing process, it can reduce ischemic damage of nerve endings or reduce the nerve-stimulating

products that cause pain (8). In spite of a lack of healing of the ulcer, the pain disappeared in those 2 patients.

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EFFECT OF DAILY HYPERBARIC OXYGEN (2 ATA) ON THE COURSE OF CHRONIC RELAPSING MURINE EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

M. R. Powell, V. Kizer, S. Hruby, E. C. Alvord, Jr, and J. Martin

The publication of the paper by Fisher et al. (1) describing their double-blind study of the effect of hyperbaric oxygen (HBO) on the short-term course of mild to moderate multiple sclerosis (MS) led to an increased awareness of this as a possible treatment modality. The uneven course of this disease and the possibility of placebo effect prompted us to examine the influence of HBO on relapsing-remitting experimental allergic encephalomyelitis (EAE) in mice, one animal model of MS.

Currently, no spontaneous animal analog of MS exists. Sensitization against myelin antigens has been the most successful induced animal model of an autoimmune demyelinating disease and was introduced in the 1930s by Rivers and coworkers. Stone and Lerner (2) produced the first model of a chronic disorder that possessed a relapsing remitting course with lesions separated in time and space (3-5).

The model of chronic relapsing EAE it animals has been applied to MS because there are many clinical and pathologic similarities between the two disorders. The former disease is produced by an injection of spinal cord homogenate and Freund's adjuvant. There ensues a latent period (which in our case averaged 21 d) before overt signs of illness are manifest, which consists of weight loss and paraparesis and sometimes progresses to quadriparesis. It is rarely fatal and remits to varying degrees, often completely.

Initial experiments with hyperbaric oxygen and EAE were performed by Prockop and Grasso (6) and Warren et al. (7). Both groups investigated the effect of hyperbaric oxygen on the acute form of EAE, and both studies indicated a mitigating effect of increased partial pressures of oxygen. Reduced severity was noted when HBO was started before the first symptoms of EAE

were noted; it was unclear, however, whether the major effect of oxygen was a) prevention of the destructive demyelination or b) prevention of the initial sensitization to myelin basic protein and Freund's adjuvant. If the mechanism was only to block desensitization, then the effect of hyperbaric oxygenation would be to function as a prophylactic before the first indication of MS was seen. For clinical use, this would entail treating those at risk for MS; this basically includes the entire population between 20 and 40 yr of age. For economic reasons, the HBO method would be of limited value.

Mokhtarian et al. (8) reported the effect of HBO on the course of EAE. Female SJL mice were sensitized with myelin basic protein and Freund's adjuvant. Lymphoid cells derived from these immunized syngeneic donor animals were injected into other mice to produce EAE by transference. These mice regularly developed paraparesis and urinary incontinence 7 to 9 d after cell transfer; most mice recovered from the initial paralysis. Hyperbaric oxygen therapy (2 ATA for 3 h) was started in the experimental group 3 d post-immune-cell transfer and continued for 2 wk posttransfer. The onset of the disease was delayed by 1 d, and the symptoms were less severe (one grade less) in the HBO group compared to the air-treated controls.

MATERIALS AND METHODS

Subjects and Sensitization

The F-1 generation of a cross between female SJL/J and male PL/J mice was utilized for these studies. EAE was induced by the direct sensitization method similar to that described by Fritz et al. (9). Injections were given (0.1 ml 3 times) in the flank; two sets of immunizations were made with a 1-wk interval. Between 19 and 25 d after the first injection, 65% of the mice displayed signs of the initial acute attack of EAE; 35% of the animals never became ill. The classic signs consisted of limp tail, hind-limb weakness or paralysis, and loss of righting reflex.

The sensitization vaccine consisted of mouse spinal cord homogenate prepared from BALB/c mice. The spinal cords were removed by insufflation and homogenized immediately in an equal weight of cold phosphate buffered saline, frozen, and stored at -70°C.

Immediately before the immunization, the appropriate volume of phosphate buffered saline was added to the homogenized spinal cord so that the final concentration 0.3 ml contained 3 mg (wet weight) of spinal cord. This was then mixed with the same volume of complete Freund's adjuvant prepared as follows: paraffin oil (hexadecane), 8.5 ml; Arlacel A, 1.5 ml; and *Mycobacterium tuberculosis* (H 37 Ra) 20 mg. The vaccine was mixed immediately before use to prepare a smooth, stable emulsion, and each mouse received 3 times 0.1 ml of the vaccine injected s.c. in both flanks on Days 0 and 7.

Disease Course

This immunization produced disease which began on Day 19. The mice were scored every other day on a clinical grading scale as follows (10):

0 = no abnormality
0.5 = flaccid tail, hesitancy in righting reflex
2 = 1 or 2 limbs obviously weak, poor gait
3 = obviously weak, very poor gait; must closely examine to determine if limb is really not paralyzed
4 = 1 or 2 limbs paralyzed
5 = 3 or 4 limbs paralyzed

Subjects destined to be treated with oxygen (n = 15) or air (n = 15) were determined by random selection and marked before the time of the first inoculations. All animals were given food and water ad libitum. Oxygen-treated and air-treated animals were held together in the same cages and identified by colored marks; the cages in which the mice were held were varied in their position in the animal room to minimize positional effects.

Animals were assessed for the severity of their clinical signs 3 times/wk and weighed at least once weekly. There was no significant statistical difference in weight between the air-treated controls, oxygen-treated animals, or other untreated EAE subjects.

Hyperbaric Treatment

Experimental subjects were exposed in a hyperbaric chamber 6 d/wk for 2.5 h at 2.0 ATA (33 fsw) beginning an day 28 after the initial injection; this was 4 to 9 d after onset of clinical signs. Control subjects were exposed to the same time-pressure regimen, but the compression gas was air. Pressurizations were generally made morning and afternoon and the sequence was varied randomly so that oxygen-treated animals were not always done in the morning and air-treated in the afternoon; this was to minimize effects based on diurnal variation. Pressurizations were continued until Day 122.

RESULTS

Clinical Course

Figure 1 shows a plot of the average clinical grades of the air-treated and oxygen-treated animals as a function of time. As indicated on the graph, oxygen therapy was initiated after the initial acute phase of EAE.

Oxygen-treated animals, although their average score before therapy was higher, had a reduced severity and a shorter duration of illness. The reduced degree of severity is indicated in Fig. 2, which is a histogram of animals with each clinical grade. This histogram is based on the clinical grades of the mice from Day 35 to 123.

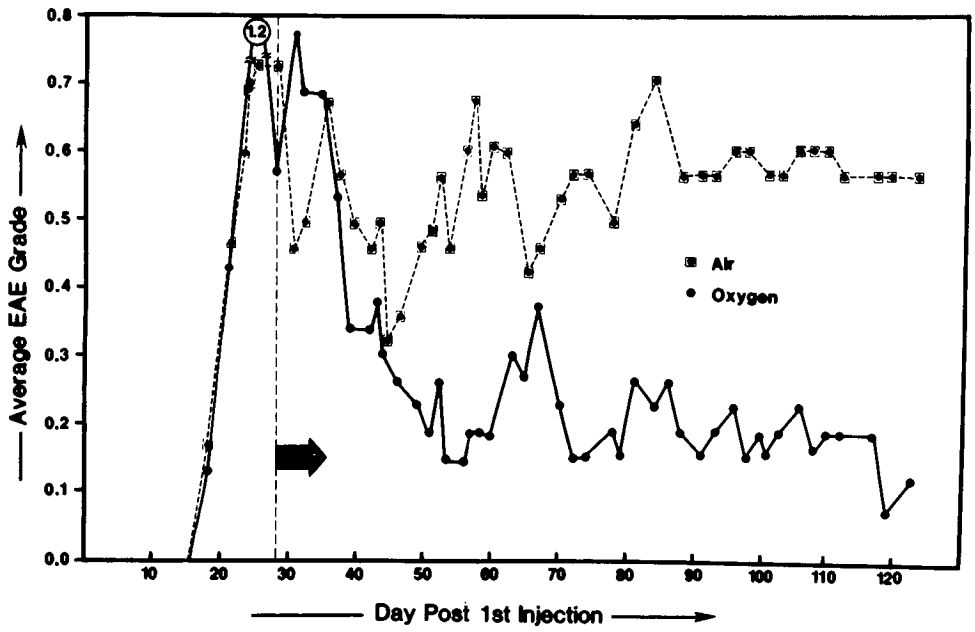

Fig. 1. The average clinical EAE grade vs. time (days) following the first inoculation. Mice were exposed to either air or oxygen (both at 2.0 ATA for 2.5 h) on a 6-d/wk basis. Pressure treatments concluded on Day 122.

Immunologic Studies

Mice were killed on Day 123 by cervical dislocation. The brain was extracted and homogenized in 2 ml of Tris buffer, pH 7.4. Blood was obtained from the vena cava and allowed to coagulate for 30 min. Blood was pooled from the oxygen-treated, air-treated, and normal, F-1 generation mice, and sera was extracted from the blood clot in each group and then frozen at-70°C.

Immunoblot

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using an 8% stacking gel and a 10% separating (running) gel. Brain preparations were sonicated for 30 s and then boiled in sample buffer for 5 min. Gels were loaded with 10 μl of protein/well; appropriate sample size was previously determined by a Bradford assay. One of the five SDS-PAGE preparations was stained for protein with Coomassie blue, and the remaining four were transferred to nitrocellulose. These four nitrocellulose sheets were treated as follows: a) treated for 1 h with 200 μl of pooled HBO-treated mouse sera, b) treated for 1 h with 200 μl of pooled air-treated mouse sera, c) treated for 1 h with normal (noninjected) mouse sera, and d) used as the conjugate control (no sera). All procedures were conducted at room temperature.

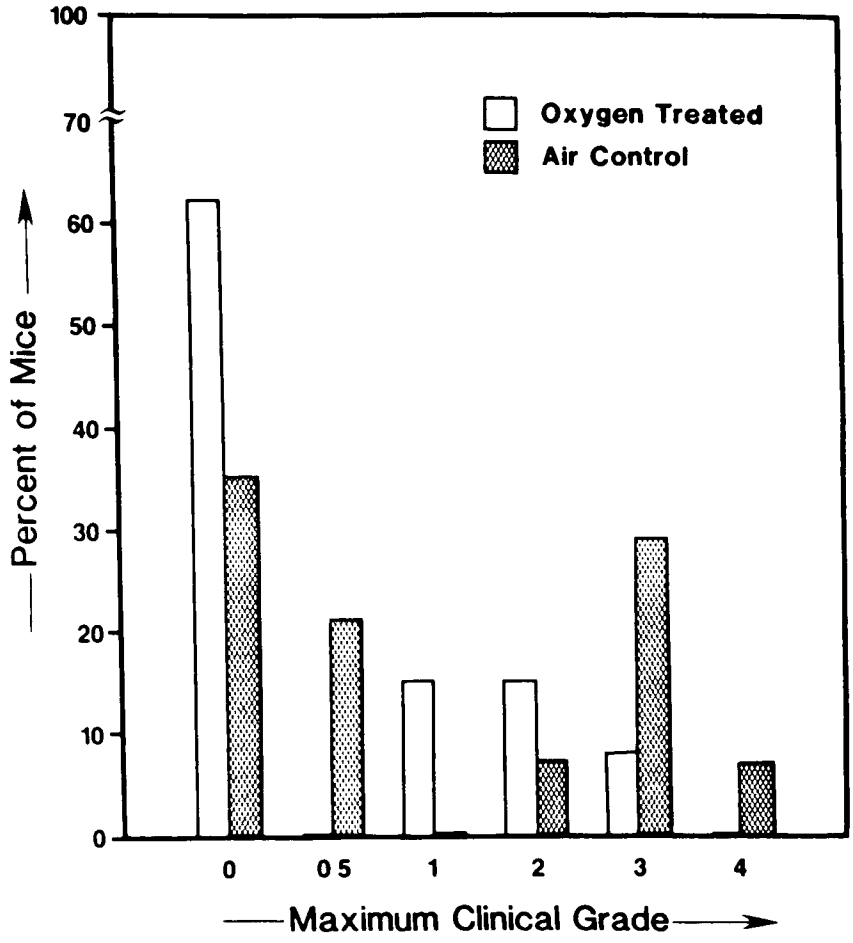

Fig. 2. Maximum clinical EAE grade that developed after the initiation of treatment (either oxygen or air). Period included in the histogram is Day 35 to 123, inclusive.

Unoccupied sites were blocked with 0.5% gelatin, 0.1% Tween 20 then washed in phosphate buffered saline with 0.1% Tween 20. Sheets were washed again in phosphate buffered-Tween. An affinity purified rabbit antimouse peroxidase-labeled IgG was applied at 1:100 dilution to each of the four nitrocellulose sheets. The sheets were again washed with phosphate buffer-Tween and then stained with 0.005% 4-chloro-1-naphthol and with 0.008% H_2O_2. Color appeared within 5 min after application.

Stains of the various proteins of the mice brain tissue separated by SDS-PAGE showed bands at 63, 57-62, and 56 K, which reacted strongly on the immunoblot assay procedure with air-treated mouse sera and the conjugate. An

immunoblot of brain proteins reacted with oxygen-treated mouse sera along with the conjugate showed that all bands reacted similarly except the 63 K band showed minimal reactivity. The entire blot suggests less reactivity of the oxygen-treated mouse sera, perhaps by limited IgG production. Brain proteins treated with normal (control) mouse sera had no reactivity to self-proteins. The conjugate control indicated no IgG present in brain tissue.

The major proteins of myelin in the central nervous system are: a) Soluble proteins A_1 or P_1 (defined as extrinsic proteins); these are encephalitogenic with a molecular weight (MW) of 18,400 daltons, and basic protein P_2, MW 15,400 daltons. b) Hydrophobic proteins, or intrinsic proteins: DM20-intermediate protein, MW 20,600 daltons; and the "Wolfgram" protein MW 50,000 to 60,000 daltons.

Wolfgram proteins are in the range of 50 to 60 K and comprise 15% of the total protein in the rodent CNS myelin; in the immunoblot assay, it was the protein components in this weight range that reacted in both the air- and oxygen-treated mouse groups. Myelin basic protein and prebasic proteins reacted at the leading edge of the immunoblot and did not separate. There was a slight reaction at 206 K, which is possibly contamination because there are no known major CNS proteins of this molecular weight.

Histologic Study

On Day 125 after the course of exposure to either air or HBO, the mice were killed by cervical dislocation and the brains and spinal cord dissected out. These were placed in phosphate buffered formalin, embedded in paraffin, sectioned, and stained with a myelin stain, gallocyanine-Darrow red. The slides were read in a blind fashion (without knowledge of the clinical state or treatment). The results were as follows:

1. Subpial demyelination was the major finding; inflammation was not prominent (samples taken approximately 120 d postinitial injection). There was no evidence of remyelination.
2. The severity of lesions correlated with the severity of the clinical disease in 88% specimens (HBO, $n = 11$; air, $n = 14$). The exception included 2 cases of mild disease without histologic evidence of disease and 1 case of slight demyelination and no clinical signs.
3. The mice that had been exposed to the HBO showed a reduced degree of demyelination ("index" = 0.55) compared to those exposed to only hyperbaric air ("index" = 0.86).

DISCUSSION

The Immune Response and Experimental Allergic Encephalomyelitis

The immune reaction in EAE is one which the T- and B-lymphocytes are sensitized against certain antigens of myelin basic protein (MBP). The process is complex, involving many steps, many of which are speculative. In general, it

is envisioned that particular MBP antigens are presented to immunocompetent T-cells by the macrophages (possibly as surface antigens following ingestion). The T-cells (helper-inducer) can now release a "helper" (interleukin-1) compound which stimulates the proliferation of both T- and B-cells followed by antibody formation by plasma cells. Normally suppressor T-cells (suppressor-cytolytic) would modulate the activity of T- and B-cells, but the necessary control signals often seem to be insufficient. T-cell lytic activity and B-cell (plasma cell) production of antibody go temporarily unchecked and myelin degradation results. T-cell encephalitogenic activity against MBP has been shown to be very species specific in EAE (11).

The initial sensitization step in EAE is the presentation of MBP to macrophages; this biomacromolecule is present in the injected emulsion. In MS, the antigen may enter into the immune system by means of a virus that releases MBP and this, in turn, stimulates the immune response.

Immunosuppressive Properties of Oxygen

On the basis of observations concerning tissue culture of donor material, there was an initial suspicion of the immunosuppressive effects of HBO (12). Mouse thyroid graft acceptance could be prolonged by tissue culturing the graft in high concentrations of oxygen (13-15). This work seems to indicate that the effect of HBO was on the "processing" stage by the macrophage (16).

The effect of HBO on contact sensitivity to dinitro-flurobenzene (DNFB) was shown to be one of suppression of the cell-mediated response (17). This occurred either with preexposure of mice to HBO before sensitization or treatment after sensitization. Hyperbaric oxygen produced a dramatic decrease in total circulatory leukocytes and lymphocytes and in spleen weight; serum cortisol levels were only mildly elevated in HBO-treated mice.

Hyperbaric oxygen has been shown to reduce the severity of several cell-mediated immune phenomenon, including allergic encephalitis, tuberculin reaction, adjuvant disease (18), and allograft rejection (15). Thuning et al. (19) showed that HBO was also able to suppress migration of lymphocyte migration factor and that it depressed the humoral response to sheep erythrocytes in BALB/c mice. Tregeubenke et al. (20) found changes in the T-lymphocyte system in guinea pigs with HBO exposure. Parameters measured were hypersensitive to tuberculin and 2,4-dinitro-chlorbenzol and prolongation of skin-flap rejection.

Reactive Oxygen Intermediates

Oxygen is utilized in the body (particularly in oxidative phosphorylation) in a series of reactions in which one electron at a time is added to the oxygen molecule. In the mitochondrium, a well-controlled series of reactions guides the electrons down energy pathways so that specific reaction products are formed. "Leaks," however, often allow reactive oxygen species to be introduced into the cytosol where untoward actions may take place either toward cellular membranes or other cellular constituents.

The concentration of reactive oxygen intermediates (ROI) under normal conditions is kept to a low level by naturally occurring enzymes and scavenging compounds. Examples are superoxide dismutase (which causes the dismutation of the superoxide anion radical to hydrogen peroxide) and catalase (which catalyzes the disassociation of hydrogen peroxide into oxygen and water). It is becoming increasingly evident that there are many conditions in which the body's natural ROI defense mechanisms can be overwhelmed, and the production of these intermediates becomes excessive and possibly injurious.

A deleterious role for ROI has been found in skin-flap preservation; ("ischemic-reperfusion injury"), tumor promotion, carcinogenesis, tissue damage after physical exercise, radiation damage, inflammation, aging, myocardial infarction, elective cardioplegia, organ preservation, intestinal ischemia, and possibly even immune deficiency diseases (21-28). There is currently no certain indication of the role that ROI play in the effect of HBO on EAE, but we can be reasonably sure of their presence.

From studies of situations in which ROI are formed, the scenario is always similar; a rapid influx of oxygen enters the tissue, reactive oxygen species are formed, and the resulting ROI concentrations are more than can be handled by the cell's natural defense and/or scavenging systems.

Reactive species of oxygen can be produced in the body in concentration that can be deleterious in any situation in which the means for the increase of their concentration exceeds the ability of natural enzymes to degrade these ROI. The diatomic oxygen molecule is a rather unreactive compound, because it is necessary either for the electron spins to flip before chemical reaction can occur or electrons must be received by the oxygen molecule one at a time. During the latter process there are a series of partially reduced intermediates (29)

(a) $O_2 \xrightarrow{e^-} O_2^- \xrightarrow{e^-} O_2^{-2}$

(b) $O_2^{2-} \xrightarrow{H+} HO_2^- \xrightarrow{H+} H_2O_2$

(c) $H_2O_2 \xrightarrow{e^-} OH^- + OH\bullet$

(d) $OH\bullet \xrightarrow{e^-} OH^-$.

In the mitochondria, cytochrome oxidase binds the partially reduced reactive intermediates in the pathway, so that water is ultimately formed at the reactive site and the intermediates do not escape free into the cytosol. Most of what is known about free radicals and ROI formation concerns this organelle, although other sites can generate reactive oxygen species, e.g., the microsomes and the xanthine oxidase system (29).

One important characteristic of reactive species is their interconvertability (30). Thus, subsequent to the formation of the superoxide anion, the hydroxyl radical and the singlet oxygen lead to the formation of peroxy radicals (RO_2), alkoxy radicals (RO), and hydroperoxides and peroxides

(ROOR).

Oxygen may function as an immunosuppressive agent in its modification of the course of relapsing EAE by interfering with any of a number of steps:

a) The blockage of antigen uptake by or cytotoxic destruction of the macrophage, and thus a reduction in the initial "presentation" or "sensitization" involving the macrophage cell surface (and major histocompatibility complex, HMC) and the T-cell. Lipid peroxidation in rat splenocytes caused by treatment with the xenobiotic cumene hydroperoxide led to a functional disability but not to cell death [Shimura et al. (31)]. The effect was a marked depression of the mitogen response blocked by the addition of alpha-tocopherol or thiourea, agents that are scavengers of reactive oxygen intermediates. The authors speculate that ROI lipid peroxidation is highly associated with depression of lymphocyte function.

b) The cytotoxic destruction of the T-cell line by reactive oxygen species during the proliferation phase. T-cells are known to be very sensitive to ionizing radiation, and the mechanism of action of radiation is known to be reactive oxygen species. The effectiveness of oxygen (i.e., ROI) may lie in its ability to function at all loci of T-cell formation and/or proliferation (31).

c) The "helper signal" (interleukin-1) for proliferation of both T- and B-cells may be blocked by reactive oxygen species; this would be a certain outcome if T-cell proliferation were blocked. Without B-cells and plasma cells, the production of the humoral component of immunity is blocked.

Any or all of these steps in the autoimmune process might be blocked by HBO (and the effector species produced). From our initial work, it seems that there is at least a reduction in the serum titer of an antibody to an unidentified myelin protein, possibly Wolfgram.

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DOUBLE-BLIND, CROSSOVER STUDY OF HYPERBARIC OXYGEN IN MULTIPLE SCLEROSIS

E. W. Massey, D. L. Shelton, C. W. Erwin, H. Saltzman, P. B. Bennett, and E. M. Camporesi

Beneficial effects of hyperbaric oxygen in multiple sclerosis (MS) have been reported (1-4). Therefore, a double-blind, crossover study of 18 patients using the multiplace chamber was undertaken. This report discusses the clinical and electrophysiologic evaluation of these patients before, during, and after this therapy.

METHODS

Eighteen patients were selected after referral by their primary neurologists for evaluation and treatment and their diagnosis of MS was established. The criteria for inclusion in the study were: a) chronic progressive MS, b) greater than 3-yr duration, c) no remissions in 3 yr, d) no complicated pulmonary problems, and e) a Kurtzke scale between 3 and 7.5.

This group subsequently evaluated included 4 males and 14 females. The mean age was 45 with the range of 29 to 64 yr. The mean age of onset was 36 with a range from 17 to 54 yr. The average duration of illness was 9 yr with the range of 3 to 17 yr.

The physical dimensions of the chamber allowed a maximum of 6 patients at a time; thus the patients were assigned to 1 of 3 groups with 6 patients in each group. At the beginning of the first series of hyperbaric exposures, 3 patients from each group were randomly selected to be treated with 100% oxygen. The remaining 3 patients received normoxic control gas of 10% oxygen (Fig. 1). Each group of 6 patients was exposed to the hyperbaric environment of 2 ATA for 90 min, 5 d/wk for 4 wk, followed by a 4-mo. interval.

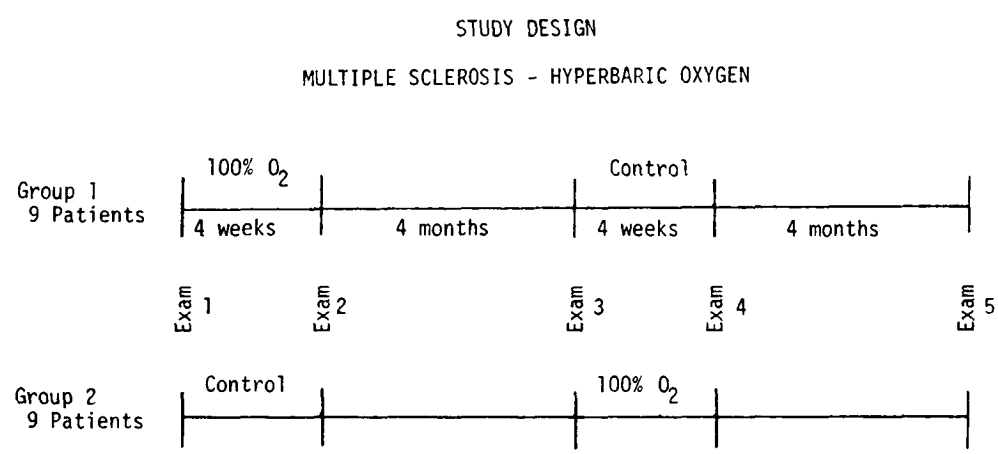

Fig. 1. Randomized, placebo controlled double blind with crossover. Two groups, with 4 wk of HBO, delay of 4 mo., then crossover.

The patients and evaluating physicians were blinded as to which patients were receiving the oxygen. At this point the groups were crossed over (i.e., the previously oxygen treated group were now the control group). They were again exposed to the hyperbaric environment of 2 ATA pressure for 90 min, 5 d/wk for 4 wk, followed by a 4-mo. interval.

All patients were evaluated before and after each series of exposures and at the end of the follow-up interval (5). Each evaluation included: a) neurologic history and examination, b) pattern reversal evoked potentials (PREPs), c) ambulatory assessment, d) visual acuity, and e) estimation of Kurtzke scale (6, 7). Each evaluation was performed by 2 independent neurologists unaware of which gases the patient had been exposed to in the previous treatment. At each evaluation a neurologist did not have access to the results of the previous examinations.

The brainstem function was evaluated by assigning a numerical value to the following parameter: facial weakness, nystagmus, gaze impairment, dysarthria, dysphagia, and other cranial nerve impairment. A value of 1 for each parameter indicated no impairment. Values greater than 1 equaled a degree of impairment. A sum of 6 for brainstem equaled normal findings. Greater than 6 equaled abnormal findings. Sensory evaluation results were likewise graded and totaled. Each limb was evaluated for sense of vibration, position, and touch or pain. A sum of the evaluations for all parameters in each limb of 12 equaled normal findings. Any sum greater than 12 equaled abnormal sensory findings. Strength was graded for both upper and lower limbs. A value of 5 for each limb equaled normal strength. Any decrease in strength resulted in a lower grade. A sum of 4 limb strength evaluations of 20 equaled normal strength. Less than 20 revealed weakness.

Patients received the appropriate breathing gas (100% oxygen or normoxic 10% oxygen) from a closed hood. Respiratory gas concentrations were measured

every 15 min during each exposure (Table 1). The mean FI_{O_2} in the oxygen treated group was 0.0987 with a range of 0.968 to 0.996. The mean FI_{CO_2} was 0.0018 with a range from 0.001 to 0.003. Respiratory gases measured in the control group revealed a mean FI_{O_2} of 0.105 with a range a 0.098 to 0.116. The mean FI_{CO_2} in this group was 0.0016 with a range of 0.0010 to 0.0029.

Table 1
Inspired Gas Concentrations Measured Every 15 Minutes During Each Exposure

	Treatment Group	Mean	Range
2 ATA 100% O_2	FI_{O_2}	0.987	0.968-0.996
	FI_{CO_2}	0.0018	0.0010-0.0030
2 ATA 10% O_2	Control Group		
	FI_{O_2}	0.105	0.098-0.116
	FI_{CO_2}	0.0016	0.0010-0.0029

Arterial blood gases were measured during each series of exposures for each group of patients (Table 2). The mean Pa_{O_2} for the oxygen-treated group was 1299 mmHG with a range from 1183 to 1403 mmHg. The mean Pa_{CO_2} was 30 mmHg with a range of 21 to 37 mmHg. The mean pH was 7.47 with a range from 7.45 to 7.54. Arterial blood gases measured in the normoxic control group revealed a Pa_{O_2} of 103 mmHg with a range from 65 to 140 mmHg. The mean Pa_{CO_2} was 33 mmHg with a range from 27 to 39 mmHg. The mean pH was 7.46 with a range from 7.36 to 7.50.

Table 2
Arterial Blood Gases Measured Once During Each Series of Exposures

	Treatment Group	Mean	Range
2 ATA 100% O_2	Pa_{O_2}	1299	1183-1403
	Pa_{CO_2}	30	21-37
	pH	7.47	7.45-7.54
2 ATA 10% O_2	Pa_{O_2}	103	65-140
	Pa_{CO_2}	33	27-39
	pH	7.46	7.36-7.50

PREPS were obtained at the time of each assessment. A four-channel montage, including an occipital-frontal (Oz-Fz) derivation, recorded responses to 28-min arc clicks presented separately to the left (OS) and to the right eye (OD). The averages were formed from 200 to 400 separate responses and both

the absolute P_{100} latency and P_{100} intraocular latency and amplitude asymmetrics were determined (Fig. 2).

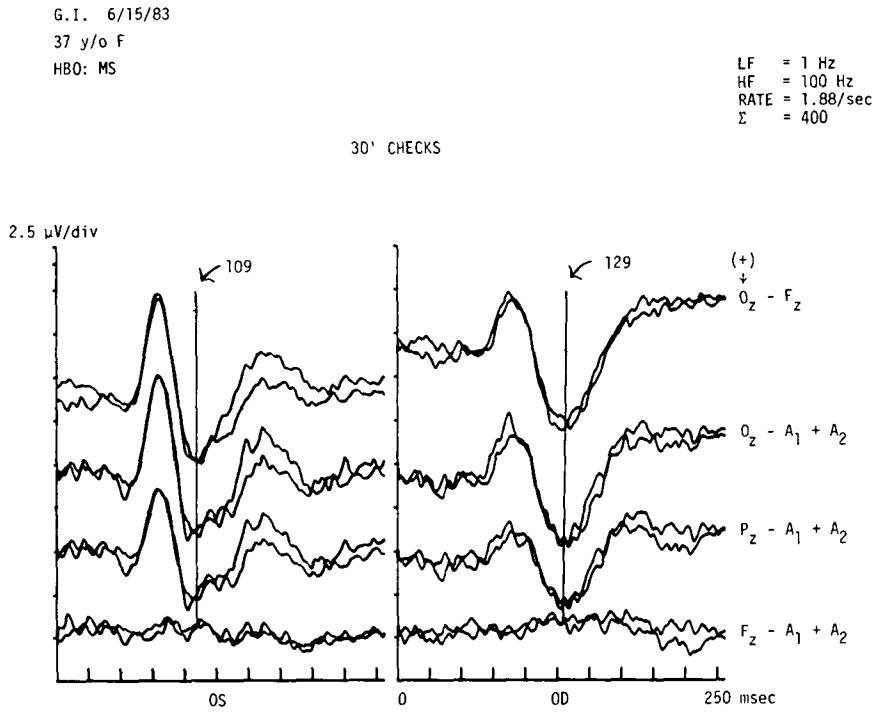

Fig. 2. A typical 4-channel PREP from a patient in the study. Stimulation and recording parameters are indicated on the figure. A standard bipolar Oz-Fz derivation is at the top. Channels 2, 3, and 4 are an ear-referenced posterior to anterior montage, useful in determining field distributions. At a 30′ arc check stimulation, our laboratory normal studies give 108 ms as the 99% upper tolerance limit of the P_{100} latency. In this patient of O.D. value of P_{100} = 129 ms is clearly abnormal as is the magnitude of interocular latency asymmetry.

At the completion of the 10 mo. crossover study, each patient was asked to return for a follow-up evaluation 1 yr later. Fourteen of the 18 patients were able to return at 1 yr. Because of deteriorating physical condition, the other 4 patients were unable to return for evaluation.

RESULTS

Clinical Evaluation

Neurologic examination performed at each evaluation revealed no significant change throughout the study (Fig. 3-5). Specifically, there was no measurable change in examination of brainstem, strength, cerebellar, or sensory function. Ambulation did not change. Visual acuity remained relatively stable

throughout the duration of the study. The Kurtzke scale remained relatively constant (Fig. 6).

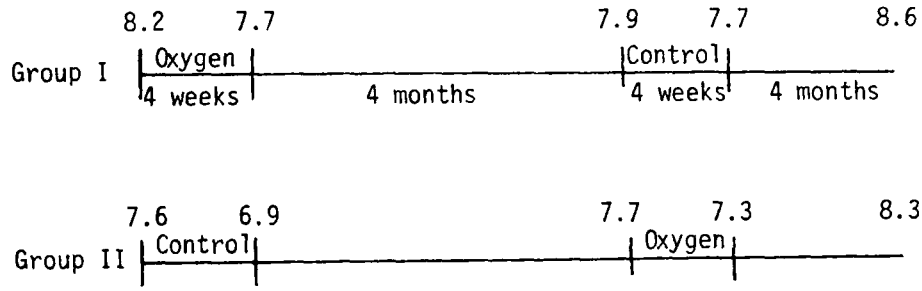

Fig. 3. Data on cranial nerve examinations; normal = 6, abnormal > 6. Number represents total values of scale when testing 6 cranial nerve functions with value of 1 being normal; all above 6 total are abnormal.

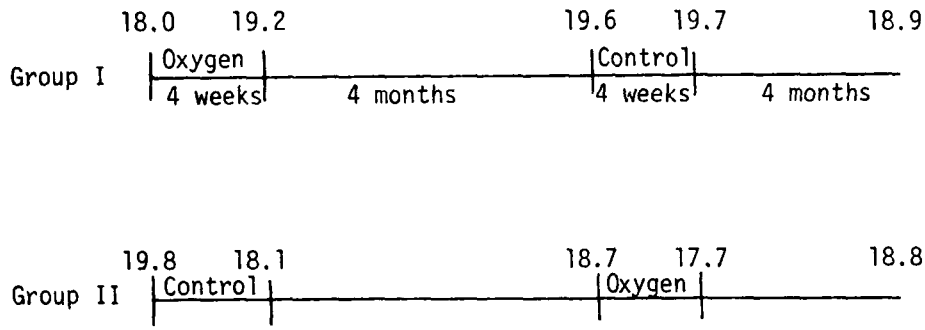

Fig. 4. Data on sensory examinations; abnormal > 12. Number represents total value for 3 sensory tests in 4 extremities (vibration, position sense, touch/pin); value of 1 is normal for each, so total of 12 is normal sensation.

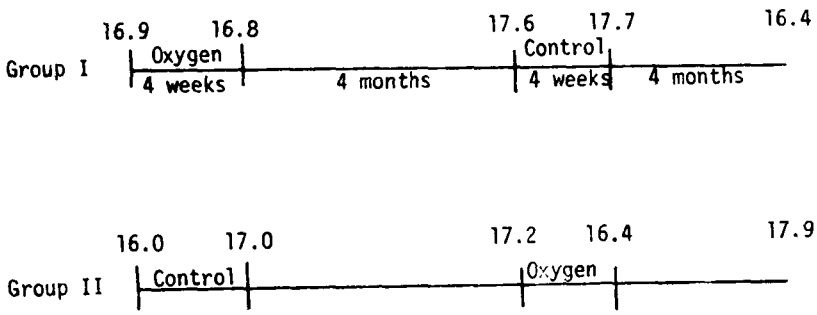

Fig. 5. Data on muscle strength (mean); normal = 20, weak < 20. Number represents total strength in 4 extremities with maximum strength as 5 in each extremity.

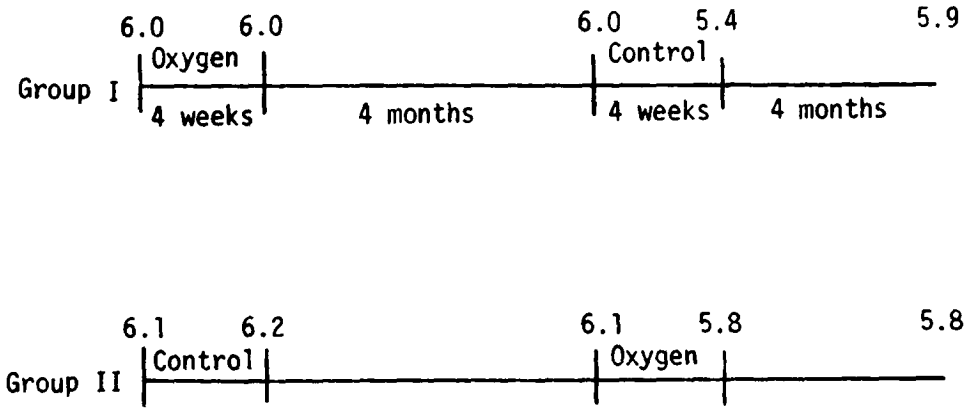

Fig. 6. Data on Kurtzke scale results (mean). Number is absolute mean value or Kurtzke scale determined at each point for total population group (9 patients each group).

Similarly, patients seen at 1-yr follow-up showed no significant changes in these areas although the follow-up numbers were small. There was, however, some evidence of stabilization in a few patients.

Electrophysiologic Results

Before treatment, 14 patients had abnormal PREP findings with significant latency prolongations in response to stimulation OS or OD. Four patients were normal, 1 of whom developed an abnormal response during the 100% O_2 treatment series. Analysis of variance indicates no statistically significant group changes from pretreatment baselines in a P_{100} latency or amplitude during either the 100% O_2 or 10% normoxic treatment periods compared to the pretreatment values (Fig. 7). In general there was remarkable stability of response during the study period. An exception was 1 patient whose P_{100} latency OS changed from a clearly abnormal 136 ms to a normal 110 ms during the 10% normoxic treatment period. One month later, during the intertreatment interval, the latency reverted to an abnormal value.

Patient Subjective Evaluations

Subjective responses to the hyperbaric oxygen treatment were: a) improved bladder control, b) increased stamina, c) increased energy, and d) increased strength and mobility in 13 of 18 patients. Based on the above subjective information, 14 of the 18 patients correctly identified the exposure series during which they received 100% oxygen. Three patients chose the incorrect series of exposure during which they received 100% oxygen. One patient was unable to make a choice.

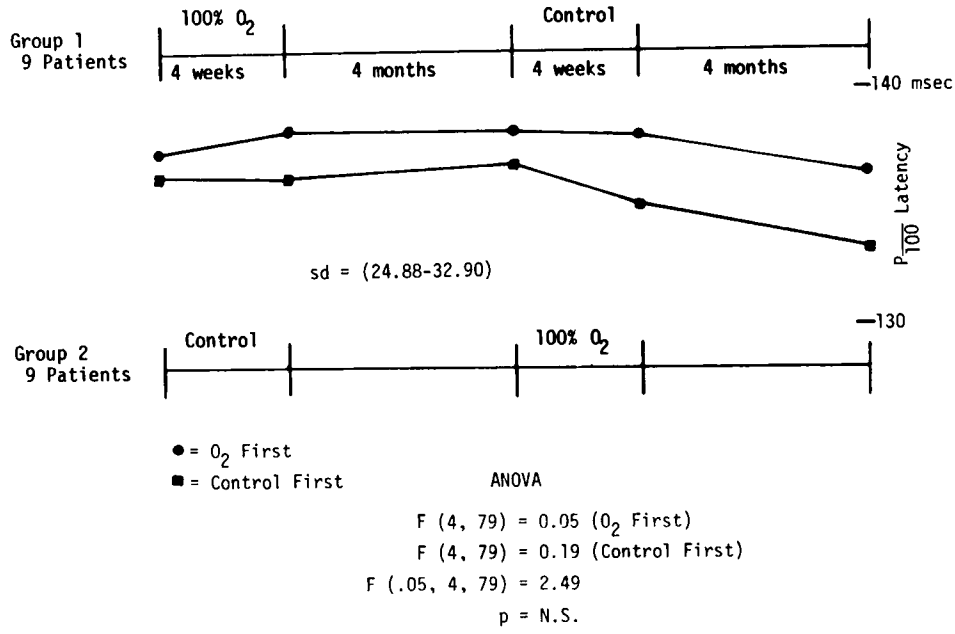

Fig. 7. This figure illustrates the overall design of the study as well as the mean P_{100} latencies taken at each measurement time in the study. The 2 groups (O_2 first and control first) are plotted separately. There was no significant difference between the 2 groups in terms of mean values at any time during the study. Group 1 latency was -140 ms; group 2 was -130 ms. Means ranged between 133 and 138 ms. SD is indicated on the figure. A significant F value of 2.49 (05, 4, 79) was not obtained by ANOVA, indicating the absence of a significant treatment effect on the P_{100} latency.

DISCUSSION

In 1957, Layton and McKay first suggested a mixture of 95% oxygen:5% carbon dioxide administered at atmospheric pressure as an adjunct to therapy for acute exacerbations of MS. Boschetty and Cernoch (8) observed a modest and short-lasting improvement in 16 of 26 patients treated with this method. Neubauer (9) noted improvement of a patient with multiple sclerosis being treated for osteomyelitis. Also Formai et al. (4) reported favorable results in treating MS patients. They rationalized their treatment on the resemblance of MS to neurologic decompression sickness. Pallotta (10, 11) also described improvement. However, Fisher et al. (1, 12) were the first to report a controlled study of MS using hyperbaric oxygen treatment, and suggested that the observed effects noted in the clinical trials were probably not the results of placebo effect. Powell et al. (13, 14) and Kizer and Powell (15) performed initial studies indicating that improvements in patients can be noted beyond those that are purely subjective. Neubauer (16) reported changes in the patterns of visual, auditory, and somatosensory evoked potentials in 22 of 26 MS patients treated with hyperbaric oxygen. He also reported changes in

magnetic resonance imaging (MRI) post-hyperbaric treatment (17). Some animal studies utilizing experimental autoimmune encephalomyelitis (EAE) and treated with oxygen therapy have been performed (18, 19). In these 2 animal studies, oxygen therapy was found to alter the course of EAE.

These studies have spawned a series of controlled clinical experiments. Barnes et al. (20) reported short-term results of a placebo-controlled, double-blind trial of 120 patients with chronic MS. Patients were given 100% oxygen at 2 ATA for 90 min daily, for a total of 20 exposures. The short-term result of this trial did not support the claim made for hyperbaric oxygen in the management of MS. Wiles et al. (21) reported 84 patients treated in monoplace chambers with either hyperbaric oxygen at 2 ATA or placebo with comprehensive double-blind assessment 1 mo. after treatment. There was no clinically significant benefit in the patients subjective opinion, the examiner's opinion, the score on the Kurtzke disability scale, or in the time taken to walk 50 m. Rosen (22) reported 12 patients with moderately advanced chronic progressive disease who showed no objective benefit in the treatment of moderately advanced MS.

The results of our present series do not show a statistical difference between oxygen and placebo groups not do they suggest a beneficial role of hyperbaric oxygen in MS. Patients in our series had chronic MS with moderately severe deficits based on their Kurtzke scale. Comparing results of patients with estimated disability status scale scores of 6 or less and those with scores greater than 6 did not reveal any difference. Conclusions from this data indicate no significant objective difference between the 100% oxygen treated and the 10% normoxic controls. There were, however, subjective reports of improvement during hyperbaric oxygen treatment, including "bladder control, stamina, energy and mobility." Based on this perceived subjective improvement, 4 of 18 patients requested continuation of intermittent hyperbaric oxygen therapy. Their neurologic status was assessed at 6-mo. intervals and there were no further significant changes in the neurologic status. The follow-up data on 13 of the 18 patients did not reveal any changes that could be statistically verified.

Therefore, despite the encouraging results of uncontrolled trials and subsequently the controlled trial of Fisher et al. (1), we have been unable to demonstrate benefit from the use of hyperbaric oxygen in the treatment of MS.

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9th International Symposium on Underwater and Hyperbaric Physiology
Undersea and Hyperbaric Medical Society, Bethesda, MD, 1987

A CONTROLLED STUDY OF HYPERBARIC OXYGEN TREATMENT IN MULTIPLE SCLEROSIS

G. Hart, M. J. Rowe III, L. W. Myers, and A. Afifi

Recurring reports in the 1970s (1-4) using hyperbaric oxygen (HBO) in treating multiple sclerosis have given rise to several controlled studies (5-8). Excepting the first controlled study by Fischer et al. (5), subsequent reports have not supported claims of significant neurologic improvement.

A preliminary study at this institution was undertaken in 1978 to treat a small number of patients with multiple sclerosis. Eight patients with chronic, progressive multiple sclerosis of at least 2 yr duration and significant neurologic deficit were treated. The protocol for this group required 2-h daily treatments with HBO at 2 atmospheres absolute for a period of 3 wk. Neurologic examinations before and after therapy revealed no objective, measurable improvement in their neurologic status.

The present study was performed in cooperation with the UCLA Multiple Sclerosis Research Center and represents a double-blind, crossover study extending from January 1984 to July 1985.

METHOD

Volunteer Selection

Volunteers were accepted with the established diagnosis of chronic, progressive multiple sclerosis of at least 2 yr duration. Entry criteria were a) be between 20 and 60 yr of age, b) abstain during the course of the study from the use of nicotine products, and c) be free of contraindications to hyperbaric oxygen treatment including:

- Claustrophobia
- Epilepsy
- Angina pectoris

- Chronic pulmonary emphysema or asthma
- Congenital spherocytosis
- Acute viral infections at time of exposures
- Females in the child-bearing age must not be pregnant at the time of exposures
- Does not require aspirin or salicylates in case of other concurrent diseases
- Is not on corticosteroids
- Not be experienced aviators or divers.

The last contraindication was included because these subjects could determine the treatment protocol from past experience with changing pressure environments.

Randomization and Control

Randomization was achieved using 2 groups of sealed envelopes and then randomly numbering before acceptance of patients. One group of envelopes was sorted by "card shuffle" technique and the other by a random number generator. Each group was then placed into separate identical envelopes. The group in the envelope decided by coin toss was used in this study.

This is a double-blind study inasmuch as the clinicians evaluating the neurologic response and the patients were not aware of the treatment received.

The crossover of the treatment (air vs. oxygen) was performed at 6 mo. when the volunteer receives the alternate exposure from that 6 mo. previous.

Treatment Schedule

All therapy was performed in a monoplace chamber. The HBO schedule was 1.5 ATA, 90 min daily for 20 consecutive treatments. The air schedule was 1.0 ATA, 90 min daily for 20 consecutive treatments with a pressure sham. The sham was created by rapid pressurization for 2 min (not to exceed 1.25 ATA air). The chamber pressure was then allowed to drift back to 1 ATA air — the sham was repeated at the end of each exposure.

Discontinuance of Protocol

A volunteer was dropped from the study if any of the following occurred:

1. Subject had uncontrollable confinement anxiety.
2. Subject requested discontinuance.
3. Subject was found to have been using nicotine products, salicylates, or corticosteroids.
4. Subject was found to have a condition normally treated with HBO such as osteomyelitis, carbon monoxide poisoning, gas gangrene, etc.

Patient Isolation

Each patient was scheduled for clinical examinations, electrodiagnostic examinations, and exposures in a way that prevented contact with other patients in the study. Each was screened before each series of exposures as to knowledge of any other participant.

Evaluation

Clinical testing. Each patient was evaluated before each exposure by the same blinded neurologist from the UCLA Multiple Sclerosis Research Clinic, and the subjects were rated on the UCLA Multiple Sclerosis Clinic Standard Examination form and on the Kurtzke Functional Systems and Disability Status Scale. The neurologic evaluation was repeated 1 wk after the first treatment series and a 6 mo., just preceding the next crossover series. The examination was repeated 1 wk after the last treatment and again at 6 mo. for a total of five neurologic examinations, including an initial control examination.

Neurodiagnostic testing. Somatosensory and brain stem auditory evoked responses were performed after the standard protocols of the electrodiagnostic laboratory, before and after each series, and after 6 mo. Responses were ranked as being normal or abnormal by specific criteria for interpeak latencies, amplitudes, and symmetry values. Follow-up studies were ranked as being the same, worse, or better by specific criteria for change in interpeak latency or amplitude values.

Electroencephalographs recorded before and after each series were analyzed by standard protocols for quantitative EEG testing in the electrodiagnostic laboratory. Quantitative frequency spectral analysis data were studied for specific responses to therapy.

RESULTS

All subjects completed the study. Eleven subjects (3 males, 8 females) (age 27-57 yr, mean 43 yr ± 9, median 47 yr) were studied. The Kurtzke Disability Scale ranking was 3 to 9 (median 6, mean 6.09 ± 1.76). Actual values were 3,3,6,6,6,6,7,7,7,7,9 before treatment. Three patients did not receive a complete EEG series because initial studies revealed no abnormalities of the EEG or of brain stem evoked responses. Four subjects refused somatosensory evoked responses due to pain experienced during initial studies or were not suitable for measurement due to involuntary tremors.

Six patients received the placebo first and 5 received oxygen (Table 1). Based on the Kurtzke scales the ranking of the subjects at the completion of the study was 3,5,6,6,7,7,7,7,7,8,9 (mean 6.55 ± 1.57).

Two patients had a worsening of the Kurtzke scale by 1 after oxygen; 2 improved by 1 after oxygen. One patient improved by 1 after the placebo and 2 worsened by 1.

Table 1
Kurtzke Disability Scale Ranking

Subject by Entry	Initial Series				Crossover				After 6 Mo.
	Placebo		Oxygen		Placebo		Oxygen		
	1	2	1	2	3	4	3	4	5
1			7	8	8	8			7
2	7	7					7	8	8
3			7	7	7	6			7
4	7	7					7	7	7
5			6	6	6	6			6
6	3	3					6	5	5
7	6	7					6	6	7
8	6	6					6	6	6
9			3	2	2	3			3
10	9	9					9	9	9
11			6	6	6	6			7

Statistical Analysis

Tests used on this limited amount of data were a) paired *t* test on difference between treatment effect and placebo effect, and b) McNemar test of equality of effectiveness of treatment and placebo.

DISCUSSION

The study was terminated when we recognized that a gradual worsening of the Kurtzke Scale was occurring in this small group. This study does not preclude a treatment response that may be achieved in the acute relapsing form of multiple sclerosis or any synergism that might be achieved with other forms of treatment. These data indicate that a clinically meaningful response to HBO in chronic, progressive multiple sclerosis is unlikely.

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SESSION 14: CLINICAL DIVING MEDICINE I

9th International Symposium on Underwater and Hyperbaric Physiology
Undersea and Hyperbaric Medical Society, Bethesda, MD, 1987

INTELLECTUAL IMPAIRMENT WITH DIVING: A REVIEW

C. Edmonds and L. Hayward

ETIOLOGY

The possible causes of intellectual impairment from compressed-air diving include neurologic decompression sickness, air embolism from pulmonary barotrauma, carbon monoxide toxicity, and hypoxia. The ill-defined and unquantified damage from other gas toxicities may involve carbon dioxide, nitrogen, oxygen, and contaminants. Repeated neuropsychologic effects of immersion, such as dehydration and hypothermia, as well as subclinical decompression sickness (silent bubbles) are problematical.

NEUROLOGIC DECOMPRESSION SICKNESS AND NEUROPSYCHOLOGIC DAMAGE

Reports from the 1950s and 1970s argued that divers who suffered severe neurologic decompression sickness were likely to sustain permanent brain dysfunction. These reports are widely cited as demonstrating the relationship between CNS decompression sickness and disruption of cognitive functioning. There are good reasons, however, for treating the findings of these reports with caution.

In 1959, Roszahegyi (1) examined 100 subjects between 2.5 and 5 yr after they had sustained neurologic decompression sickness and concluded that over one half had some form of psychologic disorder. Three quarters of these had neurologic findings on clinical examination. He noted that quiet men would frequently become irritable and uncontrolled after the injury, and that pathologic drunkenness and alcohol intolerance were frequent. He also observed an association between neurologic and EEG abnormalities with psychiatric disturbance, leading him to postulate that these consequences were organic as

opposed to psychogenic in nature.

Although these observations were of value in prompting later research on the relationship between the neurologic sequelae of decompression sickness and intellectual functioning, they do not constitute firm evidence for an association between the two. Roszahegyi's study was a clinical survey that reported his conclusions. There was no control group, no psychometric testing, and some doubt about the contamination of diagnostic categories, because many of the patients suffered from inner ear and possibly other barotraumatic lesions.

Between 1975 and 1977, a number of studies (2-4) reported neurologic and psychologic problems after decompression sickness affecting the CNS. The symptoms they attributed to decompression sickness included, in order of frequency, personality change, headache, recent memory impairment, discoordination, paresthesia and weakness, hearing loss, vertigo, urinary symptoms, and dysphasia.

They also reported a correlation between neurologic features and psychologic impairment on a range of neuropsychologic tests. Impaired divers had poor verbal and nonverbal scores on the Wechsler Adult Intelligence Scale (WAIS), deficient memory, disrupted storage of new information, slower psychomotor skills, and more distress anxiety, depression, and somatic concern than a control group.

Unfortunately, there are some difficulties in evaluating the reports. The patients studied were probably not a representative group because "litigation was pending in most cases," and it could be argued that such a situation could influence neurologic symptomatology, "soft" neurologic signs, and motivation during psychologic testing.

No attempt was made to control for the time lapse since the injury, in that patients were examined some time between 1 d and 2 yr after the incident. Inasmuch as tests performed shortly after a neurologic injury will, in many cases, overestimate the amount of damage that will remain in the future, it is important to report the time interval and, if necessary, control for it in the statistical analysis.

The way in which the control group was obtained also raises doubts about the validity of the conclusions regarding impaired performance in neuropsychologic tests in Levin's paper (3). This method was carried over by Peters et al. (4). In that study, 5 patients were allocated to the control group because they were considered not to have CNS involvement, and compared to 6 divers whose "complaints of CNS symptoms were substantiated by a test battery." Apparently the groups were determined by performance on the very tests they were later compared on. Under such circumstances, it is not surprising that the 2 groups differed on the test results. The error is analogous to dividing patients into groups of tall and short, and then using statistical tests to show that 1 group is taller than the other. A further 4 divers were excluded from the comparison "with equivocal neuropsychological findings," thereby maximizing the difference between the groups.

The concordance that was described between neurologic and neuro-

psychologic findings was also not unexpected. One would anticipate that subjects who had low results on the neuropsychologic tests would also have either recent or past neurologic damage. One would also expect concordance between the neuropsychologic findings, the litigation potential, "soft" neurologic findings, and abnormal neuropsychologic assessments.

In summary, although the above reports have been widely quoted, the only conclusions to be drawn are that some patients with neurologic decompression sickness may have neurologic findings and abnormal neuropsychologic assessments.

Værnes and Eidsvik (5) reported neuropsychologic damage after "near miss" diving accidents. They compared 9 divers who had had accidents to 15 nonaccident divers and an age-controlled reference group. The findings of Peters et al. (4) of impaired intelligence among diving accident victims, as assessed by the WAIS, were not replicated. However, they did report that 8 of the 9 diving accident victims developed a syndrome in which there appeared to be a change in cognitive functioning; most reported impaired memory capacity as the main problem. In addition, difficulty in concentration, irritability, alcoholism, and aphasia were noted. As with the earlier studies, there were irregularities that make the association between the effects of diving accidents and cognitive functioning open to question. The duration between accident and neuropsychologic testing was not reported, a problem shared with earlier studies.

Although these investigators (5) compared their diving accident group to a control nonaccident diving group and a "reference" group, the control group differed from the accident group in a number of ways. The nonaccident group had a higher mean IQ (111) and were younger on average (26 yr) than the accident group (mean IQ of 106 and mean age of 36 yr). Performance on some of the other tests of cognitive functioning that they used can be affected by age and IQ, with increasing age and lower IQ being associated with poorer performance (6). In this study, the effects of age and IQ on test performance operated in the same direction as the obtained results. The authors' attempt to dismiss this difficulty was not adequate. Some account should have been made for these differences between groups, e.g., adjustment for the effects of age and IQ could have been made using an analysis of covariance.

There is also considerable difficulty in ascertaining exactly which accidents were being investigated. In their accident table, the cause in 3 cases was listed as "carbon dioxide." Whether this is a typographical error and really refers to carbon monoxide toxicity or whether they were postulating a genuine carbon dioxide toxicity is unknown. With either possibility, the problem did not seem to be related to decompression sickness. There were a number of references to hypoxia, and in the remainder of the 9 cases the suspected accident was listed as "emboli." The one common feature among the subjects was that they had a severe neurologic problem of some sort due to diving.

The authors stated that EEGs were performed and brainstem auditory evoked potentials were taken; however the results were not reported in the

paper. They stated that a longitudinal study had been instigated, but no reference to this was made in the subsequent Stavanger symposium (7). This is unfortunate, because the results, even if negative, would have been of interest.

CIRCUMSTANTIAL EVIDENCE FOR BRAIN DAMAGE

A number of diverse reports all seem to support the association of compressed-air diving and neuropsychologic damage. Clinical decompression sickness cases among sport divers during the last decade show an increased proportion of CNS manifestations, from Hawaii (8), Australia (9), and Israel (10), than the earlier traditional navy populations.

Ingvar et al. (11) reported abnormal EEGs in 3.5% of free-ascent trainees, suggesting the presence of cerebral arterial gas emboli. Kwaitowski (12) investigated 150 professional Polish divers and found abnormal EEGs in 43%, compared to 10% in the normal population.

Extrapolation from the animal models of Leitch and Hallenbeck (13) suggests a clinical value from EEGs and evoked cortical potentials in divers, at least in the acute stages of decompression sickness.

An investigation by Gorman et al. (14) on civilian divers who had been treated for decompression sickness in the Royal Australian Navy recompression chamber demonstrated a large number of neurologic, EEG, and psychometric abnormalities during the following 1 to 2 wk. This was so even with divers who had no obvious clinical neurologic component to their decompression sickness. These manifestations seemed to diminish with time.

Calder (15) demonstrated neuropathologic autopsy findings more extensive than would have been anticipated from the mild or treated decompression sickness to which subjects had been coincidentally exposed. There was, however, more spinal than cerebral damage.

Roszahegyi (1) called attention to a chronic, progressive encephalomyelopathy resulting from repeated decompressions. Support was claimed by both Texan and Norwegian studies, and Hallenbeck (16) explained the possible etiologies.

DIVING FOLKLORE

In the symposium in Norway in 1983, there was apparent acceptance of the neuropsychologic complications of diving with compressed air at shallow depths, even though no consensus was reached regarding the long-term neuropsychologic complications of deeper diving.

The anecdotal or folklore belief developed among many occupational diving groups that a dementia (diver's dumbness or the "punch drunk" syndrome) was produced by prolonged compressed-air diving. This presumption was supported by media reports of brain damage in divers (17) during the 1980s.

In the United Kingdom, clinical observations from the Royal Navy (18)

and pilot studies from the University of Lancaster (Leach J, personal communication) have been widely quoted, and have probably heightened the concern about this topic by suggesting that it may have a basis in fact. In a report on abalone divers in Australia (19), it was stated that 30% of the divers suffered chronic ear damage, 20% had dysbaric osteonecrosis, and 10% had brain damage, but no supporting evidence was submitted.

AUSTRALIAN ABALONE DIVER SURVEYS

In 1985, an opportunity became available to investigate a very special group of divers. The Australian abalone divers were interesting because of their extremely provocative diving procedures, the high prevalence of conventional occupational diseases of diving, and the alleged presence of a punch drunk syndrome. It was presumed that if this group showed no evidence of intellectual impairment, the disorder would be an unlikely or uncommon complication of the more conventional air-diving groups. Conversely, if damage were detected, its specific nature would be more obvious in this group.

After an initial pilot survey by Edmonds and Boughton (20), special interest researchers were asked to investigate the larger population of these "excessive" divers, using their own specialized psychologic, neurologic, and electrophysiologic tests. The purpose was to employ objective investigations, standardized and extensively used on the Australian population, to indicate the existence or otherwise of brain damage.

Edmonds (21) has described the "excessive" diver population elsewhere. Of this group of 152 divers, the average duration of diving was over 16 yrs whereas over 12 yr were spent in professional abalone diving. During the time as a professional abalone diver, the average diver spent over 5 h/d on compressed air (Hookah) for 105 d each year, reaching just over 50 ft (15.25 m) on a typical day. He claimed to have been "bent" over 4 times, but probably did not recognize the less dramatic types of decompression sickness. Routinely, 58% of the divers employed a dive profile that required some time for decompression, but which was omitted. Sixty-nine instances of decompression sickness were diagnosed and treated by recompression therapy in a recompression chamber. Of these, 39 seemed to be neurologic in nature.

Because of the contradictory findings between the Texan and Norwegian studies on the WAIS, this approach was repeated by Edmonds and Coulton (22) on a larger (n = 67), "excessive" diving population, using the Multidimensional Aptitude Battery (MAB). The MAB is a group-administered counterpart of the WAIS (23-25), but avoids the observer bias that sometimes exists with that test (26).

A multiple regression analysis was made against all diving covariants and the 10 MAB subtest scores (verbal IQ, performance IQ, total IQ) and deterioration index ("dementia score"), corrected for age. Apart from very minor and unimportant associations, the analysis showed no relationship between the type of diving and these measurements of intellectual functioning. Nor was there an

abnormal profile or scatter in the divers' results, which would have been supportive of brain damage. This investigation indicated that if neuropsychologic changes were present, they would be of a more subtle nature than those detected by such multiple aptitude batteries.

Neurobehavioral researchers, who specialized in detection of minor abnormalities among occupational groups exposed to chemicals, heavy metals, and toxins, also examined a group of excessive divers. Williamson et al. (27, 28) reported two studies, one looking at whether divers differed from controls in their performance in a variety of tests, and the second looking at the relationship between the test performances and the indices of diving exposure. Thirty-three excessive divers were compared with 33 nondiving workers matched for age, sex, language ability, educational level, and cigarette and alcohol consumption. The tests were of visual perception, psychomotor coordination, sustained attention and psychomotor speed, psychomotor performance, reaction time, Sternberg tasks, and short- and long-term memory.

In the first study they found that the divers did well or better than the controls on some tests (reaction time, some memory and motor tests) and worse than controls on others (visual and short-term memory and some psychomotor learning skills). However, the way in which divers chose to complete their tests differed from the controls, in that they were more likely to take risks and substitute speed for accuracy. This sort of difference in motivation must be taken into account when interpreting test results, and may lead to difficulty in neuropsychologic assessment in other surveys. The second study of Williamson and Clark focused on neuropsychologic functioning and a number of diving-related variables, but the associations found were weak.

Another 48 excessive divers were subjected to the more conventional psychometric tests by Andrews et al. (29). This study compared excessive divers to nondiving fishermen controls living in the same locality, and used the symbol digit modalities test (written and oral), single and double simultaneous stimulation test, Wechsler memory scale, and the controlled word association test.

They found that divers did significantly less well (after correction for age and for the number of statistical tests performed) than controls on two of the eight measures of various aspects of neuropsychologic function. These were from the Wechsler Memory Scale, i.e., logical memory and paired associate learning. The differences were small, and the divers' scores were within normal limits for the general population.

In analyzing the relationships between diving parameters and psychometric results, there was a correlation between the decompression stress and the symbol digit modalities test, between the hours of diving and the visual reproduction test from the Wechsler Memory Scale, and between near drowning or carbon monoxide poisoning and the oral part of the symbol digit modality test and the controlled word association test for animal words. The Andrews survey (29) did not include the size of these correlations, and because approximately 40 correlations were calculated without appropriate statistical

adjustment, it is questionable whether they would have achieved significance, i.e., some of the reported significant correlations may have been spurious.

A valuable approach in the Andrews survey was the comparison of the "abnormally low" performance members from both the divers and control groups, and they found no evidence for a subset of divers with abnormal scores. The authors concluded that "there was no evidence for the accumulation of subclinical insults leading to a dementing process."

Hjorth et al. (30) performed neurophysiologic assessments on 20 excessive divers. Apart from a couple of minor abnormalities on EEGs, no significant findings were made. In each of these 2 cases, the divers had not suffered clinical neurologic decompression sickness, and the 6 who had suffered this illness in the past were normal. One diver who had received multiple treatments for severe cerebral decompression sickness within the preceding 2 wk was classified as borderline.

Visual evoked cortical potentials and upper and lower limb somatosensory evoked cortical potentials were all normal.

DISCUSSION

Even though it is appreciated that neuropsychologic sequelae could result from some diving diseases, it does not follow that the average compressed-air diver, who does not sustain an obvious episode of brain damage, has an increased likelihood of this problem developing.

Brain damage leading to a permanent deterioration of intellectual capacity is often described as dementia. To demonstrate that this has developed, it is necessary to measure a degree of deterioration in intelligence, or in one aspect of intelligence.

Brain function can be assessed in a variety of ways. Apart from a personal, social, and occupational history and a clinical examination, there are other, more objective measurements. These include psychometric testing, EEGs, computer axial tomography brain scan, evoked cortical potentials, nuclear magnetic resonance, and positron emission tomography. The latter investigations are likely to be more available in the future, but currently we have to rely on the simpler techniques.

Earlier studies, although contributing to the concept of neurologic decompression sickness causing permanent neuropsychologic damage, had serious limitations in their diagnostic categories, statistical analysis, and use of control groups. Unanswered questions about this damage are its likelihood, specificity, extent, and permanency. Apart from cases that have obvious and gross neurologic damage, the possibility of a cumulative intellectual impairment from repeated and excessive diving, remains unconfirmed.

In the Australian abalone diver, cross-sectional survey, tests of neuropsychologic impairment were performed on a population of excessive divers to ascertain whether this form of diving is associated with changes in intellectual functioning in the population as a whole. Edmonds and Coulton (22) found no

change in intellectual functioning in divers when compared to the general population, using conventional multiple aptitude testing. Hjorth et al. (30) compared divers and the general population with EEG and evoked cortical potentials and found no difference in brain activity. Small variations in conceptual functioning were noted by both Andrews et al. (29) and Williamson et al. (28) on the more sophisticated neuropsychologic and behavioral tests. Williamson revealed the different ways in which divers approach testing procedures — with implications for future studies and which must be considered when interpreting conventional psychometric testing.

Two other problems face future investigators. The size of the group being investigated must be large for real differences to be identified. With the number of tests and subtests performed, and the number of diving-related variables considered, chance differences are likely. The more statistical tests performed, the more the likelihood of finding differences between groups, due entirely to chance. If the sample size is reasonably large, this problem can be accounted for by tightening up the criteria for accepting that a difference is real [e.g., Bonferroni adjustment (31)]. This strategy has been used, in part, in one published study (29), even though all the studies that have control groups have carried out a large number of comparisons on the same data set.

The adverse effect of the disease and the treatment on the anxiety and self-esteem of the diver, together with attitudes of both peer and therapist groups, may well have psychologic effects. The posttraumatic stress syndrome is a possible sequel. These effects, together with the physiologic influences of sleep deprivation, hyperbaric therapy, drug administration, and noncerebral manifestations of decompression sickness, may well complicate the interpretation of psychometric tests performed soon after the incident.

The Australian abalone diver cross-sectional surveys attempting to relate brain damage to excessive air diving, without gross neurologic incidents, were not successful. Nevertheless there is ample evidence that acute and temporary neurologic insults are experienced by compressed air divers. That these were not translated into hard evidence of permanent brain damage or dementia may reflect the insufficient degree of damage or the lack of sophistication of the investigation performed. If such a deterioration does occur, it is likely to be either minor or rare.

The definitive survey should take into account the above problems with selection of divers and controls, statistical techniques, and newer investigatory procedures. Even then, a longitudinal study may be needed to demonstrate possible neuropsychologic damage (32) presenting much later than the actual neurologic damage.

SUMMARY

Earlier studies, although contributing to the concept of neurologic decompression sickness causing long-term neuropsychologic damage, had serious limitations in their use of control groups and statistical analysis.

Unanswered questions about this damage included its likelihood, specificity, extent, and permanency.

Evidence for acute and temporary neurologic insults from diving are well recognized and may be due to a variety of stresses, including gas toxicities, hypoxia, air emboli, and neurologic decompression sickness.

The possibility of a cumulative intellectual impairment, from repeated and excessive diving, also needed to be assessed. The Australian abalone diver cross-sectional surveys, attempting to relate brain damage to excessive air diving, did not confirm this association.

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EFFECTS OF RECOMPRESSION TREATMENT ON EEG IN DIVING ACCIDENTS

S. A. Sipinen and J.- P. Halonen

Reports published on diving accidents generally state that more than one-third or even one-half of severe cases of decompression sickness (DCS) and/or air emboli (AE) are associated with symptoms in the CNS, even on a supraspinal level (1-3). Electroencephalography has not been used or discussed in these investigations. Moreover, most of the reports on treatment of DCS and AE do not discuss the primary findings or late changes in EEG, even though more than a quarter of their patients had supraspinal CNS symptoms (4-6). In these previous reports a complete or almost complete cure seems to be based mainly on clinical examination without regular EEG recordings. Because there might be some connection between the recompression treatment of DCS-AE on one hand and abnormal EEG findings in cases of inadequately treated diving mishaps on the other, we compared such divers with a history of diving accidents to healthy naval divers without a diving accident history, and used EEG to detect supraspinal lesions.

MATERIALS AND METHODS

Twenty-one sport and professional divers, who had had a diving accident with clear symptoms of DCS and/or AE during the last 10 yr volunteered for the study. A standard 22-channel EEG was recorded on each diver in the Central Military Hospital in Helsinki. Thirty-seven healthy naval divers, who never had any accident with symptoms of DCS or AE, served as controls and were examined with the same method. All 58 EEG recordings with all the necessary information were sent to the Department of Clinical Neurophysiology in Turku to be assessed by the same investigator, who was unaware of accident history. Quantification of findings was made on the following

parameters: Overall normality and general disturbance were graded as normal [1], slightly abnormal [2], abnormal [3], or grossly abnormal [4]. Vigilance was graded from stable [1] to unstable [2] near sleeping [3] and sleeping [4]. Asymmetries were graded as symmetric or asymmetric and the side of the lateralization of the asymmetry as no lateralization, to the left and to the right. Partial irritation was graded like overall normality, and general irritation was graded from no irritation [1] to slightly abnormal delta waves [2], frontal intermittent rhythmic delta activity (FIRDA) [3], and spike wave complex [4].

RESULTS

The anamnestic data of diving experience could be evaluated on 15 sport or professional divers and 27 naval divers (Table 1). There were no statistical differences between the accident and the control group in the diving experience given as years or hours of diving, the common diving depth, or the deepest dives. The age range was 20 to 35 yr in both groups. The EEGs were recorded 3 mo. to 10 yr after the accidents. With the exception of vigilance on three EEG recordings (1 control and 2 in the accident group), all data could be evaluated (Table 2). The score for overall EEG normality was 1.86 + 0.19 (mean + SEM) in the accident group, and 1.27 + 0.01 in the control group. The difference between the groups is significant at $P < 0.01$. The findings in general disturbances differed in the accident group from the controls in general disturbance, 1.81 + 0.18 vs. 1.16 + 0.08 ($P < 0.05$). Vigilance was more unstable in the accident group than in the controls, 1.53 + 0.18 vs. 1.11 + 0.05 ($P < 0.01$).

Asymmetries were rare and there was no trend of lateralization of asymmetries to either of the hemispheres. Partial irritation was seen only on one EEG in the accident group.

Table 1
Diving Experience

	Accident Group $n = 15$	Controls $n = 27$	$P <$
Total diving years	11.8 + 1.9	9.2 + 1.1	NS
Total diving hours	1583.0 + 794.0	810.0 + 157.0	NS
Common diving depth, m	19.7 + 2.2	21.0 + 1.6	NS
Deepest dives, m	53.9 + 3.6	54.9 + 2.1	NS

Anamnestic data on diving experience in diving accident group and the control group (mean + SEM. NS = difference statistically not significant).

Table 2
Quantification of EEG findings

	Accident Group	*n*	Controls	*n*	*P* <
Overall normality	1.86 + 0.19	21	1.27 + 0.10	37	0.01
General disturbance	1.81 + 0.18	21	1.16 + 0.08	37	0.001
Vigilance	1.53 + 0.19	19	1.11 + 0.05	36	0.01
Partial irritation	1.05 + 0.05	21	1.00 + 0.00	37	NS
General irritation	1.33 + 0.20	21	1.00 + 0.00	37	0.05

EEG findings in accident and in control group, mean + SEM, Student's test, NS = not significant. For explanation of the numerical score, see text.

The distribution of EEG recordings is presented in Table 3. The distribution of overall normality was 8 (21.6%) abnormal and 29 (78.4%) normal EEGs in the control group, whereas the accident group had 12 (57.1%) abnormal and 9 (42.9%) normal EEGs. Of these 12 divers in the accident group with abnormal EEG only 4 were treated in a pressure chamber. In contrast, of the 9 divers in the accident group with normal EEGs, only 3 were untreated, whereas 6 were adequately treated in the pressure chamber.

DISCUSSION

Late consequences of the neurologic symptoms in DCS, like chronic encephalomyelopathy, vegetative neurosis, or psychosomatic symptoms, have already been described by Rozsahegyi (6). In a later study he found that when a caisson worker had never suffered from DCS, the amount of abnormal EEGs in a small control population (n = 12) was 25%, about the same percentage as in our present diver population, whereas of those caisson workers who had suffered from a central nervous form of DCS (n = 57), only 25% had normal EEGs (7). In 15 patients the EEG recording time varied from 2 to 60 d; in the remaining patients evaluated several years after the accident, no information was available about possible treatment. From the results of that investigation Rozsahegyi (8) also drew the conclusion that the incidence of EEG abnormality was not higher than in the normal population in workers who had not suffered from DCS.

Table 3
Effect of Treatment

	Normal EEG		Abnormal EEG	
	n	%	*n*	%
Controls	29	(78.4)	8	(21.6)
Accident group				
6 with treatment			4 with treatment	
	9	(42.9)	12	(57.1)
3 no treatment			8 no treatment	

Distribution of normal and abnormal EEG findings in controls and in accident group with and without adequate recompression treatment.

Ingvar et al. (9) examined submarine personnel undergoing training in free escape and found a pathologic postdiving EEG in 12 of 14 cases, with clinical evidence of lung rupture and AE. Each of these patients was immediately treated in a pressure chamber; untreated controls were, of course, not available. In most of the cases the EEG normalized in a few days or weeks and symptoms remained in only 1 patient. They concluded that a routine EEG has less value in screening submarine personnel but more in diagnosing and following up cases where AE has occurred.

When divers who have suffered from a diving accident are examined with detailed neurologic or neuropsychologic methods, some degree of disturbance in cerebral functions can be found (10). The investigators gave no information about the time of the accident, possible treatment, EEG findings, or the previous health of the divers. As a pilot study we examined 2 accident cases with abnormal EEGs by using neuropsychologic tests, and the results were within normal limits. Both divers were treated in a pressure chamber after the accident and had been tested by a clinical psychologist before the accident. Because the comparison between the 2 tests revealed no changes, we discontinued this line of investigation.

Since 1954, the EEG has been proposed by some investigators as useful in selecting divers, especially in the French Navy (11). Other institutions have investigated the association between the EEG response and a diver's professional capabilities, with an opposite result (12). Compared to the present investigation, the number of abnormal EEG findings in the study of Malhotra and Kumar (12) was as low as 9.2% (vs. 21.6% in our divers).

From the normal adult EEG (13) it is difficult to determine a diver's general health and fitness or unfitness to dive. It seems to lack adequate

criteria. Some authors (14), after they found 41% abnormal EEGs on professional divers when judged by average criteria, have proposed dividing the subjects into 3 groups: fit, unfit, and questionable. Using these criteria, none of the present EEGs would indicate unfitness to dive, nor would all abnormals be in the questionable group. However, an abnormal EEG, even years after a diving accident, might be a sign of a CNS involvement even when, as is usual, no control EEG recording is available.

Irrespective of the small number of accident cases in the present study, the beneficial effects of a recompression treatment resulted in a reduced number of abnormal EEGs. The opposite finding was noted when no treatment was provided.

The present results indicate that supraspinal lesions after DCS and AE are common. The effect of an adequate recompression treatment on the CNS seems to be beneficial when judged from the EEG results. The increased number of abnormal EEGs on clinically healthy divers does raise the question of subclinical DCS with CNS involvement.

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OSTEONECROSIS OF THE LONG BONE IN DIVING FISHERMEN

H. Oiwa, A. Itoh, T. Ikeda, and S. Sakurai

Among disorders associated with underwater activity, aseptic bone necrosis presents difficulty in early diagnosis due to its insidious onset and asymptomatic progress. Consequently, radiologic findings of bone necrosis are often too late for the patient to benefit.

According to past reports (1-3), skeletal distribution of bone necrosis tends to appear in three parts of the body: the upper humeral head, upper femoral head, and the knee joint consisting of the lower part of the femur and the upper part of the tibia.

Based on the classification of aseptic bone necrosis in divers by the British Medical Research Council's Panel into type A, which is juxta-articular lesion, and type B, which is shaft lesion, it is well known that lesions of the upper humeral and upper femoral head are either type A or B or both, whereas knee joint lesions tend to be mostly type B (4, 5).

Inasmuch as inadequate decompression must cause uniform inert gas bubble formation in all of the long bones of the body, it is not possible to predict the sites and type of bone necrosis.

Type A bone necrosis has been extensively investigated by many researchers due to its seriousness, but type B has been given little attention, although its prevalence in the knee joint is statistically more significant in comparison with type A prevalence in other skeletal bones (3).

We felt that further detailed examination of type B aseptic bone necrosis of a long bone adjacent to the knee joint would be beneficial to early diagnosis and prevention.

In the present study, we report results of a sample survey on aseptic bone necrosis, especially bone lesions of the knee joint, obtained from diving fishermen who reside in the Chiba prefecture of Japan.

METHODS

The survey was done on 37 of approximately 70 diving fishermen, ranging from a 19-yr-old novice to a retired 70-yr-old, who live in the Bohso peninsula area and are engaged in fishery diving along the coast of Katsuura city. They dive with a compressed air breathing helmet for sea shells, mainly abalone, making four to five dives/d between April and September. They dive to 30 to 40 m, normally for 20 to 30 min.

The survey that was conducted in February 1984 consisted of x-ray examinations of eight skeletal joints, i.e., both joints of the upper humeral head, both upper femoral heads, both elbow joints including the distal end of the humerus and the proximal end of the radius, and both knee joints including the distal end of the femur and the proximal end of the tibia. An anteroposterior x-ray was taken, being careful to include in the film as much of the shaft of the long bone as possible.

Radiologic judgment and classification of aseptic bone necrosis were based on the Ohta-Matsunaga modification of the British Medical Council Decompression Sickness Panel Classification (2).

Among patients who showed definite lesions in the knee joint by an A-P roentgenograph, 6 divers with bone necrosis in the lower part of the femur underwent computerized tomography (CT) scanning. CT scanning was performed every 1 cm over a distance of 15 cm in both distal and proximal ends of the tibia, with a window level of +850 and window width of 1200. Furthermore, all 37 diving fishermen were examined for the range of motion (ROM) of the joints to determine the functional sequelae resulting from previous decompression sickness (DCS). In addition, each diver was interviewed about his daily work schedule, including diving profiles, diving career, and medical history, including DCS.

RESULTS

Of all 37 divers, 20 (54.1%) had positive roentgenographically determined abnormalities. Of these, 7 had bone lesions classified as type A, 9 as type B, and 4 as type C. Type C means a suspected lesion showing bone cysts and bone islands that are frequently found in divers (4). The prevalence of definite aseptic bone necrosis, except for type C (43.2%), was demonstrated.

Thirty-eight bone lesions were observed in 20 divers. The skeletal distribution was separated into roughly three regions (Fig. 1), i.e., 16 (42.1%) knee joint, 13 (34.2%) upper part of the femur, and 8 (21.0%) upper part of the humerus. Of the 8 humeral-head lesions, 7 were type A and 1 was type B. In the femoral head, there were 4 each of type A and B, and 5 of type C in 13 cases.

Most of type A were A-1, spherical segmental opacities, and others were A-3, linear opacities. Most of type B were B-2, irregular calcified areas (Table 1). There was no significant association between the incidence of bone lesions

classified by diving career and groups with or without a history of previous DCS (Table 2). In knee joint bone lesions, 13 of 16 cases were categorized as type B, 3 as type C, and 0 as type A.

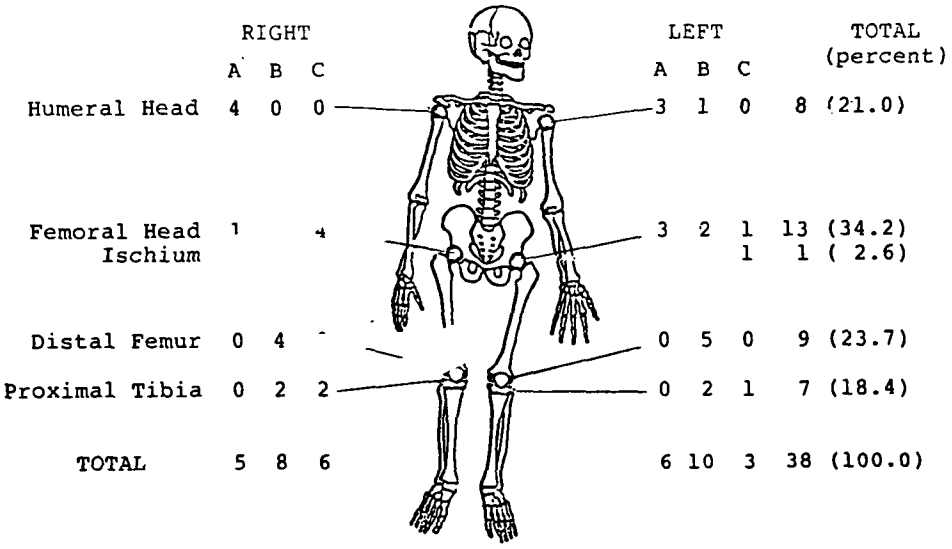


Fig. 1. Skeletal distribution of aseptic bone lesions diagnosed by roentgenography, in sample survey of 37 diving fishermen, at Chiba prefecture.

Table 1

Age Distribution and Classification of the Bone Lesions in Diving Fishermen

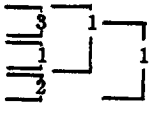
	Age, yr	20-29	30-39	40-49	50-59	60-69	70-	Total
No. of Divers		4	12	5	10	5	1	37
With Bone Lesion		2	7	4	3	3	1	20
	A-1	0	2	2	1	2	0	7
Type A:	A-2	0	3	0	1	0	0	4
	A-3	0	0	0	0	0	0	0
Sub Total		0	5	2	2	0	0	11
	B-1	0	1	0	0	1	0	2
Type B:	B-2	0	3	2	3	3	2	13
	B-3	0	1	2	0	0	0	3
Sub Total		0	5	4	3	4	2	18
Type C:	C-1	2	3	1	0	3	0	9
	C-2	0	0	0	0	0	0	0
Sub Total		2	3	1	0	3	0	9

Table 2
Classification of Divers with Aseptic Bone Necrosis According to Their Diving Career and Previous DCS

Diving Career, yr	No. of Divers	With Bone Lesion	With History of DCS	Without Bone Lesion	With History of DCS
0-10	6	3	1	3	3
11-20	10	6	6	4	2
21-30	6	5	5	1	0
31-40	8	2	2	6	4
41-50	7	4	3	3	2
Total	37	20	17	17	11
		85.0% with DCS history		64.7% with DCS history	

Among 9 cases of bone necrosis in the knee joint at the distal end of the femur found in 8 divers, only 3 were accompanied by another site of bone necrosis; 1 with type A in the humeral head and 2 with type B in the proximal end of the tibia (Table 3). Figure 2 summarizes the roentgenographic findings of 8 divers with definite knee-joint bone necrosis. Figure 2 shows that no characteristic difference exists between age and diving career in the knee joint bone necrosis in comparison with bone necrosis in other joints in the body.

Table 3
Most Common Sites of 29 Definite Aseptic Bone Necroses is 37 Diving Fishermen

Sites	No. Lesions	With Bilateral	Commonest Sites*
Humeral head	8	2	
Femoral head	8	3	
Distal femur	9	3	
Proximal tibia	4	0	

* Lesions accompanying other joints.

Table 4 outlines the influence of repetitive DCS at the knee joint on bone necrosis. Of 8 divers with bone necrosis around the knee, 7 had repetitive DCS of the knee joint. Of 8 divers with bone necrosis of other joints, 5 had repetitive DCS of the knee; 17 divers without bone lesions, 7 had a history of repetitive DCS.

The high density areas that appeared in CT scanning examination of 6 divers who had 9 cases of definite bone necrosis in the distal end of the

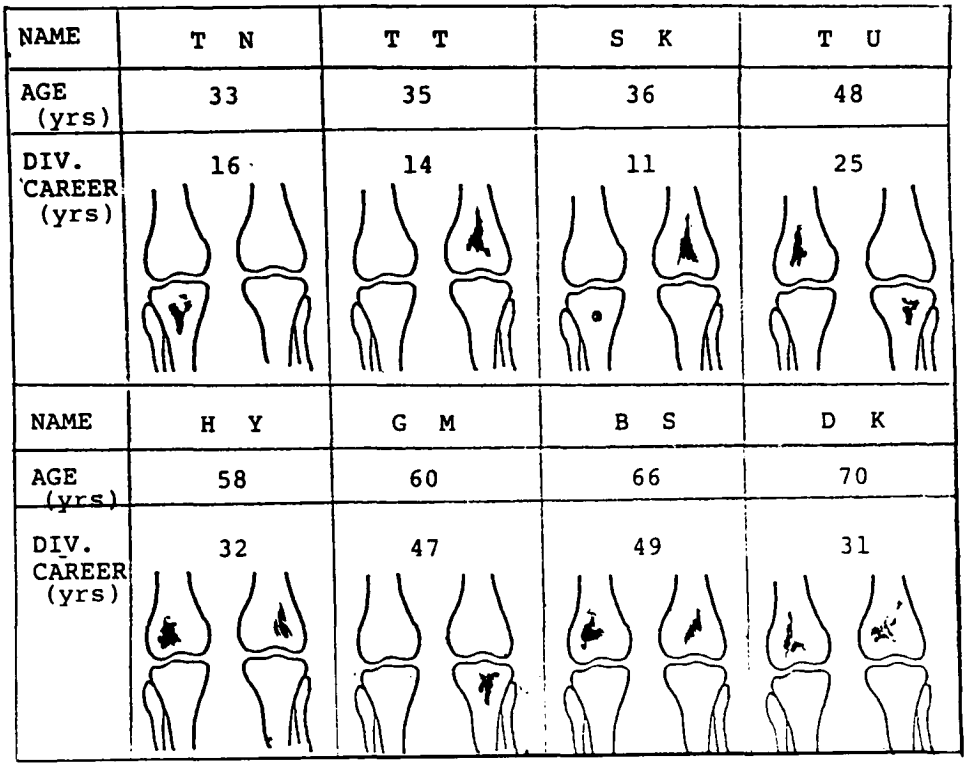


Fig. 2. Roentgenography of aseptic bone necrosis at the knee joint in 8 divers.

Table 4
The Influence of Repetitive Type I DCS at the Knee Joint an Aseptic Bone Necrosis

	No. of Divers	With History*
Definite ABN** at the knee	8	7
Definite ABN at other joint	8	5
Suspected ABN	4	3
With no ABN	17	7

* No. of divers who have experienced repetitive type I bends at the knee. ** Aseptic Bone Necrosis.

femur at the knee joint coincided with sclerosis found in antero-posterior roentgenographs. A gradation could be seen in all cases where the high density area began, as if subcortical thickening occurred and progressed toward the

center of the bone. In 5 of 9 cases, sclerotic changes of the metaphysis of the femur were conspicuous in the lateral condyle. In no case were sclerotic changes spread broadly in both condyles or seen in only the medial condyle side (Fig. 3). In all cases of aseptic bone necrosis, the epiphyseal area of the distal femur and the structure of osteon were intact (Fig. 4).

Fig. 3. Case BS, aged 66, 49 yr diving career, a and b, calcified area in the distal end of right femur.

DISCUSSION

Although the occurrence of aseptic bone necrosis depends on the decompression procedure, the best decompression table that prevents divers from

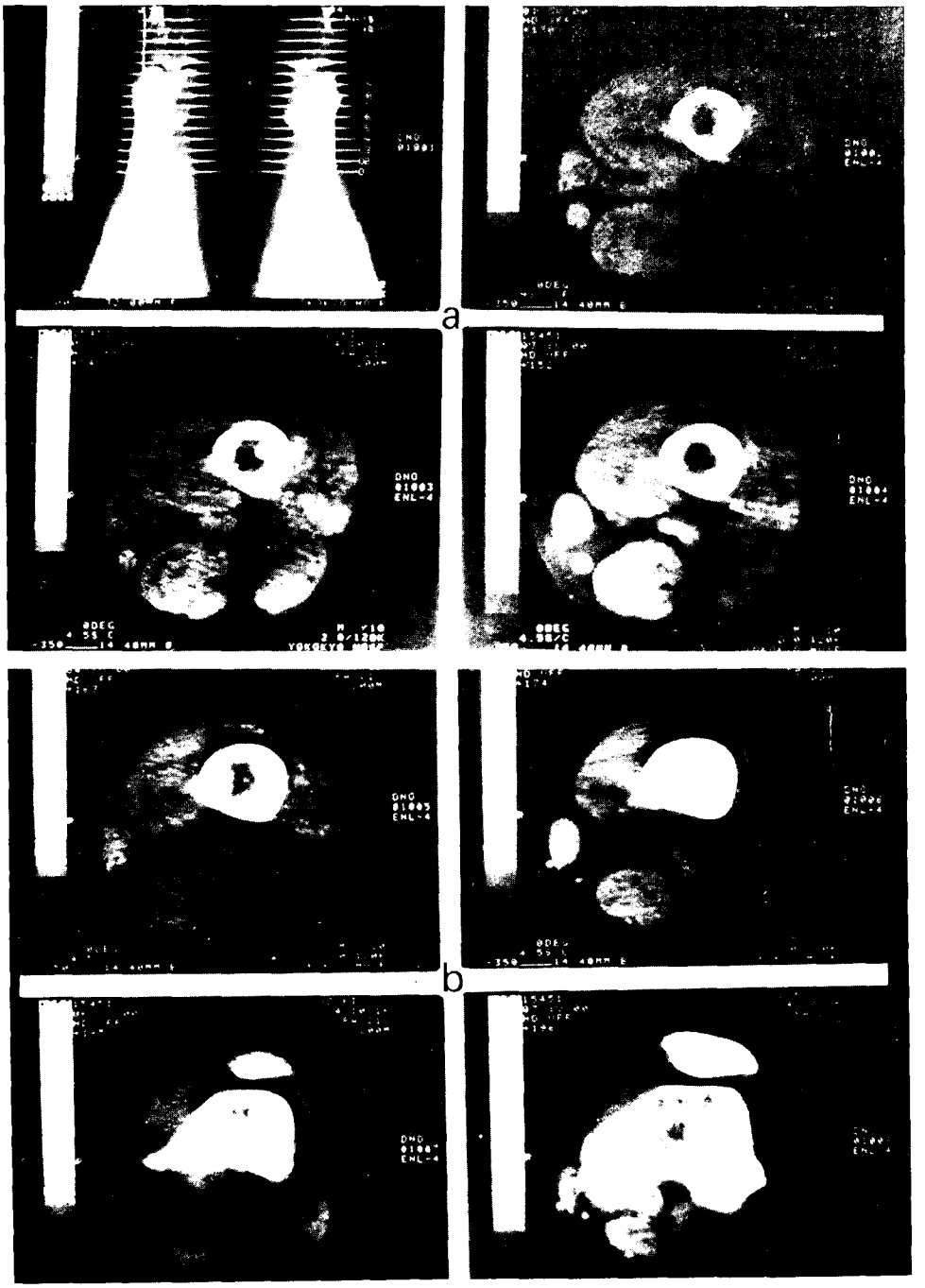


Fig. 4. A CT scanning at lesions of femoral shaft (a) and condyles (b) of the knee. The same diver whose hip is shown in Fig. 3.

developing bone necrosis has not been generally used (6). When bone necrosis is found it is often too late to be remedied, not only because the onset of bone necrosis is insidious but because roentgenographic diagnosis does not detect early stages of bone necrosis.

Type A bone necrosis of the juxta-articular lesion has been intensively investigated due to the importance of orthopedic treatment. On the other hand, the origin of type B lesion in a long bone has been paid little attention when compared with type A, although the prevalence of type B is high in long bones of the body. The reason for the paucity of reports of type B is due to its better prognosis, even though study of this necrosis is as important as type A in terms of pathogenesis related to microbubbles.

Even among navy divers who adhere to strict diving procedures and are given the best recompression treatment, there has been a 3 to 5% incidence of bone necrosis, most of which is type B (7-9).

In the present survey, 16 type B lesions of 38 cases of bone necrosis were found in long bones around the knee joint in the distal femur or proximal tibia. In the case of type B, the ratio of lesions in the knee joint area to other joints was 13:5. This value is very similar to that found in the report from the DCS Control Registry and Radiological Panel (3).

Most bone necrosis found in knee joints was type B; type A was very rare. This is probably due to the difference of blood flow in the bone head area between the mortar joint seen at the shoulder and hip and the hinge joint seen at the elbow and knee.

The knee joint is well perfused by an extensive popliteal artery network. These perfusion characteristics do not predispose to type A lesions in the distal end of the femur and proximal end of the tibia. However, type B lesions may occur because of the characteristics of the blood supply of long bones. The main artery perfusing long bones enters the center of the bone shaft and runs diagonally toward the end of the bone. After running through Haversian canals, the arteries form venous sinuses. The venous return parallels the arterial system. Because of these perfusion characteristics, blood flow is slower in places remote from the center of long bones.

Furthermore, the greater the bone marrow cavity at the end of a long bone, the less blood flow per unit volume of the marrow. Bove et al. (10) in inadequate decompression studies in dogs, demonstrated this well. Another possible explanation for type B bone necrosis frequently found in long bones is, as Hills and Straley (11) pointed out, that an increase in marrow pressure and decrease in blood flow during decompression tend to hinder inert gas elimination.

In comparison with the femoral head, the distal end of the femur forms a complicated perfusion network expanding into both condyles. The medial and lateral condyles of the distal femur may have different perfusion characteristics caused by different body weight bearing. The weight-bearing mechanical axis is close to the lateral side of the medial condyle; therefore, it is reasonable to consider that the medial condyle has a better blood flow supply than

the lateral condyle at the distal femur (12).

It is interesting to compare the anatomic locations of diving-related osteonecrosis of the knee to spontaneous or steroid-induced osteonecrosis. Spontaneously occurring necrosis is found mainly in the medial condyle, whereas steroid-induced necrosis can occur in both condyles (13, 14). This difference indicates that aseptic bone necrosis seen among divers is strongly related to perfusion dynamics in the bone, when compared with other types of bone necrosis.

An increase in fatty marrow induced by aging facilitates inert gas uptake during hyperbaric exposure. During decompression, microbubbles disrupt fatty tissue in the bone marrow, causing small fatty emboli to form (15). These emboli may result in intermittent ischemia in long bones and disrupt bone marrow recovery. If this situation persists over a long period, aseptic bone necrosis may be induced (15, 16).

In the present study, 7 of 8 divers with definite knee-joint osteonecrosis have experienced repetitive type 1 DCS at the knee joint, and about 50% of divers with bone necrosis at other joints or without bone necrosis have had repetitive knee-joint bends. Consequently, it is an unsatisfactory explanation to consider aseptic bone necrosis solely as a sequelae or reflection of repetitive knee-joint type 1 bends.

That many divers over 30 yr old, with over 10 yr of diving experience and the risky decompression procedures of diving fishery, do not suffer from bone necrosis in a long bone, may be due to different physical characteristics. Further research on this subject is needed.

Type B bone necrosis in long bones has been found frequently in divers engaged in deep-sea and saturation diving conducted under strict diving regulations (3). Therefore, the frequent use of roentgenography for bone necrosis of long bones, especially type B, is needed for better evaluation of diving safety.

SUMMARY

In this study of Japanese diving fishermen, type B aseptic bone necrosis was a common radiographic finding, particularly about the knee. By computerized tomography, these legions appeared to progress from the subcortical region centrally. The relationship between type 1 DCS and the radiographic findings in these patients was not statistically significant.

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EVALUATION OF WORKLOAD FOR SAFETY DIVING WORK

Y. Mano, M. Shibayama, T. Mizuno, and H. Furuhashi

In August 1985, we studied 2996 professional divers during actual diving conditions. Diving profiles and real working conditions have been analyzed and it is obvious that some of the work tasks required workloads that imposed a heavy burden on the divers. It has been estimated that divers consume about 1.8 liters/min STPD of oxygen ($\dot{V}O_2$) with a 40 liter/min BTPS expiratory gas volume/min ($\dot{V}E$). Because it is difficult to measure $\dot{V}O_2$ in relation to actual fatigue in underwater activity, we looked for an efficiency index to evaluate the diving workload. Research on the work of diving has been studied in our laboratory since 1981; we developed regression equations that give us a value for the work of diving, using the heart rate as an index.

METHODS

Seven healthy males were chosen as subjects (mean age 34.4 yr and mean dive history 7.3 yr). The performance time of their maximal work was measured in each subject with a bicycle ergometer, and maximal oxygen consumption ($\dot{V}O_{2max}$) was recorded (group 1). Each subject used a bicycle ergometer according to Ikari's all-out test (1).

A warm-up exercise was imposed at a rate of 2.0 kpm/min for 3 min. After a rest of 2 min, the load was increased by 0.5 kpm/min until exhaustion. The pedaling rate was fixed at 60 rpm. When the subject could not adjust the rhythm of the metronome, he was considered to have reached exhaustion. This method is the so-called "all-out test." During the exercise, cardiopulmonary functions were monitored (heart rate [HR], $\dot{V}O_2$, $\dot{V}CO_2$, respiratory quotient [RQ], $\dot{V}E$, respiratory frequency [f], and VT) continuously.

A J-valve (Fukuda Industry Co. Ltd.) was used for gas sampling with group 1 for 3 different experiments.

In the next series of experiments (group 2), the same performance test was employed; however the respiratory attachment was not a J-valve but a scuba regulator (Type TR-60, Tabata Co. Ltd.), which is generally used for diving in the open sea in Japan. The difference between groups 1 and 2 was the respiratory pressure resistance, due to the mechanical difference between the J-valve and the demand valve.

In group 3, a fin-swimming load was used in the pool. Each subject was fully equipped with a wet suit, a 12-liter scuba tank, and 5-kg weights, breathing air through a J-valve, as in group 1. Since there was no way to use the all-out test in fin-swimming, a load similar to the group load was used. The subjects initially started to swim 25 m/50 s on the surface of the pool and then increased the swim rate to 25 m/5 s. Only the legs were used. The observer and assistants accompanied the subjects at the poolside and encouraged them during the experiments.

These so-called all-out tests forced the subjects into exhaustion. It became easier when the tests were repeated frequently. The tests were carried out once a week for each subject.

Analyses of respiratory gas were done by oxygen and carbon dioxide analyzers (RAS-31 and RAS 41, AIC Co. Inc.). V_T and $\dot{V}_E$ were measured by dry method gas meter (NDS-5A-T, Kansai Gas Meter Co. Inc.). ECG was measured continuously by a Life Scope (OEC-5201, Nihonkoden Co. Inc.), but in group 3, a waterproof housing was used for the transmitter and the data were recorded during the experiments. Respiratory frequency in group 3 was not recorded but V_E was obtained from sampling the gas by a Douglas bag each minute.

RESULTS

Initially, data were obtained from 10 male subjects for the performance tests of the 3 different groups. Three subjects could not complete all of the series and were excluded from the data. Physical characteristics and diving history are shown in Table 1.

Results by different respirators between groups 1 and 2 are shown in Table 2. Inspiratory resistances were the same in both groups, but expiratory resistance was greater in group 2 than in group 1.

The relationship between $\dot{V}_E$ and V_T of these 2 groups is described in Fig. 1.

When V_T was less than 1170.8 ml, the $\dot{V}_E$ of group 2 was greater than group 1, according to both regression equations (Fig. 1).

Table 3 shows the cardiopulmonary functions under different maximal workloads for the three groups. Maximal HRs for groups 1 and 2 were the same, but there was a difference in maximal $\dot{V}O_2$ between the 2 groups. Maximal HRs of group 3 were decreased less than the other 2 groups, and maximal $\dot{V}O_2$ in group 3 was the smallest in the 3 groups. Group 1 showed the biggest value of $\dot{V}O_{2\text{-max}}$. Group 3 reached only 74.4% of the $\dot{V}O_{2\text{-max}}$ when

Table 1
Physical Characteristics and Diving Histories of Subjects

Subjects	Age, yr	Height, cm	Weight, kg	$\dot{V}O_{2\text{-}max}$, ml/kg	VC, liter	FEV, 1.0%	Experience, Yr
M.S.	33	177	82	46.0	5.76	90.0	15
N.D.	28	171	60	50.5	3.83	89.4	2
S.S.	28	173	64	45.7	5.17	85.0	9
S.N.	20	179	75	55.0	6.07	77.0	2
K.Y.	35	158	60	44.0	2.92	89.0	8
J.T.	49	170	69	39.2	4.11	79.0	7
K.A.	48	170	70	46.8	4.91	77.0	8
mean	34.4	171.1	68.5	46.7	4.68	83.8	7.3
± SD	10.7	6.7	8.0	5.0	1.12	6.0	4.4

Table 2
Results by Different Respirators at the Maximal Workload

	Type of the Respirator		Allout Time, min-s	Resistance, mmH_2O Expiratory Pressure	Resistance, mmH_2O Inspiratory Pressure
Group 1	J-valve	$\bar{X}$	7-53	85.1	35.9
		SD	1-04	17.4	1.9
Group 2	Scuba	$\bar{X}$	7-29	121.2	35.7
	Regulator	SD	0.50	49.9	7.1

compared with group 1.

Very heavy fin-swimming work resulted in maximum $\dot{V}O_2$ at exhaustion. This meant that $\dot{V}O_2$ during diving work (fin-swimming) is determined by local kinetic energy (skeletal muscle metabolism) of the legs and not by whole body work, and even when the divers work to exhaustion the $\dot{V}O_{2\text{-}max}$ of group 1 could be reached. Therefore the diving load was apparently not maximum because the value was less than the $\dot{V}O_{2\text{-}max}$.

It is suggested that where evaluating diving workload, the relationship between percent $\dot{V}O_{2\text{-}max}$ and HR be taken into consideration, and second, the value percent $\dot{V}O_{2\text{-}max}$ can be determined by the regression equation. Three different regression equations between HR and percent $\dot{V}O_{2\text{-}max}$ are shown in Fig. 2.

According to these regression equations, it is possible to determine the ratio, as percentages, when compared to $\dot{V}O_{2\text{-}max}$ and when the HRs are obtained. Therefore, if the HRs during exercise can be obtained, percent $\dot{V}O_{2\text{-}max}$ can be estimated, which means that HR can become an index to estimate workloads.

Table 3
Cardiopulmonary Functions Under 3 Different Maximal Workloads

			HR, beats/min	$\dot{V}O_2$, liter/min STPD	$\dot{V}CO_2$, liter/min STPD	RQ	$\dot{V}E$, liter/min BTPS	f Breath/min	V_T, Liter
Group 1	bicycle*	$\bar{X}$	184.6	3.22	3.80	1.18	98.4	44.0	2.46
		S.D.	18.8	0.58	0.95	0.18	17.2	4.3	0.42
Group 2	regulator**	$\bar{X}$	183.4	2.77	3.31	1.19	80.4	31.1	2.66
		SD	14.3	0.57	0.77	0.14	13.2	7.4	0.49
Group 3	fin-swimming†	$\bar{X}$	167.2	2.33	2.29	0.99	62.1	—	—
		SD	19.7	0.36	0.59	0.12	7.1	—	—

* Workload by bicycle ergometer.

** Workload by bicycle ergometer using the regulated apparatus of scuba for their breathing.

† Workload by fin-swimming.

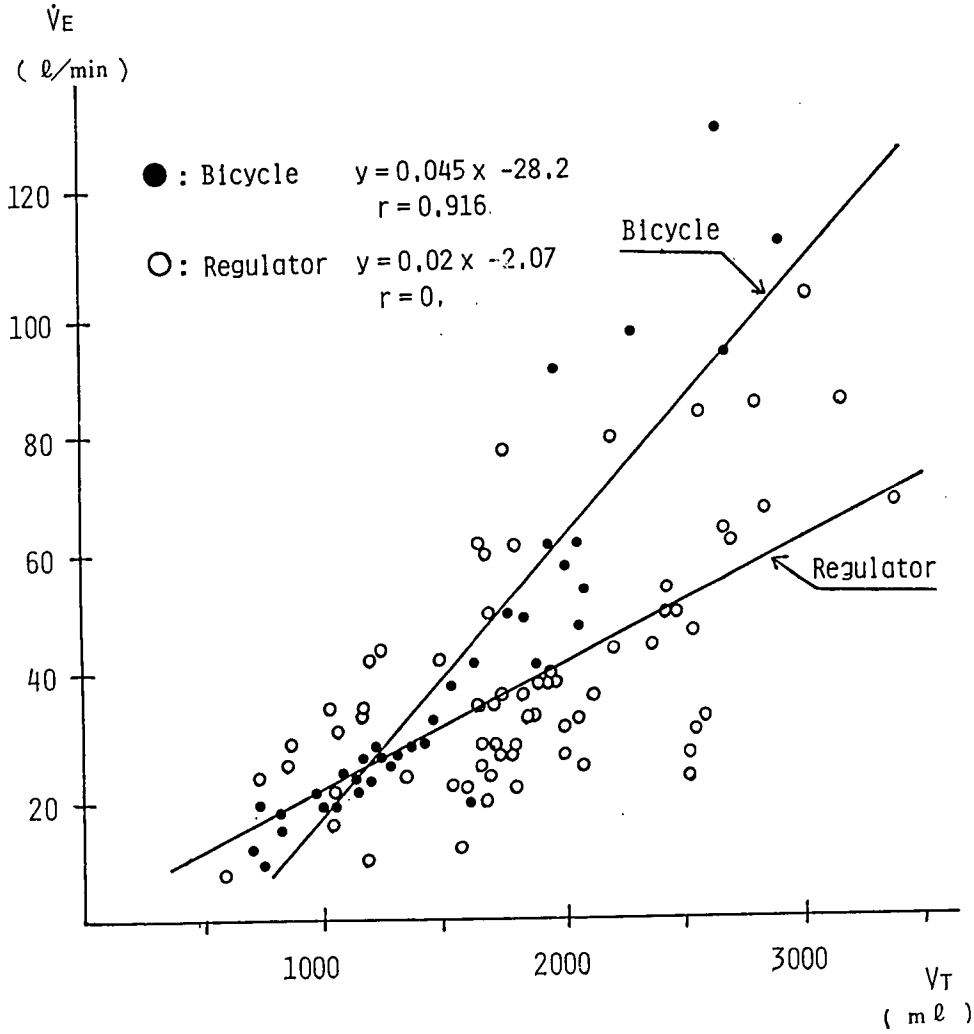

Fig. 1. Relation between VE and VT by two different tolerance tests.
Group 1. Bicycle: workload by bicycle ergometer.
Group 2. Regulator: workload by bicycle ergometer used demand valve type regulator for scuba dive.

DISCUSSION

It is generally recognized that HR is an index of workload, and percent $\dot{V}O_{2\text{-}max}$ is a popular method for estimating the ratio of kinetic energy (2). But there are few reports on the degree of diving work, because it is more difficult to calculate $\dot{V}O_2$ in the water than in the dry exercise laboratory.

According to the U.S. Navy diving manual, the amount of oxygen actually

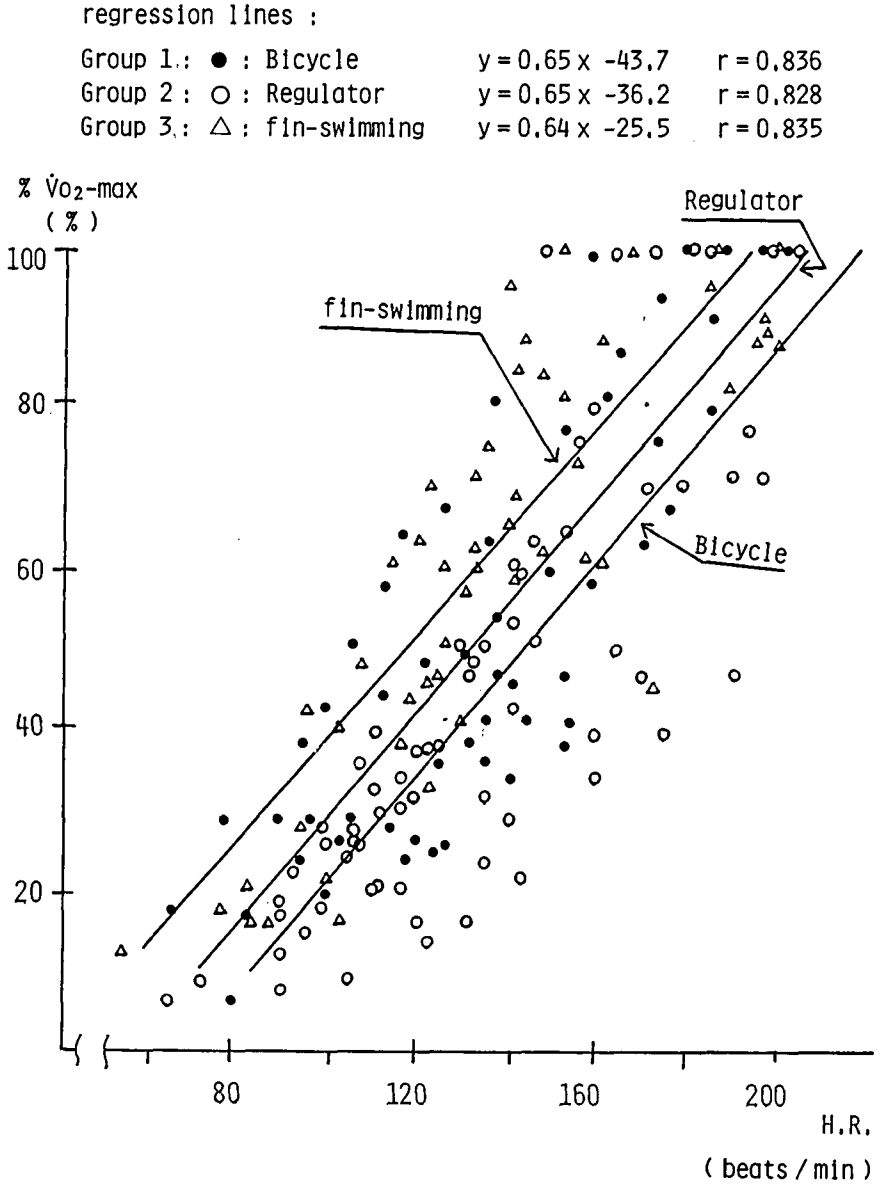

Fig. 2. Relation between percent $\dot{V}O_{2}$-max and HR by three different workloads. (subject number: 7).

Group 1. Bicycle: workload by bicycle ergometer

Group 2. Regulator: workload by bicycle ergometer using the regulated apparatus of scuba for their breathing

Group 3. fin-swimming: workload by fin-swimming

consumed by the body in a period of time is not influenced by depth, although the volume of the gas will vary with the depth. A man who is working hard enough to consume 2 liters of oxygen/min at the surface would consume only 1 liter when measured at a depth of 33 ft (10 m), but he would still be using the same number of oxygen molecules (3).

This means that the diving depth has no influence on $\dot{V}O_2$, so the value at the surface of the ocean by fin-swimming can be estimated and applied to the $\dot{V}O_2$ for underwater scuba diving work. Generally speaking, diving should not produce heavy work because of the lower level of $\dot{V}O_2$ attainable.

A diver's HR of 140 beats/min is equivalent to 47.3% $\dot{V}O_{2\text{-}max}$ according to the regression equation. However, the value was found to be 64% if one compares the value with the $\dot{V}O_{2\text{-}max}$ of fin-swimming in group 3.

Based on real physiologic calculations of oxygen consumption in group 1, this value was 1.52 liter/min for a light-to-moderate work load. A value of 2.06 liter/min was recorded with the $\dot{V}O_{2\text{-}max}$ for fin-swimming in group 3 with a heavy workload, based on their actual feeling of fatigue.

It is difficult to calculate $\dot{V}O_2$ during diving, and we found that the value was not in proportion to the actual fatigue. Thus, $\dot{V}O_2$ predicts a lower workload and closely approximates fatigue. $\dot{V}O_{2\text{-}max}$ differs between groups 1 and 3.

Dwyer (4) obtained the relationship between HR and $\dot{V}O_2$ at the surface and at 10, 20, and 30 m depths in open sea. He fixed the workloads to correspond to 1.5, 2.0, 2.5, 3.0 liter/min of $\dot{V}O_2$ at each depth and recorded the HRs. Dwyer (4) found that $\dot{V}O_{2\text{-}max}$ was equivalent to a 2.5 liter/min $\dot{V}O_2$ and 83% of $\dot{V}O_{2\text{-}max}$ was equivalent to 3.0 liter/min of $\dot{V}O_2$. This value of 83% of $\dot{V}O_{2\text{-}max}$ is almost impossible for divers in actuality, and the value of 69% $\dot{V}O_{2\text{-}max}$ is also too high for real diving work (2). A value of 70% $\dot{V}O_{2\text{-}max}$ is more reasonable for increasing the maximal $\dot{V}O_2$ and as training for sport divers.

Dwyer calculated 144 ± 14.4 for HR under 2.5 liter/min of $\dot{V}O_2$ loads and 30 m depth, and this load was equivalent to 69% $\dot{V}O_{2\text{-}max}$.

This HR is equal to 66.7% of $\dot{V}O_{2\text{-}max}$ if one uses the regression equation of group 3. The value of 66.7% approximates the actual value of Dwyer's data (4). $\dot{V}O_2$ in diving has not been considered to be influenced by depth, as mentioned before, so this regression equation obtained at the surface for fin-swimming can be used to obtain approximate values in scuba diving work.

In scuba diving, HR decreases at the deeper depth because of increased inspiratory oxygen partial pressure. However, airway resistance increases conversely by the increasing density of air. This relationship must be considered carefully, because the phenomena are opposite relations and each effect counteracts the other (5, 6).

The regression equation of group 3 in Fig. 2 cannot be considered identical with estimating the actual diving workload, but the relationship between HR and percent $\dot{V}O_{2\text{-}max}$ approximates Dwyer's data between the surface and 30 m depth.

There are no proper indexes to know the workloads in actual diving, so we suggest this system of using HR when one considers the diver's safety and their occupational health.

This calculation system gives us useful information about diver fatigue and their workload, and this system may be used as an indicator in the future to signal impending exhaustion during heavy workloads in diving.

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HYPERBARIC ENVIRONMENT AND AUDITORY TUBAL FUNCTION: A STUDY BY VALSALVA'S METHOD

I. Watanabe and J. Okubo

There are two ways of accomplishing human auditory tubal ventilation: active opening and passive opening, but the former is predominant in adults. Professional divers experience a difference in pressure in the middle ear cavity during diving, which varies with depth, and to eliminate this difference in pressure they repeatedly adjust the pressure via the auditory tube. In a previous study using an auditory tube opening test (by means of an apparatus for sonometric auditory tubal function test) (1-3), the opening incidence with spontaneous swallowing under atmospheric pressure was 85% for normal adults, and 60% for professional divers, that is, divers showed a lower incidence. This finding suggests that in professional divers frequently exposed to hyperbaric environments, the auditory tube might not open and close with each swallowing movement under atmospheric pressure, or the auditory tube and the middle ear might have adapted to a condition where they no longer need the opening with each swallowing movement under atmospheric pressure. We compared the intranasal pressure at the opening of the auditory tube by Valsalva's method in divers with that of normal adults under atmospheric pressure to determine auditory tubal function of divers often exposed to hyperbaric conditions.

METHODS

Valsalva's Method With Sonometric Auditory Tubal Function Test (Fig. 1)

A microphone is first set in the external auditory meatus on the test side, a pressure sensor is pressed onto the orifice of the nostril on the ipsilateral side, and a loudspeaker is fixed to the orifice of the nostril on the contralateral side. The loudspeaker and pressure sensor are set to prevent air leaving the nostrils, and when the auditory tube is opened by Valsalva's

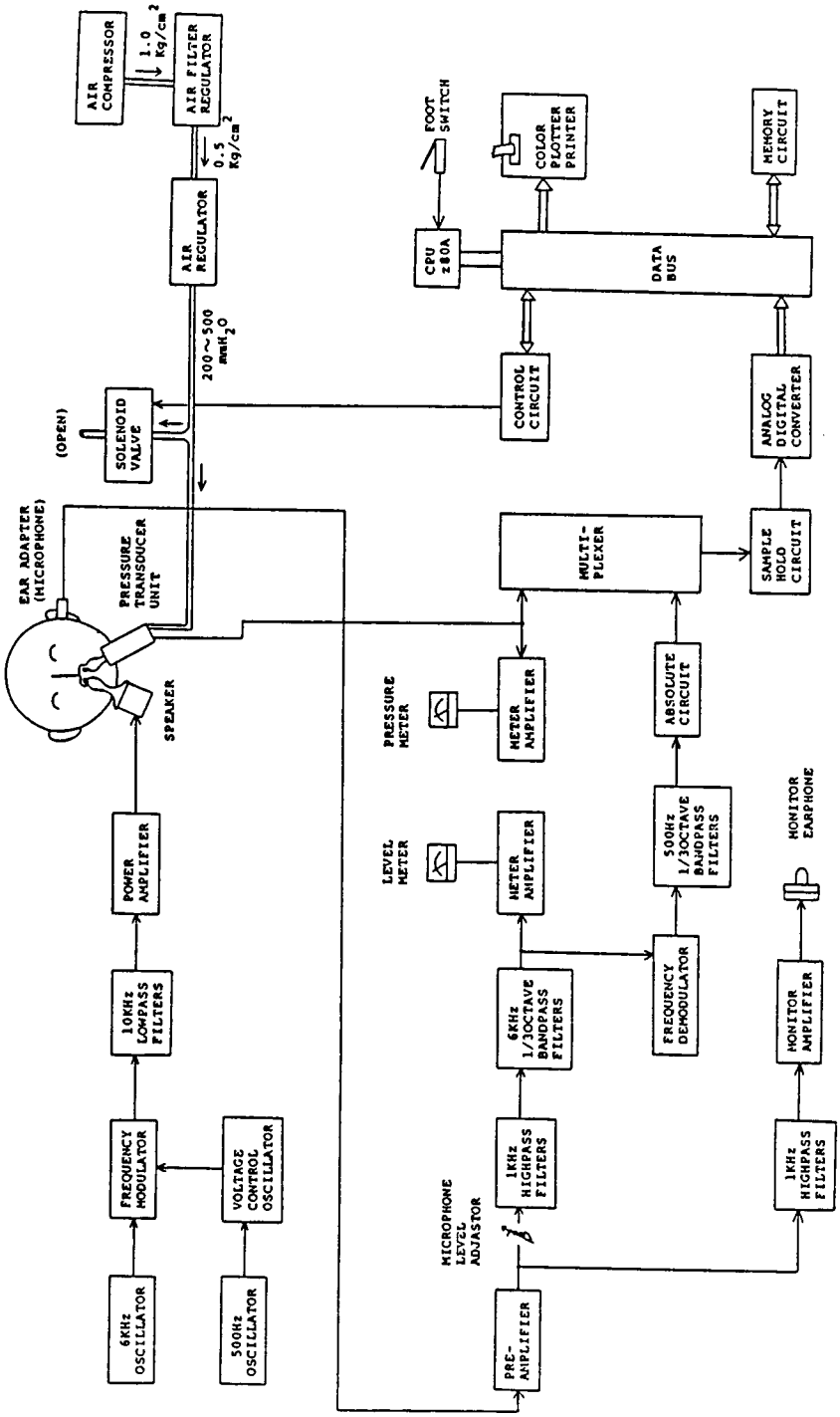

Fig. 1. Valsalva's method by means of sonometric auditory tube function test.

method, the intranasal pressure is recorded as the opening pressure.

RECORDING

Figure 2 shows the records with the auditory tube open (*top*), and those with the auditory tube closed (*bottom*). When the auditory tube is opened by Valsalva's method, the sound pressure change of tubal opening is recorded as a wave pattern, and the apparatus reads the intranasal pressure. When the auditory tube is not opened the maximum pressure, as the intranasal load by Valsalva's method, is recorded.

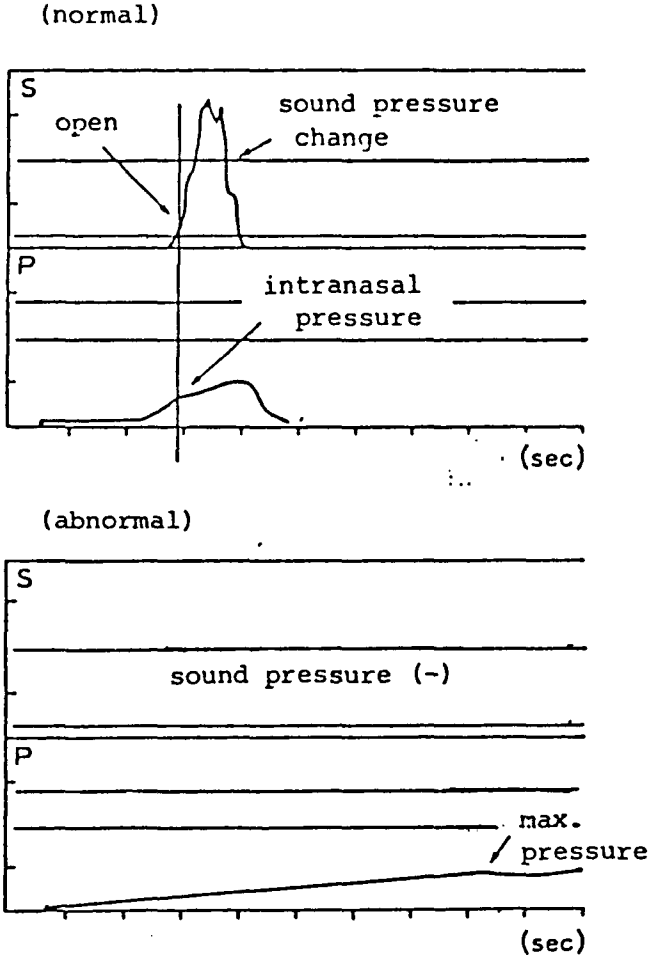

Fig. 2. Top section is the records with the auditory tube opened, and the bottom section, those with the auditory tube not opened.

RESULTS

Thirty-three professional divers were enrolled, ranging in age from 19 to 50 yr (mean 32.1), with diving experience ranging from 1 to 30 yr (mean 13.4). The auditory tube opening incidence with the swallowing movement under atmospheric pressure was as low as 60%. The tympanogram was of type A for 61 ears, type B for 2, and type C for 2 (one ear, perforated otitis media). In other words, the pressure in the middle ear cavity was maintained at atmospheric pressure in 92% of the ears. Fifty-eight normal adults were chosen as controls, and the tympanogram was of type A for 116 ears.

Comparison With Normal Adults (Table 1)

The auditory tube was opened by Valsalva's method in 88 ears (75.9%) of the normal adult controls, and in 51 (77.3%) of the 66 ears of the 33 professional divers. No significant difference was found in the positive rate between the 2 groups. The mean auditory tube opening pressure was 386.4 mmH_2O for the normal controls, and 540.6 mmH_2O for the professional divers. In other words, the latter tended to require a higher pressure for opening the auditory tube.

Table 1
Comparison of the Intranasal Pressure by Valsalva's Maneuver

Normal Control, $n = 58$	Profession Diver, $n = 33$
41/52 (78%)	22/26 (84.6%)
322.6 ± 140.8 (20s)	460.9 ± 169.8 (20s)
10/14 (71.4%)	18/26 (69.2%)
398.0 ± 196.0 (30s)	572.8 ± 233.6 (30s)
24/32 (75.0%)	11/14 (78.5%)
480.0 ± 218.0 (40s)	625.5 ± 334.6 (40s)
13/18 (72.2%)	
515.0 ± 263.0 (50s)	(mmH_2O)

COMPARISON OF AUDITORY TUBE OPENING PRESSURES BY AGE GROUP

Comparison of the auditory tube opening pressures by age group revealed that no significant difference occurred in the auditory tube opening rate by Valsalva's method to the tested number of ears between the age groups, whereas the professional divers in each age group required a higher opening

pressure than the counterpart of the normal controls. With the advancement of age to the 30s and 40s, the mean pressure was higher and associated with greater variations.

In Fig. 3, the mean pressure for each age group, at *right*, are high and associated with significant variation. Pressures for normal adults are shown on the *left*. Even in the latter group, the auditory tube opening pressure is higher, the higher the age group, but this tendency for the auditory tube opening pressure to increase with age is absent beyond 40 yr of age. This pattern is also observed in the professional divers and seems to result from the aging of auditory tubal function. This finding is interesting because clinical evidence suggests that the incidence of secretory otitis media increases in the 40s and higher age groups.

Postural Changes and Auditory Tube Opening Pressure in Professional Divers (Table 2)

Profession divers take various postures while they are diving, and in rapid diving they often take a head-down posture. It is reported that rapid descent is likely to be associated with the difficulty in depressurizing the middle ear. For this reason, we measured intranasal pressure on opening the auditory tube by Valsalva's method with the professional divers in sitting, lateral, and head-down positions. The auditory tube opening incidence was 98.0% for the sitting position, and 84 (up side) to 54% (down side) for the lateral position, with a higher mean pressure required to open the auditory tube of the ear on the top side than on the bottom side. The opening incidence for the head-down position was as low as 40.0%, with a higher mean opening pressure required.

DISCUSSION

The auditory tube connects the middle ear cavity with the retronasal region (the nasopharynx), and opens and closes with swallowing as its ventilation function in adults. Under atmospheric pressure, the auditory tube opens and closes with mere swallowing. With a change in atmospheric pressure, a closed feeling of the ear occurs, and people try to accommodate the pressure by repeated swallowing. However, when the tubal pressure accommodation is disturbed, disorders such as round window rupture result. This disturbance, damages the inner ear, leads to sequelae such as deafness, tinnitus, and dizziness, and should be treated as early as possible. To predict this auditory tube insufficiency, we devised a "sonometric auditory tube function testing apparatus" which captures as a sonic marker the opening and closing of the auditory tube with spontaneous swallowing. With this apparatus, we found that ventilation of the auditory tube by opening and closing itself with swallowing took place in 85% of normal adults, and in 117 of 178 ears (65.7%) in 89 children aged 4 to 12 yr (Fig. 4). In children about age 10, the opening rate of the auditory tube with swallowing was about 80%, and the duration of opening

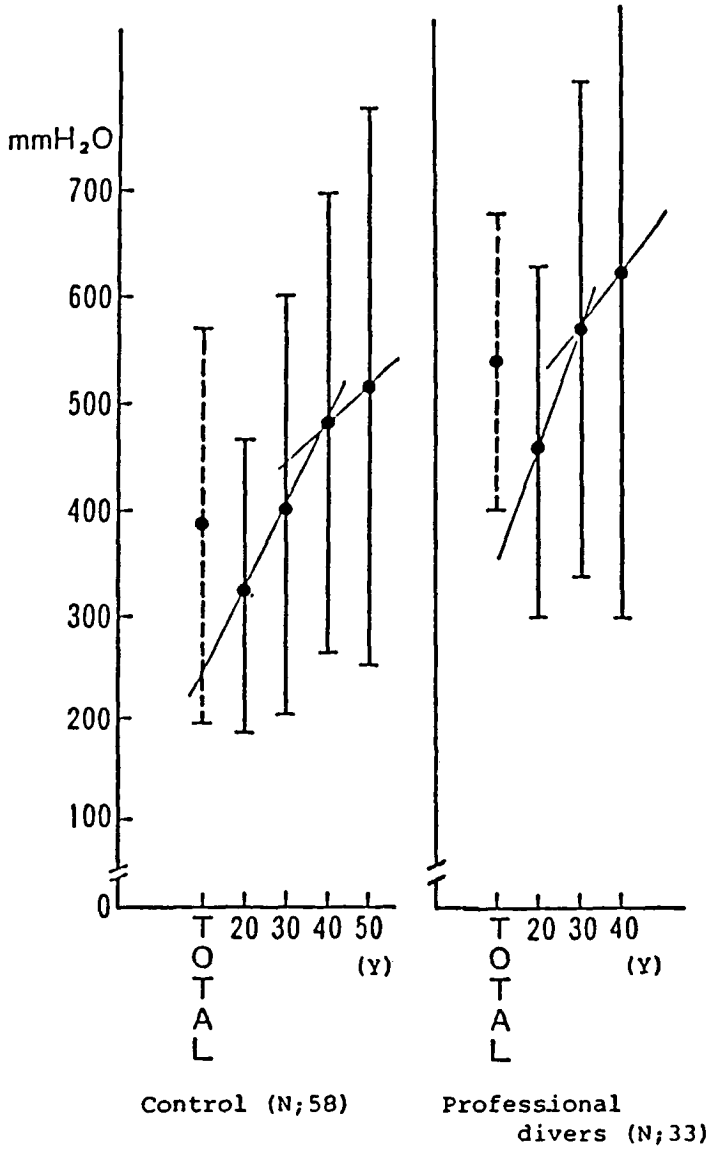

Fig. 3. Comparison of auditory tube opening pressures by age group.

of the tube tended to be close to the duration in adults.

In a study of auditory tube opening rate with spontaneous swallowing in occupational divers always exposed to hyperbaric environment (diving), Takahashi et al. (4) reported auditory tube opening in about 60%, which is close to the opening rate in children. One of the reasons for this low opening rate is that people frequently exposed to hyperbaric environment equilibrate

Table 2
Valsalva Maneuver*

	Ears of Positive Response	Mean Value of Opening Pressure
Sitting	49/50 (98.0%)	373.3 ± 184.3
Supine	31/50 (62.0%)	501.9 ± 224.4
Prone	29/50 (58.0%)	519.7 ± 251.2
Lateral, up	42/50 (84.0%)	532.9 ± 213.4
Lateral, down	27/50 (54.0%)	536.3 ± 232.0
Head down	20/50 (40.0%)	614.0 ± 225.3

* Observation of each head position, repeated the Valsalva maneuver twice, $n = 25$.

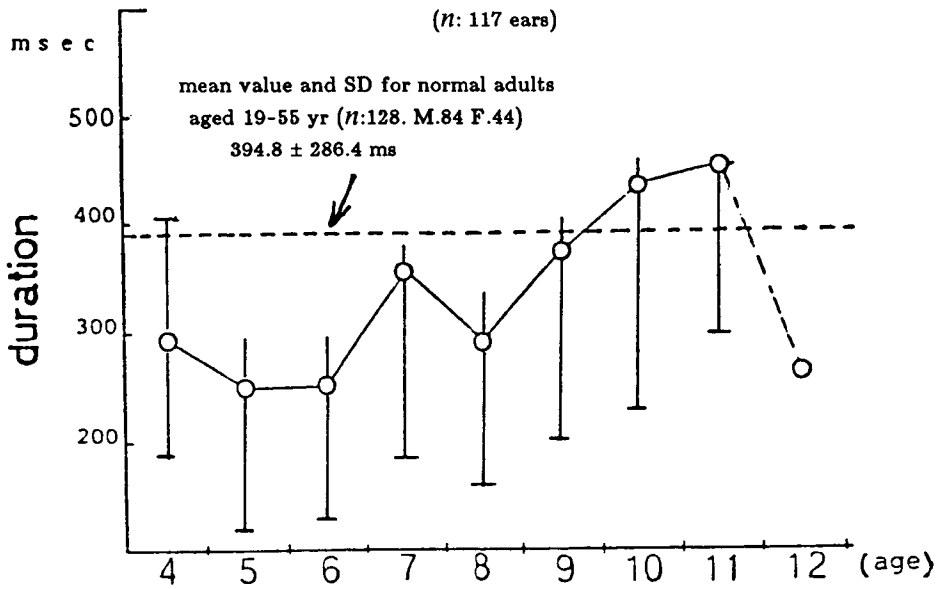

Fig. 4. Sonometric tube function test in 89 normal children aged 4-12 years.

the middle ear cavity pressure by opening and closing the auditory tube with the assistance of pressure. The tube is likely to open with a simple swallowing. This training seems to adapt the auditory tube function to that of children (4), who have both passive and active openings. In other words, it may be presumed that because occupational divers repeatedly dive and surface rapidly, they do not accommodate the air pressure in the middle ear cavity at each diving and surfacing. Rather, they have adapted to ventilating the middle ear cavity by opening the auditory tube with swallowing or Valsalva's method once every several repetitions of diving and surfacing when there has arisen a difference in pressure between the middle ear cavity and the ambient pressure

(5, 6). For this reason, it is possible that when the auditory tube opening and closing function is trained in hyperbaric environment, the activity of the musculus tensor veli palatini easily opens the Eustachian tube due to the effect of air density than in normobaric environment. In this study, auditory tube opening was performed by Valsalva's method under atmospheric pressure, but practically no difference occurred in the auditory tube opening rate between occupational divers and normal controls. This finding indicates that normal adult controls and occupational divers do not differ in the physiologic rate as to whether the auditory tube opening can be done well by Valsalva's method, but that only the mean auditory tube opening pressure in occupational divers tends to be higher than in controls. Auditory tube opening pressure by age also indicates that it rises slowly as we grow older, and reaches a plateau in the 40s. Since this tendency was observed in both normal adult controls and occupational divers, there appears to be a change with aging in auditory tubal function, as seen with Valsalva's method. Moon and Swanson (7) reported that the pressure for passive opening of the auditory tube in children is close to the adult value at the age of about 8 to 10 yr. Such a change in auditory tubal function with aging in occupational divers and normal adult controls, compared with that in children, is of interest. Posture is also known to exert various effects on the body during diving. The tendency to enhance the opening of the auditory tube on the upper side compared with the lower side in a lateral decubitus position is interesting.

CONCLUSION

Professional divers showed a lower auditory tube opening rate with swallowing movement than the normal adult controls; they required a high pressure for opening the auditory tube by Valsalva's method. However, 92% of them showed the type A tympanometric pattern. It seems that the auditory tubal function of such workers in hyperbaric conditions is acclimatized to the hyperbaric environment. The aging effect was observed on the auditory tube opening pressure of both normal adult controls and professional divers. The aging effect appeared in the 40s. It was obvious that the auditory tube opens less readily in the head-down position.

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BRONCHIAL PROVOCATION TEST IN FINER SELECTION OF SPORT SCUBA DIVERS

M. Schiavon, R. M. A. Osti, C. Schiraldi, and F. Rusca

The examination of a scuba diver's respiratory system must be done carefully to reduce the incidence of pulmonary barotrauma. During ascent with scuba gear, serious overpressure due to air trapping can develop in susceptible pulmonary structures. The most important risk factors are considered to be smoking, allergic diseases, asthma, and airways obstruction. Smoking and decline in pulmonary function have been correlated in scuba divers (1).

Allergic subjects require special surveillance in case of allergic rhinitis with bronchial responses which may be undetected with basic pulmonary function tests. Active asthma is an absolute contraindication for divers (2).

Airways obstruction is sometimes detected in sport scuba divers (8% out of 247, 5.4% out of 628 subjects) (3, 4). In the cases we examined spirographic data were not accounted for in the clinical history. In some, lung volumes seemed to be high, and there was a reduction in the forced expiratory flow. In other cases allergy sufferers and/or smokers showed no appreciable alterations in first level spirometry. Therefore, we devised a means to select sport scuba divers who had histories of slight alterations in respiratory function. The U.S. National Institute for Occupational Safety and Health (5), British (6), and Norwegian (7) standards provide disqualifying spirometric limits for divers. However, the few spirometric parameters taken into consideration identify only serious pathologic alterations. We sought a test capable of simulating in the laboratory, the airways environment encountered during immersion. Sport scuba divers inhale cold air, and water that often remains in the mouthpiece of the regulator is later nebulized. Such environmental conditions emulate the nonspecific test of bronchoreactivity (8-10) using ultrasonically aerosolized distilled water. Hence this test was chosen for our survey.

MATERIALS AND METHODS

Twenty-two subjects were chosen out of 645 sport scuba divers examined over 4 yr to verify fitness for recreational diving activities. The 22 divers were initially examined at the Center for Sports Medicine (National Health Service U.L.S.S., 21 Padua, Italy). Medical examinations and associated surveys were carried out according to a modified version of the protocol suggested by European Diving Technology Committee for commercial divers (11) and according to guidelines in Italian laws concerning medical evaluation of sport scuba divers (12). Information about family, physiology, illness, and sports activities was gathered through a questionnaire. The 22 divers were examined when they were not suffering from acute or infectious illnesses; moreover they had taken no medicine for over a week.

Pulmonary function was performed with a dry wedge bellows (Vitalograph S-Model Spirometer 20.410 with function analyzer 21.300 A, Buckingham, England). Vital capacity (VC), forced vital capacity (FVC), forced expiratory volume at 1 s (FEV_1), FEV_1-FVC x 100, and forced expiratory flow at 25 to 75% of FVC (FEF_{25-75}) were measured.

The subjects were examined further at the Department of Pneumology (National Health Service U.L.S.S.). This second examination of pulmonary function was performed with a variable pressure body box plethysmograph (SMB 611, Fenyes & Gut, Basel, Switzerland) and light bell, computerized spirometer (Baires 80-Biomed-In, Padua, Italy). VC, FEV_1, FEV_1-VC x 100, FEF_{25-75}, residual volume (RV), peak expiratory flow (PEF), maximal expiratory flow after exhalation of, respectively, 50% ($\dot{V}_{max50}$) and 75% ($\dot{V}_{max75}$) of FVC, airways resistance (R_{aw}), and the specific airways conductance (sG_{aw}) were measured. The best effort was always selected for analysis, corrected to BTPS. CECA (13) and Knudson et al. (14) spirometric values were used. Data are expressed as percentage of predicted values.

Nonspecific bronchoreactivity was obtained with an ultrasonic aerosol of distilled water (De Vilbiss 65 Comesa, Milan, Italy) at a 2 ml/min flow for 10 min. After the bronchial provocation test, FEV_1, FEF_{25-75}, $\dot{V}_{max50}$, $\dot{V}_{max75}$, and sG_{aw} were measured. Data are expressed as percent of baseline values. Changes of $FEV_1 \geq 15\%$, $FEF_{25-75} \geq 30\%$, $\dot{V}_{max50} \geq 35\%$, $\dot{V}_{max75} \geq 40\%$ were considered positive. Student's *t* test was applied for the analysis of data.

RESULTS

The 22 sport scuba divers, 10 experts and 12 beginners, had mean (± SD) values for age, height, and weight of 29.1 ± 8.1 yr, 174.5 ± 6.6 cm, and 69.6 ± 8.4 kg, respectively. Ten had clinically significant allergic symptoms and 12 showed evidence of bronchospasm. Four of the divers were current smokers, 3 former smokers, and 15 had never smoked. None was exposed to occupational lung-damaging conditions.

Baseline values of lung functions and percentages of predicted values are

shown in Table 1. The parameters are divided into spirometric (stage 1) and plethysmographic (stage 2) studies.

We found mean percent FVC and RV to be higher by 11 and 9%, and FEF_{25-75} to be lower by 19% with changes comparable to those indicated by other authors for commercial and military divers (1, 15).

In the 12 subjects (2 smokers, 2 ex-smokers, and 8 who never smoked) with bronchospasm, the values of VC, FVC, and FEV_1 (mean ± SD) respectively, 100.2 ± 14.5; 113.0 ± 13.7, and 105.4 ± 8.8) were normal compared to predicted values. The FEV_1-FVC x 100 (93.2 ± 4.3) showed a slight reduction. The FEF_{25-75} (63.6 ± 8.6) was the only value significantly lower than normal. Percent variation from baseline values after the bronchial provocation test is shown in Fig. 1.

Bronchial reactivity did not change significantly for FEV_1: 98.5 ± 4.5, FEF_{25-75}: 96.8 ± 10.3, $\dot{V}_{max50}$: 94.1 ± 9.6, $\dot{V}_{max75}$: 97.1 ± 9.8. A highly significant ($P < 0.001$) variation was evident in sG_{aw} (responders: 40.3 ± 13.8, nonresponders: 99.6 ± 15.3) as shown in Fig. 2. There was no correlation between bronchial provocation test responders and allergy sufferers and bronchial obstructed groups. A positive reaction of sG_{aw} was considered a disqualifying condition for diving.

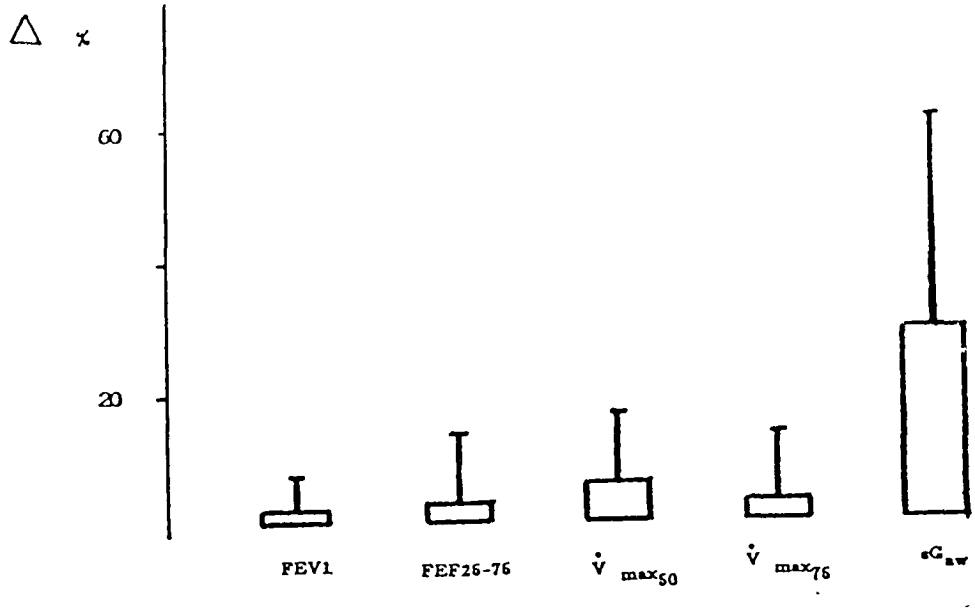

Fig. 1. Percentage variation in comparison with baseline values after bronchial provocation test (mean ± SD).

Table 1
Pulmonary Function Basic Data in 22 Sport Divers with Airways Abnormalities*

	Stage 1 Spirometry		Stage 2 Plethysmography	
	Measured	Percent Predicted	Measured	Percent Predicted
VC**	5249.5 ± 606.8	99.4 ± 13.7†††	5485.9 ± 861.4	101.5 ± 15.8††
FVC**	5473.1 ± 667.2	111.9 ± 13.2†††		
FEV_1**	4362.7 ± 611.1	106.5 ± 8.8†††	4282.2 ± 622.1	100.6 ± 14.7††
$FEV_1/FVC \times 100$	80.1 ± 6.9	96.7 ± 7.2†††		
FEF_{25-75}***	4152.3 ±1296.3	81.0 ± 25.4†††	78.6 ± 6	99.6 ± 7.4††
RV**			4102.3 ± 900.1	81.4 ± 15.4†††
PEF†			1637.7 ± 639.4	111.9 ± 45.2††
V_{max50}†			10.2 ± 1.4	117.1 ± 22.8†††
V_{max75}†			5.5 ± 1.7	92.5 ± 28.4†††
R_{aw} (kPa-1/1s)			2.5 ± 1.2	80.8 ± 36.6†††
sG_{aw} ($s^{-1}kPa^{-1}$)			1.1 ± 0.8	
			0.26± 0.1	

* Data are mean ± SD. ** = ml; *** = ml/s; † = liter/s. Predicted values: †† CECA (13), ††† Knudson et al. (14).

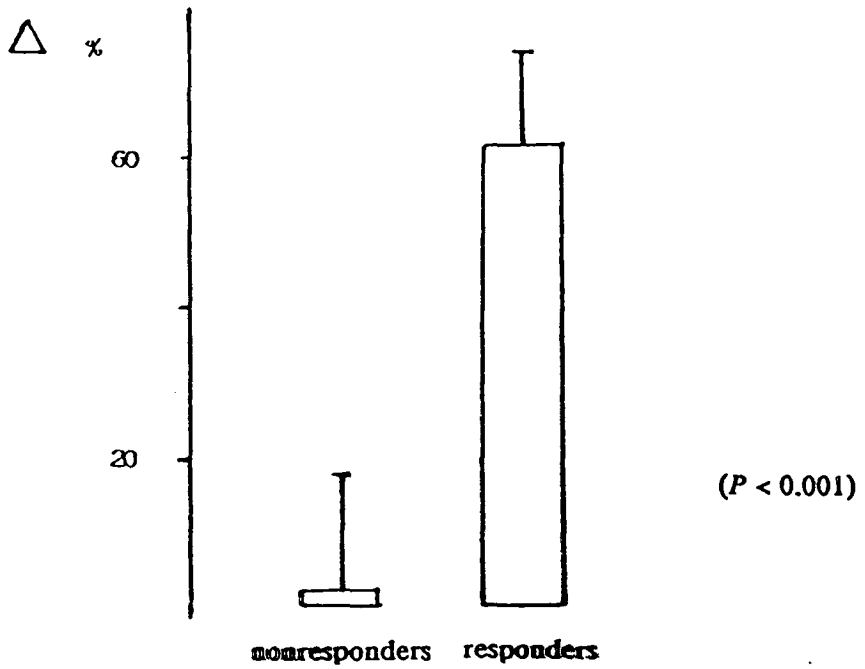

Fig. 2. Percentage variation of sG_{aw} in responders and nonresponders (mean ± SD).

DISCUSSION

The risk factors for pulmonary barotrauma that we considered (smoking, allergic diseases and asthma, bronchial obstruction) lead to serious damage to the respiratory system which can only be detected by x-rays late in the progress of the disease. Therefore, functional testing becomes extremely important in the selection and periodic evaluation of scuba divers. By comparing the data obtained from the various methods, the validity of stage 1 testing (spirometry) to screening for abnormal pulmonary function has been confirmed. The FEF_{25-75} was the most sensitive parameter in the early detection of bronchial obstruction.

Statistical comparisons among different groups (smokers, nonsmokers, allergy sufferers, nonallergic subjects, subjects with or without bronchial obstruction) provided no meaningful result because of the limited number of cases. We would like to point out however, that 8 subjects suffering from bronchial obstruction were neither smokers nor allergy sufferers. We cannot explain the presence of bronchial obstruction with either symptoms or justification from the clinical history. Such alterations were detected in numerous subjects engaged in other sports. At present, we are analyzing these subjects.

A finer selection of divers suffering allergy or airways obstruction is made possible by examining the sG_{aw} responses to inhalation of ultrasonically nebulized solutions of distilled water. Such a method simulates an immersion;

moreover it is simple, quick, and inexpensive.

We suggest that protocols including both a stage 1 (spirometric) survey and a bronchial provocation test be developed for allergy sufferers, smokers, and subjects with reasonable (however slight) bronchial obstruction on baseline evaluation. Periodic reevaluation of these subjects would also be of interest.

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SESSION 15: HYPERBARIC OXYGEN THERAPY II

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IATROGENIC AIR EMBOLISM: A REVIEW OF 34 CASES

H. Takahashi, S. Kobayashi, H. Hayase, and K. Sakakibara

In general, air embolism is supposed to occur following three basic mechanisms: a) air may enter the systemic circulation through injured blood vessels, b) air may remain within circulated blood when the opened circulatory system is closed after any surgical procedure, and c) air may be directly injected into both the arterial and venous system during invasive diagnostic or therapeutic procedures. In the past, the most typical situation for air embolism was thoracic injury accompanied by severe damage to a pulmonary artery, or as a complication of a mishandled delivery. However, with the increase of both arterial and venous systems for vascular access to perform medical treatment, it is not uncommon to encounter in hospital air embolism as a fatal complication of diagnostic or therapeutic medical procedures. Over 15 yr, from 1970 to 1985, 34 cases of air embolism were seen. All were iatrogenic air embolism; none of them were traumatic cases. In fact, all cases originated from cerebral angiography (CAG), craniotomy in the upright sitting position, neck surgery, open heart surgery, arteriography, hemodialysis, and others.

This paper reviews the clinical results of 34 iatrogenic air embolism cases to confirm the effect of HBO as a definitely effective treatment for such a tragic complication, and to clarify several key issues.

CLINICAL EXPERIENCE

As Table 1 shows, there are two noteworthy characteristics. One is that , as stated above, all cases resulted from medical procedures. The other is that, although the recognition of insufflated air into the circulation is indispensable to establish an accurate diagnosis and to perceive the need for emergency treatment, there was an apparent difference, depending on whether air invasion could be confirmed. Table 2 shows the 34 cases arranged according to the causes of air embolism and the number with confirmed air infusion. Among 9

Table 1
Iatrogenic Air Embolism

Case No.	Sex	Age	Original Disease	Causes of Air Embolism	Confirmation of Air	Patterns	Time Until HBO, h	Instances of HBO	Results
1	M	65	embolism of int. carotid artery	CAG	-	cerebral	4.5	14	fair
2	M	55	carotic cavernous sinus fistula	CAG	-	cerebral	4	8	excellent
3	F	60	Tolosa Hunt synd.	CAG	-	cerebral	19	14	excellent
4	M	64	Grawitz's tumor brain metastasis	CAG	-	cerebral	2	4	excellent
5	M	41	CVA?	CAG	+	cerebral	30	84	excellent
6	M	40	AV malformation	CAG	-	cerebral	5	226	fair
7	M	67	ASO (left arm)	arterio (sub-clavian a.)	-	cerebral	22 - 23	268	fair
8	M	64	lung tumor	arterio (int. mammary a.)	-	cerebral	6	1	dead
9	M	64	A-V block coronary disease	coronary angio	+	cerebral	2 - 3	7	dead
10	M	23	chronic renal ins.	dialysis	+	cerebral	25	4	excellent
11	M	38	chronic renal ins.	dialysis	+	cerebral	40	1	excellent
12	F	8	Fallot's tetralogy	open heart surg.	-	cerebral	24	2	dead
13	M	26	Mi + Ai	open heart surg.	-	cerebral	24	4	dead
14	F	36	ASD	open heart surg. valve replace	-	cerebral	27	47	fair

15	F	39	ASD	open heart surg.	-	cerebral	27	23	excellent
16	F	47	endocardial cushion defect	open heart surg.	-	cerebral	25	8	dead
17	F	37	Ms + Ai	open mitral	-	cerebral	120	171	fair
18	M	44	Ms + Ai	open mitral	-	cerebral	20	58	excellent
19	M	36	chronic renal ins.	dialysis	+	pulmonary	25	7	excellent
20	F	54	chronic renal ins.	dialysis	+	pulmonary	2	1	dead
21	F	47	chronic renal ins.	dialysis	+	pulmonary	2	7	excellent
22	M	34	chronic renal ins.	dialysis	+	pulmonary	4	2	excellent
23	M	54	chronic renal ins.	dialysis	+	pulmonary	4.5	13	excellent
24	F	62	chronic renal ins.	dialysis	+	pulmonary	1.5	3	excellent
25	M	54	chronic renal ins.	dialysis	+	pulmonary	0.5	2	excellent
26	M	50	chronic renal ins.	dialysis	+	pulmonary	3.5	3	excellent
27	M	49	chronic renal ins.	dialysis	+	pulmonary	2 - 3	5	excellent
28	M	61	chronic renal ins.	dialysis	+	pulmonary	24	12	excellent
29	F	29	cerebellum teratoma	craniotomy in sitting position	+	pulmonary	4.5	2	excellent
30	M	50	auditory nerve tumor	craniotomy in sitting position	+	pulmonary	3	13	dead
31	M	36	cerebellum hemangioblastoma	craniotomy in sitting position	+	pulmonary	3.5	5	dead
32	F	9	pineal body tumor	craniotomy in sitting position	+	pulmonary	2	8	excellent
33	M	2	neck hemangiolymphangioma	neck surgery	+	pulmonary	24	8	excellent
34	M	80	sigmoid colon cancer	IVH	-	pulmonary	uncertain	4	excellent

cases of arteriography, air infusion could be identified in only 2 cases; 1 case of CAG (case 5 on Table 1) and one after left ventriculography (case 9). The latter was a tragic accident in which a massive amount of air was injected directly into the left ventricle in place of contrast medium. Although the patient was carried to a hyperbaric chamber and received HBO within 2 to 3 h, he died after 8 d.

Seven cases were associated with open heart surgery. These were unlike previous cases where air was sucked from a malfunctioning extracorporeal circuit or pumped into the circulation during perfusion. In the present cases, air bubbles within the cardiac cavities or aortic roots were believed to be removed when the cardiotomy or aortotomy was closed. In these cases, the cause of a severe, persistent postoperative central nervous system (CNS) disorder was inductively attributed, after eliminating other possible factors, to air bubbles that might have remained in the left heart. Therefore, confirmation of air infusion was impossible, even if the computed tomography scan could be introduced earlier in diagnosis.

Contrary to this situation, in 12 cases of hemodialysis, 4 cases of craniotomy in the upright sitting position, and 1 case of neck surgery, it was confirmed that air entered the systemic circulation. This specific feature was in striking contrast to the former group of arteriography and open heart surgery. This difference in time to diagnosis seemed to relate closely to the time lag between recognition of a CNS abnormality and the start of HBO, and contributed significantly to the prognosis.

As a rule, patterns of air embolism can be divided clinically into 2 major categories: cerebral and pulmonary air embolism.

When air bubbles are pumped into the arterial system, clinically significant symptoms are produced from organs such as the brain, which has minimal collateral circulation, and blood is supplied by so-called "end-arteries." Of course, coronary air embolism will produce severe ischemic damage to the myocardium. However, it never manifests as a sole symptom but as accompaniment of cerebral air embolism. Thus, nearly all arterial air embolisms commonly are manifested as cerebral air embolisms.

On the other hand, in venous air embolism, the infused air will be brought to the lung by blood flow and primarily embolize the pulmonary arteries. Thus, all venous air embolisms can be explained as pulmonary embolisms. The severity of its symptoms largely depends on the amount of insufflated air. In some cases, venous air may cross a foramen ovale and produce cerebral air embolism.

Cerebral and pulmonary embolism allow a clarification of the pathophysiology of all cases and are useful for predicting prognosis. The *far right column* of Table 2 shows patients grouped according to these 2 categories. Nine cases of arteriography, 7 of open heart surgery, and 2 out of 12 cases of hemodialysis are classified as cerebral air embolism. Ten cases of hemodialysis, all of craniotomy and neck surgery, and 1 intravenous hyperalimentation (IVH) were categorized as pulmonary air embolism.

Table 2
Iatrogenic Air Embolism and Confirmation of Infused Air

Causes	No. of Cases	Patterns of Air Embolism	Those Confirmed Air Infusion, %
Arteriography			
CAG	6	cerebral	1 (16.6)
Subclavian	2	cerebral	0 (0)
Coronary a.	1	cerebral	1 (100)
Open heart surgery	7	cerebral	0 (0)
Dialysis	12	2 cerebral	
		10 pulmonary	12 (100)
Craniotomy	4	pulmonary	4 (100)
Neck surgery	1	pulmonary	1 (100)
IVH	1	pulmonary	0 (0)
Total	34		19 (55.9)

SYMPTOMS AND DIAGNOSIS

Tables 3 and 4 show the signs and symptoms of 34 cases. All of them were accidental, and initially differed according to the underlying clinical disorder and the amount of infused air. Detailed symptoms will be explained according to the above-mentioned categories.

Cerebral Air Embolism

In 18 cases of cerebral air embolism, air infusion was confirmed in only 4; 2 cases of arteriography and 2 of hemodialysis. The other cases usually took many hours before being diagnosed as air embolism. However, clinical manifestations suggested further division into 3 groups according to their features because it seemed more convenient to comprehend how the clinical scenario developed.

In 7 cases of open heart surgery, the possibility of air embolism was finally suggested, after long hours of examination, as the cause of postoperative neurologic impairment. In these cases, even if the actual cause was air embolism that occurred during perfusion under general anesthesia, it would be almost impossible to detect initially. Furthermore, it seemed quite natural to

Table 3
Symptoms of Cerebral Air Embolism

Case No.	Causes	Progress of Major Manifestations
1	Arteriography (CAG)	incomplete hemiplegia → complete hemiplegia, unconsciousness → semicoma, aphasia
2	Arteriography (CAG)	transient unconsciousness, bilateral visual disturbance → bil. hemianopia
3	Arteriography (CAG)	bilateral visual disturbance, light reflex(-), memory disturbance, acalculia, aphasia
4	Arteriography (CAG)	disorientation, memory disturbance, complete hemiplegia, aphasia
5	Arteriography (CAG)	transient unconsciousness → semicoma, complete hemiplegia, aphasia
6	Arteriography (CAG)	hemiplegia, consciousness disturbance, impaired light reflex, eye deviation
7	Arterio. (subclavian)	hemiplegia, slight aphasia, Babinski(-), light reflex(+), anisocoria(-)
8	Arterio. (subclavian)	semicoma, respiratory distress, miosis, light reflex(+), eye deviation(+)
9	Coronary angio.	deep coma, light reflex (weak & retarded), anisocoria(+), foot clonus(+), Babinski(-)
10	Dialysis	rigid extremities, convulsion, coma, light reflex(-), dilated pupil, irregular resp.
11	Dialysis	convulsion, unconsciousness → coma, light reflex(-), impaired miosis
12	Open heart surgery	spastic leg → rigid arm → convulsion, coma, respiratory distress → apnea, miosis
13	Open heart surgery	apnea, drop of blood pressure, anisocoria → dilated, light reflex(-), Babinski(+)
14	Open heart surgery	semicoma, hemiplegia, foot clonus(+), Babinski(+)
15	Open heart surgery	coma, hemiplegia, general convulsion, miosis, light reflex(-)
16	Open heart surgery	coma, irregular and weak respiration, frequent convulsion, miosis, light reflex(+)
17	Open heart surgery	coma, pain reflex(-), anisocoria, light reflex(+) → complete hemiplegia
18	Open heart surgery	coma, pain reflex(-), foot clonus(+) → convulsion on extremities

Tables 4

Symptoms of Pulmonary Air Embolism

Case No.	Causes	Progress of Major Manifestations	
		Early Period	Later Period*
19	Dialysis	chest discomfort, cough, chest pain	progressive unconsciousness → coma, convulsion, dyspnea → apnea
20	Dialysis	stupor, pulse(-) → cardiac arrest	
21	Dialysis	chest pain, consciousness disturbance → semicoma, light reflex(-), rales(+)	
22	Dialysis	chest pain, cough, dyspnea	
23	Dialysis	cough, precordial oppression	
24	Dialysis	cough, precordial oppression, chest pain	
25	Dialysis	cough, precordial oppression	
26	Dialysis	precordial oppression, headache, transient unconsciousness, rales(+)	
27	Dialysis	cough, dyspnea, precordial oppression	
28	Dialysis	chest pain, cough → coma, convulsion	
29	Craniotomy	blood pressure(-), extrasystole(+), bubble sound(+)	coma, light reflex(-), rales(+)
30	Craniotomy	cardiac arrest, blood pressure(-)	rigid extremities, anisocoria, coma
31	Craniotomy	drop of blood pressure, extrasystole, bubble sound(+)	cyanosis, unstable blood pressure, light reflex(-), convulsion, coma
32	Craniotomy	bradycardia, drop of blood pressure, bubble sound(+), semicoma	
33	Neck surgery	respiratory distress, bubble sound	dyspnea, coma, impaired dilated pupil, hemiplegia
34	IVH	dyspnea, sampling of air from right heart	

* For cases 21 to 28 and 32, HBO was administered early so there are no descriptions of later manifestations.

misdiagnose the cause of such a disorder because of the delayed recovery from the effects of anesthesia, perfusion, or cardiac surgery itself.

Contrary to this, in 9 cases of arteriography and 2 cases of hemodialysis, air infusion was confirmed in cases 5, 9, 10, and 11, and in the other 5 cases it was much easier to establish a diagnosis because there were no other factors to be considered, as stated above. All symptoms could be easily diagnosed because the air bubbles lodged in the carotid artery circulation. If air embolized one common carotid artery, relatively slight disturbances of consciousness or transient unconsciousness was most typical. Other common manifestations included incomplete or complete hemiplegia, aphasia, visual disturbance, or hemianopia.

In 2 cases of hemodialysis in patients 10 and 11, air was pumped into an artery during dialysis with high pressure through an internal or external shunt on the left forearm. Air was then thought to move in reverse through brachial, axillar, and subclavian arteries against the blood flow. Furthermore, part of the air might have reached the basilar artery through the vertebral artery and caused a fatal result. In such cases, symptoms were severe and progressed rapidly. Disorder of consciousness resulted in deep coma; general convulsions started immediately after air embolization and were accompanied by anisocoria, delayed light reflex of the pupils, and respiratory distress, all suggesting air bubbles within the circulation involving the cerebellum and brainstem region. This concept seemed to explain why the prognosis of 3 cases of arteriography (cases 7, 8, and 9) was so severe. Although many factors should be taken into account, the varied manifestations of postoperative disorders of cardiac surgery could be well understood as a complex of symptoms originating from cerebrum, cerebellum, and brainstem lesion.

Pulmonary Air Embolism

In Table 4, the major manifestations of 16 cases of pulmonary air embolism are shown. In 10 cases, patients 19 to 28, air was pumped into the venous line from an extracorporeal circuit during hemodialysis. In 4 cases (29 to 32), air was sucked from an open cranial sinus during craniotomy with the patients in upright sitting position. In case 33, air was insufflated by the same possible route; from an open neck vein during surgery for cervical hemangioma and lymphangioma. In the one case of IVH, air might have been sucked continuously through the night from a disconnected IVH catheter, but the exact timing could not be determined. The next morning a nurse recognized the difficulty, the patient complained of dyspnea, and the diagnosis was confirmed when 2 to 3 ml of air was obtained from the right heart through the intact IVH line.

Contrary to cerebral air embolism, except for the 1 case of an IVH accident, air inflow was visible and completely confirmed in all cases. That is, in those cases of hemodialysis, air infusion was usually found by the medical staff. Even in the case of cranial surgery, diagnosis was established by the sound of air being sucked into the open vein, auscultation of air bubbles

sounds through a stethoscope placed on the chest, abnormal echo-on-echo detector, and sampling of air through a CVP catheter positioned in the right heart. Accordingly, these cases (Table 4) show in detail the actual clinical course of pulmonary air embolism.

The most commonly seen symptoms with pulmonary air embolism were cough and chest pain similar to pleural irritation, followed by precordial discomfort and respiratory distress. If the amount of sucked air was large, as seen in cases 29, 31, and 32, sudden drop of blood pressure and frequent extrasystoles and bradycardia were seen. These symptoms seemed to be brought on by acute cardiac failure due to severely decreased cardiac output and a secondary decrease of coronary blood flow.

In almost all cases, consciousness disorders were due to hypoxic damage to the brain. This was caused by impaired cerebral blood flow secondary to low cardiac output. On the other hand, abnormally elevated pulmonary arterial pressure might have opened arteriovenous shunts of lung or a patent foramen ovale, and then air bubbles would enter the left heart and manifest as cerebral air embolism.

Diagnosis of pulmonary air embolism was also supported by auscultating discontinuous rales and an increased pulmonic second sound, and by diffuse shadows on chest x-ray. Figures 1 to 4, show improvement of these diffuse shadows on chest x-ray by repeated HBO (case 31, Table 1).

HYPERBARIC OXYGEN THERAPY

For these 34 cases, HBO was administered without exception as the first-choice therapeutic procedure. Almost all cases required intensive care for respiratory and circulatory support even during the period of HBO. HBO was administered in a multiplace chamber with the assistance of the medical staff (1). Hyperbaric oxygen was administered as early as possible under 3 to 3.8 ATA for no more than 90 min, depending on the severity of symptoms. From the 2nd d after admission, 2 ATA of HBO for 75 min was given in the morning and 3 ATA for 90 min in the afternoon and repeated everyday during the first 7 d. Following that, HBO therapy was modified according to the progress of the patient. If results were favorable with HBO, physical therapy was usually introduced. Other supplemental procedures, such as administration of steroids or depressants for cerebrospinal pressure (D-mannitol or glycerin) were introduced when judged indispensable. Heparin was employed in very few cases.

RESULTS

Results are summarized on Tables 5 and 6. Table 5 shows the relationship between the results of HBO treatment and the time elapsed between symptoms and the start of HBO.

Among 9 cases of arteriography, the start of HBO was delayed severely in

Fig. 1. A 36-yr-old male patient of hemangioblastoma on right hemisphere of cerebellum. This chest x-ray was taken before operation.

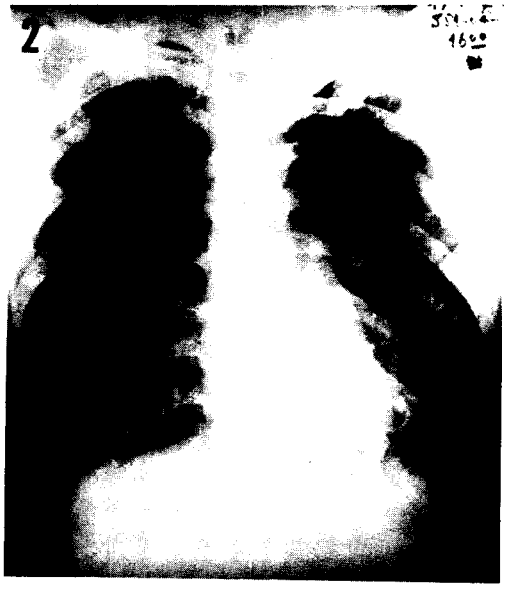

Fig. 2. Five hours after air was sucked from injured cranial sinus during craniotomy in upright position. Diffuse shadow has already been recognized on both lung fields. X-ray taken before the first HBO treatment.

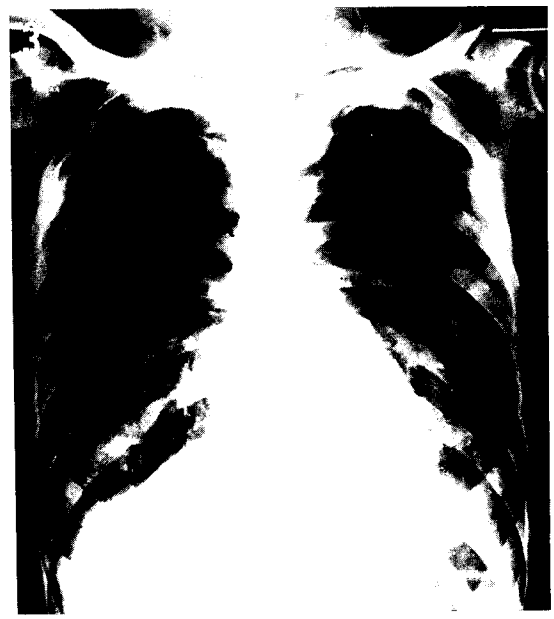

Fig. 3. The day after the operation. HBO has been administered 3 times, and diffuse shadow on lung has gradually disappeared.

Fig. 4. Two days after operation. After 4 HBO treatments, this chest x-ray shows that the lung is almost normal.

Table 5
Time Required Until HBO and Overall Results

Causes	Cases		Time Required until HBO, h	Overall Results			Patterns
				Excellent	Fair	Dead	
Arteriography							
CAG	6		2 - 30	4	2	0	cerebral
Subclavian a.	2		6 - 23	0	1	1	cerebral
Coronary a.	1		2 - 3	0	0	1	cerebral
Open heart surgery	7	6 cases:	20 - 27	2	1	3	cerebral
		1 case:	120	0	1	0	
Dialysis	12	3 cases:	20 - 25	3	0	0	2 cerebral
		8 cases:	0.5 - 6	7	0	1	and
		1 case:	40	1	0	0	10 pulmonary
Craniotomy	4		2 - 5	2	0	2	pulmonary
Neck surgery	1		24	1	0	0	pulmonary
IVH	1		uncertain	1	0	0	pulmonary
Total	34			21	5	8	

3 cases. For example, case 5 was one in which air inflow could be confirmed, but it took 30 h to transfer the patient from a distant hospital. Case 3 showed only visual disturbances and there was no evidence of unconsciousness. As a result, diagnostic evaluation was directed toward another possible disorder, and 19 h elapsed before the patient was referred for HBO treatment with suspected cerebral air embolism as a possible cause. Case 7 involved arteriosclerosis obliterans (ASO) of the upper extremities. This patient had just received a radiologic examination including arteriography. A catheter was advanced from the right femoral artery to the left brachial artery. Just after completion of arteriography, the patient became unstable and hemiplegia was recognized on the right side. First, he was misdiagnosed as acute cerebral infarction and 22 to 23 h elapsed before he was suspected to have cerebral air embolism. Although the employment of HBO was unusually delayed for these 3 cases, all of them improved except one with a slight left hemiplegia.

Table 6
Overall Results of HBO According to Patterns of Air Embolism

Patterns	Causes	Cases	Excellent	Fair	Dead
Cerebral	Arteriography	9	4	3	2
	Open heart surgery	7	2	2	3
	Dialysis	2	2	0	0
Subtotal		18	8	5	5
Pulmonary	Dialysis	10	9	0	1
	Craniotomy	4	2	0	2
	Neck surgery	1	1	0	0
	IVH	1	1	0	0
Subtotal		16	13	0	3
Grand total		34	21	5	8

On the other hand, of those who received HBO within 2 to 5 h after the accidents occurred patients, 1 and 6 had residual left hemiplegia and/or major speech disturbances, and patients 8 and 9 died.

The first symptom in 7 cases of open heart surgery was prolonged unconsciousness, and it usually took a full day to eliminate other factors that cause neurologic damage such as unstable postoperative hemodynamics, the effect of anesthesia, unbalanced cardiopulmonary bypass, and other invasive effects of surgery. In these cases, the start of HBO was usually around 20 h after open heart surgery. In patient 17, persistent low cardiac output syndrome prevented early transportation and the first HBO was administered 120 h (5 d) later. Among these 7 patients, 4 survived and 3 (cases 12, 13, and 16) died. Of survivors, 2 (patients 15 and 18) showed excellent recovery, but patient 14 had a motor disturbance of the left leg and patient 17 showed motor impairment of the left arm and some memory disturbance. These 2 were judged as fair responses. As for the 3 deaths, patient 12 showed temporary cardiac arrest during chest closure and another cardiac arrest 40 h later did not respond to resuscitation. Patient 13 demonstrated prolonged hemodynamic instability after operation, and large amounts of vasoconstrictor agents were needed to maintain blood pressure. In this case, ventricular fibrillation occurred around 48 h postop, and the patient died 90 h after surgery. Patient 16 died 6 d after surgery due to unstable hemodynamics and repeated general convulsions. In these cases, low cardiac output seemed to be the most prominent factor causing death.

Among 12 cases related to hemodialysis, 2 patients (10 and 19) received HBO after all other treatments were ineffective, so HBO started 25 h after

embolization but recovered completely. Once these results were known, almost all subsequent patients were transported between 30 min to 6 h after the accident occurred, and were satisfactorily resolved, except case 20 in which a massive amount of air infused into the right heart cavity and occluded blood circulation almost instantaneously. Twenty minutes after air embolism from hemodialysis, cardiac arrest occurred during the ambulance ride to the hyperbaric institute. Although this patient was resuscitated after 2 h of treatment, he died 18 h later.

In 4 cases of craniotomy in the upright sitting position, air entrance was identified and all received HBO within 13 h after the accident, but 2 patients died. Among the 2 deaths, patient 30 showed cardiac arrest with ventricular fibrillation just after air was sucked from the open venous sinus, and it took many hours to regain normal blood pressure. In patient 31, more than 80 ml of air was aspirated from the right heart cavity through a CVP catheter, and it required about 90 min to stabilize blood pressure. In these 2 cases, severe hypoxia occurred during the period of poor circulation, resulting in irreversible damage to the CNS and finally death. Judging from these results, the time factor from accident to beginning of HBO seemed to have a minimal affect on prognosis. Good results could be expected even if the start of HBO treatment was moderately delayed.

Table 6 shows the overall results according to the difference in pattern of the air embolism. Those related to arteriography, cardiac surgery, and 2 out of 10 hemodialysis resulted in cerebral air embolism. All of the other cases were due to pulmonary air embolism.

Among those 16 cases of pulmonary air embolism, 13 had excellent results and 3 died. Thus, the resolution rate was 81% and the mortality rate was 19%. On the other hand, in the group with cerebral air embolism, 13 out of 18 patients benefited somewhat, and 5 died. The resolution rate of this group was 72% and the mortality rate was 28%. In the former group, in almost all cases, air inflow was clearly identified and the time lag between the embolism and HBO therapy was short. This resulted in a good resolution rate, compared to the latter group.

Mortality rate of cerebral air embolism was somewhat higher than mortality from pulmonary air embolism, because in almost all arterial cases it was difficult to identify the insufflation of air bubbles. Even in severe cases of cerebral air embolism however, HBO provided favorable results for the victims, who otherwise might have remained in a prolonged vegetative state. Based on these results, HBO appears to be effective for iatrogenic air embolism.

DISCUSSION

The circumstances of iatrogenic air embolism may be hard to grasp accurately, because sometimes it may cause legal problems. Many cases will be treated and never appear on the statistics. Individual reports can be found, but

there are few systematic reviews of the topic. Peirce (2) summarized previous reports of cerebral gas embolism that were considered to be iatrogenic accidents. Noteworthy is the report of Stoney et al. (3), which dealt with the incidence of air embolism in open heart surgery. During the 6-yr period from 1972 to 1977, those responding to his questionnaire performed 347,819 operations using a pump oxygenator. The incidence of arterial air embolism was 429 (0.11%); there were 92 deaths (21%) and 61 permanent injuries to CNS (14%). Mills and Ochsner (4) reported, during 8 yr (1971 to 1979) that among 3620 cardiopulmonary bypass operations in a single institution, there were 8 instances of massive air embolism (0.22%). They included 5 additional cases from other institutions, and reported mortality rate was 4 out of 13 (30%). According to these reports, the incidence of such unfavorable complication seems to be low (1 or 2 per thousand). However, if we take account of such cases that have not been reported because the results were not fatal and left only slight neurologic impairment, the real incidence may be greater. In fact, some medical staff attending dialysis centers for renal failure patients say that minor accidents were not so rare (personal communication). Even though the incidence may be relatively low, medical personnel should be acquainted with how to deal with victims of air embolism, because it often leads to a fatal result.

Until 1964, in the United States which has a long history of diving medicine research, serious decompression sickness was treated by air recompression therapy starting at 6 ATA, which required 19 to 38 h before return to surface pressure. Later, the treatment plan was modified to provide alternative administration of oxygen and air at 2.8 ATA during decompression. Decompression time was reduced to between 2.5 and 5.5 h. In 1973, Kindwall (5) reported a case of massive air embolism stemming from open heart surgery. He treated the patient with a brief exposure to 6 ATA followed by HBO, and stressed the superiority of this method. Due to the wide use of monoplace chambers and because both oxygen and air supply cannot be provided separately, continuous HBO was introduced afterward for the treatment both of decompression sickness and air embolism. In 1974, Hart (6) reported HBO at 3.0 ATA as being superior to former recompression therapy.

Of interest is the study describing the pressure at which infused air bubbles disappear in vivo. In 1967, Waite and Mazzone (7) placed a lucite calvarial window in the cranial bone of dogs. Air bubbles were infused from the carotid artery. The changes in the occluded local circulation were examined. According to their observations, air bubble reduction was noted between 1 and 2 ATA and all bubbles were removed and circulation restored by 4 ATA. This report showed that high environmental pressure alone compressed air bubbles. Other reports suggested that sole employment of increased air pressure was not enough to eliminate all the effects of air embolism.

For example, Brown et al. (8) showed gas volume and dimension change according to Boyle's law: when environmental pressure reaches 4 ATA, volume of air bubbles will be reduced 75%, i.e., become one quarter the original

volume, but the sphere diameter is reduced by only 37%, or 63% of the original diameter. Even when pressure is increased to 6 ATA, sphere diameter will reach only 45% of the original volume and it is far more difficult to reduce the diameter to 50%. Here exists the significance of HBO. In addition to compression of air bubbles, which helps to remove obstructive effects, HBO can replace nitrogen with oxygen and hasten reabsorption of bubbles. Grulke and Hills (9), using their experimentally prepared animals, showed direct visual evidence that oxygen breathing, even at 1 ATA, can produce rapid and progressive dissolution of air emboli. This result suggested to us the benefit of combined use of oxygen and high pressure, because the greater the pressure gradient between air and oxygen, the easier bubbles can be resorbed. Furthermore, HBO can improve secondary hypoxic damage resulting from the blocked circulation. Among 34 cases, several showed uncertain benefit with the first HBO treatment. However, after the 2nd and 3rd treatment favorable effects were evident even in these cases. This clinical course seemed to indicate that the damage of air embolism could be improved continuously within 2 or 3 d.

In Japan, the Japanese Society for Hyperbaric Medicine (JSHM) established Safety Standards for HBO in 1969, which have been accepted as an authorized guideline for installing and administering hyperbaric facilities, and also for performing the clinical practice of HBO (10). Among indicated disorders for HBO contained in those standards, air embolism was accepted as an acute condition based on the above-mentioned rationale. Based on the authors' experience, the following conclusions can be drawn:

1. For acute and severe air embolism which requires adjunctive treatment, a multiplace chamber is preferred.
2. Three ATA with 100% pure oxygen is recommended for the treatment.
3. When pure oxygen is administered, 1 treatment with HBO should not exceed 3 h.
4. According to the severity of the illness, HBO can be repeated within the same day with an appropriate surface interval.

Although the efficacy of HBO has never been compared with that of recompression therapy, no detrimental effects were experienced in treating iatrogenic air embolism using the above protocols. Because air embolism is included in the accepted indications for HBO therapy (11), early employment of HBO is definitely indicated for this disorder. As Warren et al. (12) stated, the pathogenesis of the ill effects of air embolism "can never be attributed solely to the space occupying effect of the air bubbles" but if it is not treated quickly it may later produce vascular endothelial edema damage, platelet aggregation, abnormal capillary permeability, loss of vascular autoregulation, edemas and finally, "no-reflow-phenomenon," even after occlusive air bubbles are diminished. These secondary changes lead to irreversible degeneration of involved tissue.

However, in our series, a number of cases took many hours to establish a final diagnosis or did not allow for transfer of the patient in the acute phase due to unstable hemodynamics.

Fries et al. (13) produced cerebral air embolism by injecting various amounts of air or oxygen into the carotid arteries of dogs, using slow or rapid infusions. He demonstrated in autopsy that almost all animals that were killed 48 h after gas injection exhibited visible air bubbles in pial vessels. This fact suggests that, once infused, air bubbles will persist within the embolized blood vessels for a long time and trigger secondary damage. As shown in the tables, all cases of open heart surgery were diagnosed within 24 h after surgery; even these cases obtained good results and demonstrated reduced mortality. Mader and Hulet (14) reported that a cerebral air embolism which was treated by HBO 29 h after the accident recovered completely. Therefore, even cases where a full day is lost should not be excluded from HBO treatments. The time limit allowed for such delayed cases may be around 24 h, regardless of the background.

The key point for achieving good results in treating accidental air embolism is to reduce the time lag between the accident and the start of HBO. For this purpose, early establishment of an accurate diagnosis and rapid transportation of the patient to a HBO facility are strongly recommended. However, there is another problem: lack of propagation. Peirce (2) stated that even today, some physicians refer their patients unwillingly to HBO, not expecting any beneficial outcome from the treatment, and regard HBO as the treatment of "last resort."

Inasmuch as the employment of a multiplace hyperbaric chamber is advisable for severe cases, medical chambers, including monoplace and multiplace, should be distributed uniformly nationwide. Furthermore all medical personnel should know where and how to obtain HBO treatment for their patients. These are not medical but indispensable points which seriously affect the clinical outcome.

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HYPERBARIC OXYGEN THERAPY IN 72 EYES WITH RETINAL ARTERIAL OCCLUSION

Y. Miyake, M. Horiguchi, M. Matsuura, S. Awaya, K. Sakakibara, H. Takahashi, and S. Kobayashi

Retinal arterial occlusion is an acute ischemic eye disease with poor visual prognosis. We previously reported that some patients with this disease were treated effectively by hyperbaric oxygen therapy (HBO), and we studied the mechanisms of HBO's effect on the ischemic retina using electrophysiologic techniques (1-3).

In this paper, we report on the 72 eyes with retinal arterial occlusion that underwent HBO during the past 13 yr in Nagoya University Medical Hospital.

MATERIALS AND METHODS

Seventy-two consecutive patients (51 men, 21 women) with retinal arterial occlusion were treated by HBO. Among the 72 patients, 53 had central retinal arterial occlusion and 19 had branch retinal arterial occlusion. Most patients were hospitalized during the treatment period. HBO was applied once or twice a day at 2 or 3 atmospheres absolute (ATA) for 2 h, depending on the severity of the ophthalmologic findings. HBO was continued every day except Sunday until the visual functions stabilized. In addition to HBO, vasodilators were prescribed for all patients, and stellate ganglion block (SGB) for 13 patients, by injecting 5 ml of 2% carbocaine.

RESULTS

The patients' visual acuity (VA) before HBO was plotted against that after HBO, as shown in Fig. 1. To evaluate visual results, we used the

following criteria. Visual acuity (VA) of eyes in the effective treatment category improved two levels or more on the VA scale shown in Fig. 1. Under these criteria, treatment was proved effective in 32 eyes (44%) and ineffective in 40 eyes (56%).

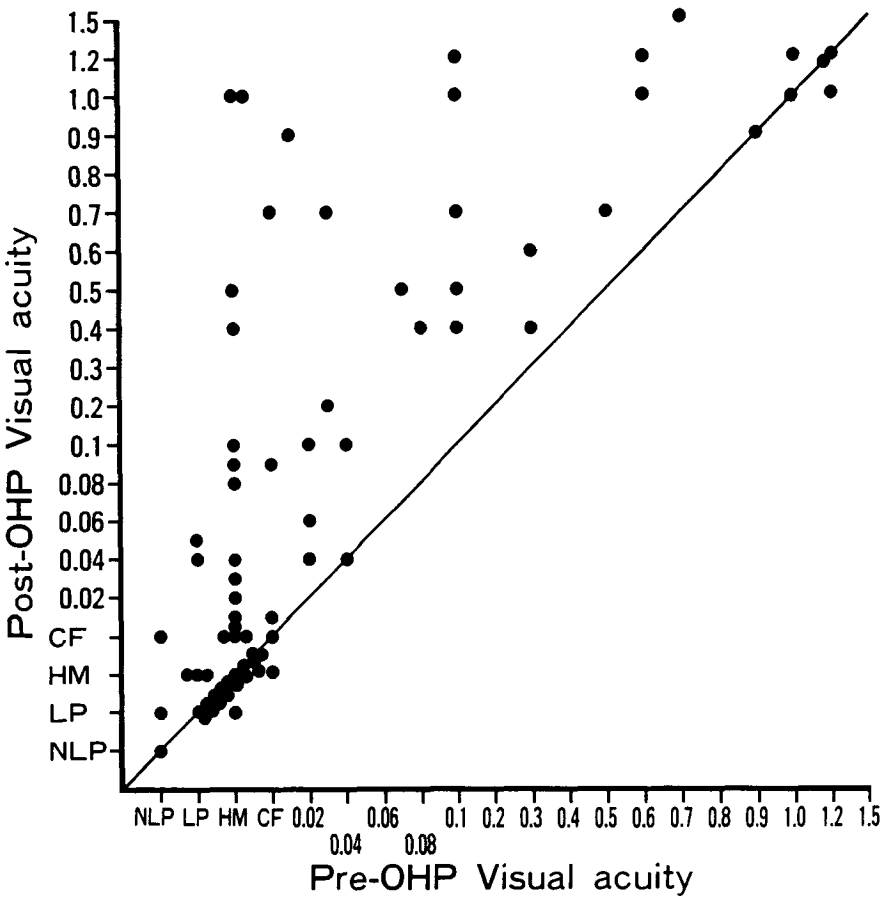

Fig. 1. A comparison of pre-HBO visual acuity (horizontal axis) and post-HBO visual acuity (vertical axis) in 72 patients with retinal arterial occlusion. CF = counting fingers, HM = hand motion, LP = light perception, NLP = no light perception.

We analyzed the age of the patients and the duration of retinal arterial occlusion before HBO was applied as possible prognostic factors in treatment outcome. Figure 2 shows the results.

In patients aged 50 yr or older, 21 of the 53 eyes (40%) were effectively treated. In the group of patients younger than 50 yr, 11 of the 19 eyes (58%) were effectively treated. Although this difference was not statistically

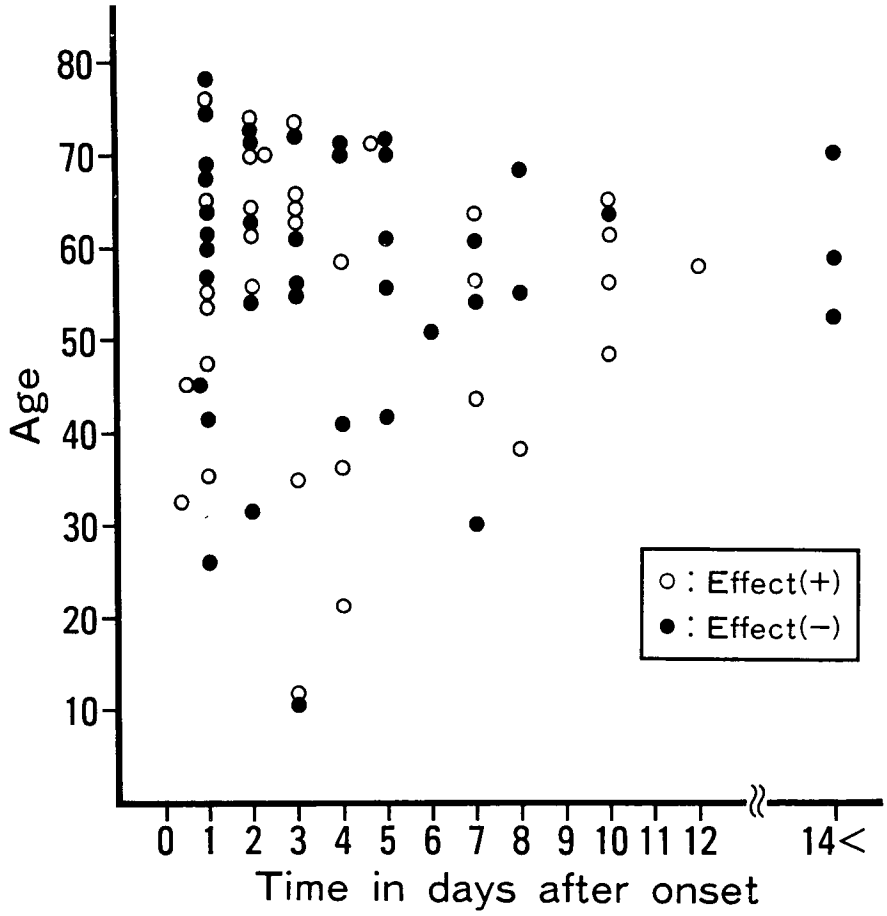

Fig. 2. Treatment outcome in 72 patients with retinal arterial occlusion by age and duration after onset before HBO. The vertical and horizontal axes indicate the patient's age and the elapsed time before treatment, respectively. Open circles indicate effective treatment and closed circles indicate ineffective treatment.

significant, there was a tendency for HBO to be more successful with younger patients than with older ones.

In all but 3 patients, HBO was applied within 12 d after the onset of retinal arterial occlusion. In these 3 patients, who had relatively longstanding retinal arterial occlusion, HBO was not effective. In the other patients, there was essentially no correlation between the duration after the onset and the results of treatment.

Thirteen of the 72 patients underwent SGB in addition to HBO. We compared the results of treatment by HBO alone with those of HBO and SGB. Of the 13 patients who had HBO and SGB, 7 (54%) were treated effectively. On the other hand, of the 59 patients who had HBO alone, 25 (42%) were treated

effectively. Although this difference was not statistically significant, the result suggests that the combination of HBO and SGB can work more effectively than HBO alone in improving visual acuity.

DISCUSSION

In the human retina, oxygen is supplied by two independent vascular systems, the central retinal artery and the choroidal artery. The central retinal artery supplies oxygen primarily to the middle and the inner retinal layers, whereas the choroidal artery supplies oxygen to the outer retinal layers.

The rationale for giving HBO therapy to patients with retinal arterial occlusion is based on a study done by Dollery et al. (4). They found that, under HBO, the choroid alone could supply oxygen to almost the entire retina, even when the central retinal artery was completely occluded. It is postulated that this treatment relieves the retinal anoxia temporarily by supplying oxygen from the choroid, and therefore could be effective unless the retinal arterial occlusion has been permanent or the retinal tissues have already suffered irreparable damage. Our results suggest that the therapeutic prognosis of HBO may be influenced more by the severity of the circulatory disturbance than by the duration of the occlusion, as shown in cases where HBO was applied within 12 d after the onset. Indeed, we found that when the electroretinogram showed a reduced a-wave in addition to a reduction of the b-wave and oscillatory potentials, HBO was ineffective regardless of the time that had elapsed before treatment (3). Since the a-wave amplitude reflects the state of choroidal circulation (5), this may indicate that intact choroidal circulation is necessary for this therapy to be effective.

To eliminate possible confusion with the effects of other treatments or with spontaneous recovery, we previously reported on 4 patients examined while under HBO (3); the 3 who responded favorably showed the following features (3). The first examination of visual acuity and visual fields, performed 10 min after the desired pressure was reached, showed a marked improvement. In the following 60 min, while the patient was under HBO further improvement was slight. This result indicates that HBO per se plays a great role in improving visual function (6).

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HYPERBARIC OXYGEN AS AN ADJUNCT TO THERAPEUTIC LUNG LAVAGE IN PULMONARY ALVEOLAR PROTEINOSIS

E. M. Camporesi and R. E. Moon

Lung lavage was introduced as a therapeutic modality by Ramirez-R in 1965 (1), and it represents the only effective treatment for symptomatic patients with pulmonary alveolar proteinosis (PAP) when progressive deterioration of ventilatory function develops. The procedure requires general anesthesia and intubation of the trachea with a dual-lumen tube to separate the two lungs and allow filling and rinsing one lung with saline, while the other is being ventilated with oxygen. General techniques for the lavage have been reported (2). One of the most challenging problems during the anesthetic management of these patients is the hypoxemia that may result during one-lung ventilation. In fact, despite the use of 100% O_2, gas exchange abnormalities in the ventilated lung and blood shunt through the saline-filled, lavaged lung may contribute to arterial blood desaturation. Extracorporeal membrane oxygenation (ECMO) has been employed during this period to improve oxygenation (3), but it is not a simple technique. It requires systemic heparinization even during partial cardiopulmonary bypass and surgical preparation of large vessels (usually femoral artery and vein) for vascular access. Another technique described for improving oxygenation requires percutaneous insertion of a balloon-tipped catheter in the pulmonary artery of the lung being filled with saline to occlude transiently the lumen of the vessel with inflation of the balloon (4). This requires fluoroscopy for positioning of the catheter, which cannot always be successfully implemented, and the catheter may migrate during the procedure. We describe here an alternative technique to prevent hypoxemia during lavage, using hyperbaric O_2 to maintain oxygenation during one-lung ventilation. This technique was used successfully during 11 separate lavage procedures in 5 patients.

METHODS

Patients were selected for lung lavage under hyperbaric conditions on the basis of poor oxygenation while breathing room air: an arterial $Po_2 \leq 50$ Torr while breathing air spontaneously with both lungs was considered too low to allow prolonged exposure to one-lung anesthesia. An additional test was designed to produce reversible hypoxia in the lung to be lavaged. This lung was filled with air and then the bronchial lumen clamped for 5 min; the other lung was simultaneously ventilated with 100% O_2. If the arterial Po_2 was less than 60 Torr at the end of this 5-min period, we would perform the procedure at pressure. Alternatively, in 1 case the first lung was lavaged at 1 ATA, but Po_2 values at the end of this procedure were too low to warrant lavage of the second lung at 1 ATA, and compression to 1.61 ATA was begun.

Informed consent of the patient was obtained, and the procedure was approved by our Institutional Review Board. The procedure was performed inside a large hyperbaric chamber. Anesthesia and paralysis were provided with benzodiazepine, ketamine (3.5 mg/kg induction; followed by constant infusion of 50-100 $\mu g \cdot kg^{-1} \cdot min^{-1}$, and pancuronium or metocurine administered intermittently to maintain muscle relaxation. The degree of paralysis was monitored with conventional techniques. A left-sided double lumen tube (Bronchocath, 37 or 39 F) was placed in the trachea and isolation of the two lungs demonstrated with an underwater seal. The patients were continuously ventilated with O_2 and maintained supine. Standard monitoring was implemented along with invasive arterial pressure and periodic arterial blood gas (ABG) determination. Monitoring signals were transmitted through the chamber hull, processed on the outside, and displayed to the anesthesia team through a porthole. ABG was measured inside the chamber with electrodes calibrated at the pressure of measurement. Calibration for O_2 and CO_2 was performed at the depth of measurement with known gas mixtures bracketing the physiologic values expected. Each ABG value measurement was preceded and followed by a calibration gas determination. Before chamber compression a Pa_{O_2} value was measured while ventilating both lungs with 100% O_2 at 1 ATA. The required depth of compression was then estimated using both room air and the 100% Pa_{O_2} values as a guide. Bilateral myringotomies were performed on the anesthetized patients before compression. At depth, sterile normal saline at 37°C was used with 30 cm of pressure head to flow in and out of the lavaged lung. Total saline volumes ranged from 8 to 19 liter/lung, depending on the amount of proteinaceous material in the effluent flow. Vigorous chest percussion by a physiotherapist was performed during expiration of the saline. After completion of the lavage and an adequate Pa_{O_2} was ascertained, the chamber was decompressed. The depth-time exposure was not sufficient to induce decompression sickness in the denitrogenated patient or in the medical team. However, as a precaution, all the medical crew breathed O_2 via BIBS masks during decompression.

RESULTS

Results are summarized in Table 1. Each line records the information pertinent to lavage of an individual lung. Bilateral lavages were performed sequentially during procedures 1, 4, 7, 8, 9, 10, and 11. All Po_2 values are in torr. Pa_{CO_2} and pH remained within an acceptable range. With one exception (procedure 8) Pa_{O_2} values measured at pressure were all higher than pretreatment room air measurements (173 ± 63 vs. 43 ± 3 Torr, mean ± 1 SEM). We calculated for each PA_{O_2} at pressure the corresponding 1 ATA value with the assumption of a constant Pa_{O_2}:PA_{O_2} ratio (5) and assuming a respiratory quotient of 0.8 for PA_{O_2} estimate. These values are reported in Table 1 as Calculated Pa_{O_2} at 1 ATA.

No complications occurred in the procedures or in the hyperbaric exposures. All patients were discharged with a satisfactory Pa_{O_2} value during air breathing.

DISCUSSION

Arbitrarily, it may be said that a Po_2 of 60 Torr is safe because O_2 saturation at that Po_2 is usually greater than 90%. In 10 of 16 cases, Pa_{O_2} would have been less than 60 Torr had the procedure been done at 1 ATA. In the 3 cases in which Po_2 would have been less than 60 Torr despite hyperbaric oxygen, the 1 ATA Po_2 would have been less than 25 Torr. We propose that hyperbaric oxygen is a useful and safe means of preventing hypoxia during therapeutic lung lavage in patients at risk. The use of increased ambient pressure may therefore allow a longer time for lavage and the use of larger volumes of saline in patients for whom Pa_{O_2} would be marginally safe during the procedure.

Table 1
A Summary of Hyperbaric Exposures During Lung Lavage For Pulmonary Alveolar Proteinosis in 5 Patients

Procedure	Name Date Age	Lavaged Side	Pa_{O_2} Air 1 ATA	Pa_{O_2} 100% O_2 1 ATA	Pressure, ATA	Time at Pressure, min	Pa_{O_2} at Pressure	Calculated Pa_{O_2} at 1 ATA	Pa_{O_2} After Decompressing	Time to Extubation, h	Pa_{O_2} at Hospital Discharge
1	K.I. 11/30/83 34	R	46	146	1.61	80	90	52	210		
		L			1.30	40	61	45	133	11	72
2	K.I. 6/22/84 35	R	29	81	1.91	115	78	38	70	14	
3	K.I. 6/22/84 35	L	54 Suppl. O_2	265	1.61	35	683	407	280	4.5	53
4	K.I. 2/26/85 36	R	36	280	1.61	45	66	39	82		
		L			1.61	45	106	62	141	5	61
5	R.R. 2/10/84 44	R	47	285	2.0	140	48	22	285	10	
6	R.R. 2/14/84 44	L	43	205	1.91	45	335	166	200	6.5	71

7	R.G. 6/4/85 47	R	46	90	2.0	43	180	84	72		
		L		72	2.0	41	80	37	215	3.7	85
8	R.G. 4/10/86 48	R	58	136	2.0	55	41	18	410	19	67
		L	1 ATA								
9	C.L. 6/6/86 38	R	53 100% O_2 10 cm PEEP	275	2.0	40	44	20	245		
		L	245		2.0	40	78	36	215	3 days	78
10	C.L. 7/30/86 38	R	84 225 35% O_2		1.91	37	344	164	54		
		L	54		1.91	25	145	69	180	3	75
11	M.D. 7/10/86 36	L	35	395	1.0						
		R	155		1.61	71	64	37	280	0.5	78

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EARLY OBSERVATIONS ON THE USE OF ADJUNCTIVE HYPERBARIC OXYGEN THERAPY IN THE TREATMENT OF THERMAL INJURY

P. E. Cianci, G. J. Petrone, J. Ross, R. L. Shapiro, H. Lueders, and H. Lee

Thermal burns represent a serious management problem for the surgeon and a devastating injury to the patient. Each year there are more than 70,000 patients injured severely enough to warrant admission to a burn center in the United States. There are over 12,000 deaths per year from burns. The acute injury is a dynamic process characterized by a nonuniform wound showing a central zone of coagulation, surrounded by a region of stasis, bounded by a region of hyperemia (1). At the time of injury some tissues are totally coagulated; others are seriously damaged but salvageable. An intense, inflammatory reaction leading to rapid edema formation, increased microvascular permeability, and sluggish blood flow/stasis results in thrombosis, progressive dermal ischemia, and advancing necrosis (2). This progressive ischemia process may increase by a factor of 10 during the first 48 h after injury (3) and is responsible for the ongoing tissue destruction seen in thermal injury (4). Control of dermal ischemia is, then, a goal of therapy.

The basic problems in repair of burns include susceptibility to infection, prolonged healing, and excessive scarring (1). Infection is greatly increased due to loss of the integumentary barrier to invasion, the ideal substrate provided by the burn wound, and the compromised or obstructed microvasculature which prevents humoral and cellular elements from reaching burned tissue. Qualitative and quantitative deficiencies in the immune system, manifested by decreased circulating polymorphonuclearcytes, defective polymorphonuclear killing, and diminished levels of immunoglobulins, are additional problems with host defenses (5-7).

Regeneration within the burn wound cannot take place until equilibrium is reached, an event delayed by the ongoing damage as the burn wound

extends its margins due to failure of the surrounding tissue to provide oxygen and nutrients necessary to sustain viability and cell function (1). Prolongation of this healing process leads to excessive scarring (8). The therapy of burns is directed to minimizing edema, prevention of dermal ischemia, preservation of marginally viable tissue, enhancement of host defenses, wound closure, and functional rehabilitation. A significant body of animal data clearly supports the efficacy of hyperbaric oxygen in the treatment of thermal injury. A reduction in fluid requirements, less conversion of partial-to-full thickness injury, improved microcirculation, reduction in edema, faster epithelialization, less inflammatory response, and safety of treatment have all been documented (9-13). The additional observations of increased neutrophil killing in the presence of an elevated O_2 tension is a promising application of adjunctive hyperbaric oxygen therapy to the later treatment of burns (14).

Clinical experience has paralleled laboratory observation. Wada et al. (15) noted faster healing of burned miners being treated for carbon monoxide poisoning from a coal mine disaster. Hart et al. (16), in their randomized, double-blind study, observed a reduction of fluid requirements, reduced healing time, and an improvement in mortality. Grossman and his colleagues (17-19) and Wiseman and Grossman (20) have amassed a very large clinical series showing reduction in hospital stay, deaths, and complications. Waisbren et al. (21) reported a small, retrospective series showing decreased renal function, decreased circulating PMNs, and an increase in positive blood cultures. Neither a deleterious nor a salutory effect on mortality was observed. The amount of skin grafting, however, was markedly reduced (1237 ± 1465 cm^2 vs. 5035 ± 1882 cm^2 in the non-HBO group). Preliminary observations at our burn center (22) are in agreement with the work of Hart and Grossman and show a significant reduction in length of hospital stay and cost of therapy when HBO-treated patients are compared to those who did not receive this additional therapy. We report here our experience in severely burned patients treated with adjunctive hyperbaric oxygen therapy as part of a comprehensive program of burn management at a burn center.

PATIENT POPULATION

All patients ages 14 to 42 suffering burns over 40 to 80% of their total body surface area (TBSA) were included in the study. There were 6 patients in the non-HBO control group and 5 patients in the hyperbaric oxygen-treated group. The mean age in the non-HBO group was 33.3 yr, with a range of 14 to 42 yr. The mean burn surface area was 49% total body surface, with 26% felt to be full thickness injury. The range was 40 to 60% total body surface and 7 to 50% full thickness injury. Inhalation injury was present in 4 of the 6 patients. There ware 8.7 operative procedures, with a range of 3 to 13. The length of stay averaged 111 d, with a range of 47 to 184 d. In the hyperbaric oxygen-treated group the mean age was 24.6, with a range of 20 to 30 yr. The mean burn surface area was 58%, with a full thickness injury felt to be 28%.

The range of total body surface area burned was 40 to 80%, with full thickness injury of 10 to 50%. Inhalation injury was present in 4 of the 5 patients. The mean number of operative procedures was 4.6, with a range of 2 to 7. Length of stay averaged 70 d, with a range of 47 to 95 d (Table 1).

Table 1
Comparison of Control and Hyperbaric Oxygen-Treated Groups

	Non-HBO	HBO
Age, mean	33.3	24.6
Range	14-42	20-30
BSA, mean		
TBSA, %	49	58
FT, %	26	28
Range, TBSA	40-60	40-80
FT	7-50	10-50
Inhalation injury	4/6	4/5
Operative Procedures, mean	8.7	4.6
Range	3-13	2-7
LOS, mean	111	70
Range	47-184	47-95

METHODS AND MATERIALS OF ADMINISTRATION OF ADJUNCTIVE HYPERBARIC OXYGEN THERAPY

Oxygen was administered in a monoplace (Sechrist) chamber pressurized to 2 atmospheres for 90 min (plus descent and ascent time) twice daily. Patients were ventilated and monitored as appropriate when undergoing therapy. Medications and fluids were administered through the hull of the chamber as required. No changes were made in fluid protocols because of hyperbaric oxygen treatment. Patients received 40 U of Vitamin E, P.O. before each oxygen treatment. Evaluation of patients on a daily basis included review of pertinent laboratory data, including complete blood count, arterial blood gases, renal and liver function test, chest x-rays, blood cultures, and other appropriate laboratory studies as indicated. Physical examination included vital signs, cardiopulmonary, and middle ear status. Particular attention was given to temperature greater than 100°F and appropriate measures were taken to control fever. Patients were not treated within 2 h after tubbing as temperature control during this period was a problem. Wounds were inspected frequently with the burn center team to assess progress.

ECONOMIC ANALYSIS

The mean cost of care for the non-HBO control group of patients was $292,000, with a range of $114,000 to $602,000. The daily cost of care averaged $2,630/case. In the hyperbaric oxygen-treated group, the mean hospital bill was $197,000/case, with a range of $110,000 to $318,000. The daily cost averaged $2,820. The savings per case in the hyperbaric treated patients was $95,000. The cost of hyperbaric oxygen therapy averaged $17,500 in the severely burned patients, with a range of $9,000 to $25,000 (Table 2).

Table 2
Economic Analysis

	Non-HBO	HBO
Cost of care, mean	$292,000	$197,000
range, thousands	$144-602	$110-318
Daily cost	$2,630	$2,820
Cost with non-survivors	$2,833	—
HBO-treated cost, mean		$ 17,500
range, thousands		$9-25
Savings with HBO per case		$ 95,000

DISCUSSION

The treatment of thermal injury with adjunctive hyperbaric oxygen therapy is not widely utilized. However, the nature of the burn injury, intense inflammation leading to vascular permeability; rapid edema formation with compromised circulation and sluggish blood flow; and stasis, resulting in progressive thrombosis and dermal ischemia with an end result of tissue necrosis, seem to be beneficially affected by adjunctive hyperbaric oxygen therapy. It would be logical to utilize a modality of treatment that can attack these problems directly (9, 11, 12). An additional consideration may be the preservation of high energy phosphate bonds necessary to sustain cell viability and capillary integrity (23).

Early observations at our burn center (22) are in agreement with the work of Hart and Grossman and show a significant reduction in length of hospital stay and cost of therapy when HBO patients are compared to those who did not receive this additional treatment. In our series of severely burned patients we have been impressed with the results of therapy in reducing edema, preventing conversion of partial-to-full thickness injury, and reducing the number of surgical procedures which has resulted in a shorter hospital stay and more rapid functional recovery. We are additionally gratified with the excellent results obtained in burned faces, hands, and ears where salvage of

tissue that might otherwise have been last has been the rule. Our experience in this small series encourages us to continue the use of adjunctive hyperbaric oxygen therapy in the treatment of thermal injury, particularly in those instances where the patient can be referred for treatment within the first 72 to 96 h.

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BURNS TREATED WITH ADJUNCTIVE HYPERBARIC OXYGEN THERAPY: A COMPARATIVE STUDY IN HUMANS

A. K. C. Niu, C. Yang, H. C. Lee, S. H. Chen, and L. P. Chang

Ketchum et al. (1) reported that healing time was reduced 30% in burned rabbits treated with hyperbaric oxygen (HBO) compared to untreated rabbits, and the overall infection and bacterial growth rates were reduced. Hart et al. (2) and Wells (3) showed that fluid requirements in the first 24 h after injury in serious burns treated with HBO were 35% less. Comparing data from the U.S. National Burn Information Exchange to those from the Sherman Oaks Burn Center, Grossman (4) found a decrease in the mortality of burn victims, and noted a reduction in hospital stay and an improvement in morbidity. Grossman and Yanda (5) demonstrated that when using HBO in burns there was less edema, fewer tracheal secretions, and faster epithelialization of second degree burns compared with non-HBO-treated patients. The same was true with regard to skin graft donor sites. Experimenting with dogs, Wells and Hinton (6) showed that burned dogs treated with HBO required less fluid which resulted from less plasma extravasation and improved oxygenation.

PATIENTS AND METHODS

The use of HBO as an adjunct to treatment in burns was introduced at the Naval General Hospital, Tsoying, Taiwan, in 1980. Because some hospital surgeons elected to use HBO as an adjunct, whereas other surgeons did not due to its controversial nature, we were afforded the opportunity to study a group of burn patients treated with HBO and to compare them with a non-treated group, managed concomitantly at the same burn unit and treated with the same surgical procedures and ancillary therapy. Although patients were not randomly allocated to treatment and control groups, the 2 groups were essentially similar as can be seen from the age ranges and surface area of the burns.

To date, more than 400 burn cases have been treated with adjunctive HBO therapy. However, since some physicians did not prescribe HBO according to protocol and because of transfer delays, only 266 cases could be used for purposes of this study. Both treated and control patients included in the study were treated since 1981.

Patients treated with HBO adjunctive therapy were placed in the upper part (Igloo chamber) of a wet pot (Fig. 1) in an atmosphere of 2.5 ATA oxygen for 90 to 120 min 2 or 3 times for the first 24 hours, and then once or twice a day for adults; children were treated at 2 ATA in oxygen for 60 min once or twice a day. The percentage of oxygen in the atmosphere of the chamber was observed to reach 90% by the end of each treatment. Patients were transferred to a monoplace chamber when their condition stabilized. Children treated in the Igloo chamber could be accompanied, if necessary, by a parent through the entire course of therapy. Critical patients were more easily managed in the Igloo chamber with ventilation, etc. Grounding and fire proofing of the Igloo chamber was considered critical.

Using urinary output as a guide, the Parkland fluid resuscitation formula could be reduced by 30% and, when hypertonic solutions were used, fluid requirements could be reduced by an additional 20%. Colloid was started 12 to 16 h after injury. Blood gases were corrected before the patient entered the chamber. An attempt was made to keep the patients' temperatures under 38.8°C, but in septic patients whose temperatures rose higher, the pressure was lowered to 2 ATA of oxygen to prevent oxygen toxicity. Myringotomy was performed as necessary.

Dependent areas, such as the posterior thighs, the arms, and the back, with definite full-thickness burns were debrided intermittently, starting on the 2nd d when the patient's general condition permitted. Not more than 15% total body surface area (TBSA) was removed at one time. Homografts, if available, or biological dressings were applied to the exposed areas after the dead skin had been excised. Using HBO, tangential excision could be delayed for up to 5 d, especially in the areas of suspected deep, partial-thickness burns. Operative time was limited to no longer than 2 h for each surgical procedure, and the temperature of the operating room was kept above 27°C so that heat loss and surgical stress could be reduced to a minimum.

Contraindications, such as viral infections, untreated pneumothorax, serious otolaryngologic conditions, and claustrophobia, were ruled out or controlled. We did not put septic patients transferred from other hospitals or patients who had not been stabilized into the chamber for test treatment. The remainder of the burn management of patients treated with HBO is basically the same as that for non-HBO-treated cases.

RESULTS

Comparing 266 burn patients treated with adjunctive HBO therapy with 609 non-HBO-treated cases, mortality was 28 deaths in the HBO group (10.5%)

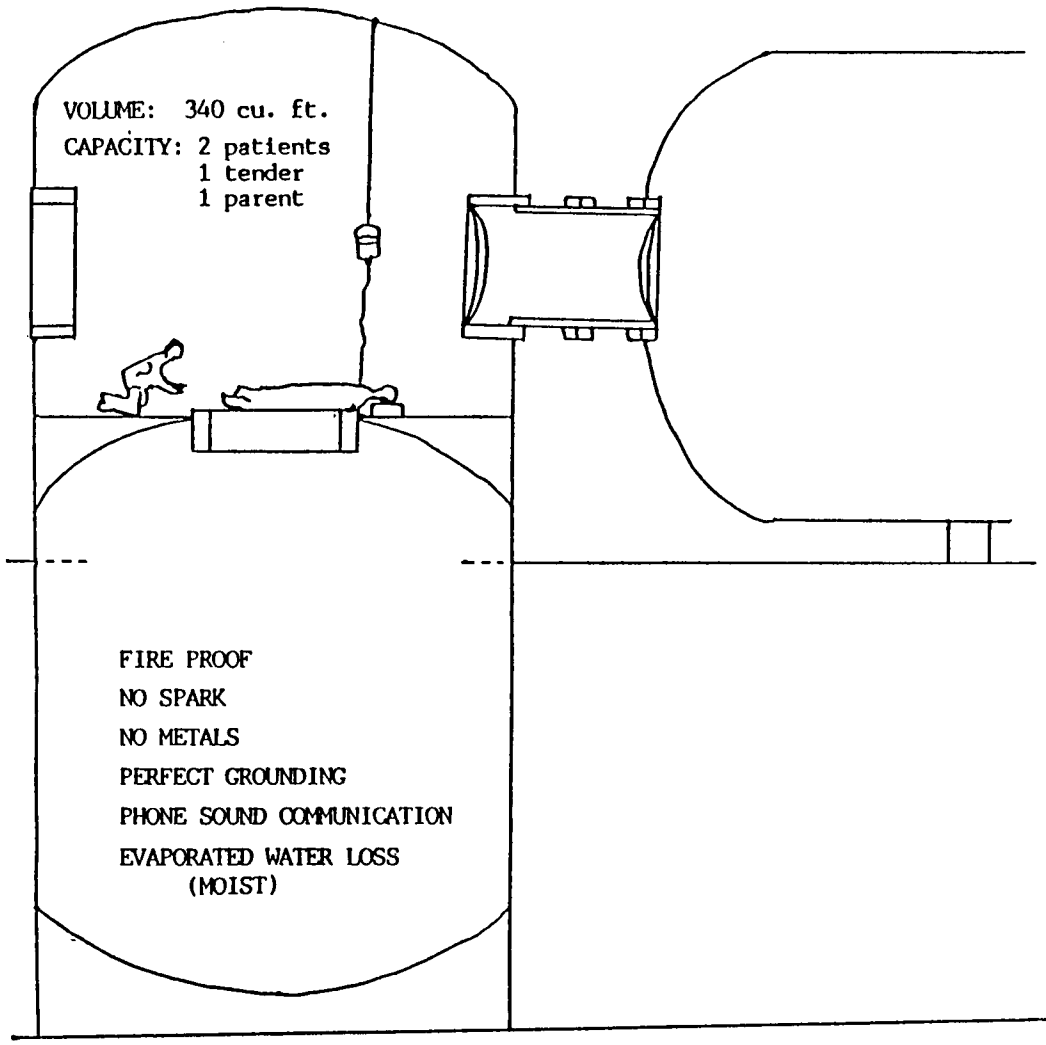

Fig. 1. Burns treated in Igloo chamber.

and 79 deaths in the non-HBO group (13.0%). Mortality rates were almost the same ($P > 0.05\%$) (Table 1). The difference in mortality in high-risk patients with TBSA burns of 35 to 70% and aged 15 to 45 yr, showed a statistically significant difference between the HBO and non-HBO-treated groups: 8 deaths in 117 cases (6.8%) in the HBO group vs. 25 deaths in 169 cases (14.8%) in the non-HBO group, giving a highly significant P value of 0.028 using Fisher's exact test. The average number of hospital days in the high-risk patients treated with HBO was less than that of the high-risk, non-HBO group (47 vs. 59 d), but this was not statistically significant ($P > 0.05$).

Table 1
Comparison of the HBO-Patient Group and the Control Group

Parameter	HBO	Non-HBO
Total number of patients	266	609
Age, mean	2-82 (27) yr	7/12-80 (26)yr
TBSA, mean	7-90% (34%)	5-85% (36%)
Deaths*	28 (10.5%)	79 (13.0%)
Patients with TBSA 35-70% aged 15-45 yr		
Number of patients	117	169
Survived	109	144
Deaths**	8 (6.8%)	25 (14.8%)
Hospital days, average*	47 d	59 d

* $P > 0.05$. ** $P = 0.028$ (Fisher exact test).

The mortality rates were high because many patients were not primary referrals and were septic or severely dehydrated on arrival. For this reason and because of the small sample, comparison of the number of hospital days in the 2 groups was not reliable.

Much faster epithelialization and healing of the burn wounds was observed in HBO-treated cases. For example, in treating a group of 10 patients burned in a military accident, we noted that the severely burned cases treated with HBO (about half) were discharged at least 20 d earlier than those in the non-HBO group. The HBO group had burns involving a greater percentage of TBSA (20-30%) compared to the non-HBO treated group (10-20%).

DISCUSSION

By providing better oxygenation and reducing edema, HBO may reopen subcutaneous capillaries earlier and enhance the speed of epithelialization. Therefore, tangential excision may be delayed for up to 5 d until the suspected deep, partial-thickness burns are satisfactorily epithelialized. Without being concerned about partial-thickness burns converting to full-thickness burns with resulting sepsis, we were able to be conservative during the first debridement on the 2nd or 3rd d and the second debridement on the 6th d. With HBO, failure to remove dead skin may cause catastrophic infection later, especially in dependent areas over the posterior thighs and arms.

Adequate excision and homograft covering of the exposed tissue coupled with less chance of partial-thickness burns converting to full-thickness burns gave patients with large burned areas a better chance to survive. To preserve residual dermis, we suggest bloodless tangential excision be performed so that the remaining skin has a better chance to regrow under a covering of silver

sulfadiazine in a well-oxygenated environment.

Not only was the fluid requirement reduced by 30 to 35%, but the hemodynamics were improved in the HBO-treated group. With earlier completion of fluid resuscitation and less generalized edema, there was a more rapid recovery of the mesenteric circulation, as evidenced by an earlier return of bowel sounds. We were able to institute aggressive nasogastric hyperalimentation immediately after the first 24 h or even sooner, which was an aid to the patients' metabolism and host defenses.

Because of difficulties in instrumenting our monoplace chamber and the occasional need to treat mass casualties, we used a 340-ft^3 Igloo chamber above a wet pot, filled with 2.5 ATA of pure oxygen with up to 2 patients, 1 tender, and sometimes 1 parent inside (Figs. 2 and 3). The use of a 100% oxygen environment in a multiplace chamber, however, is inherently very dangerous and is *not* recommended for the standard single- or double-lock facility.

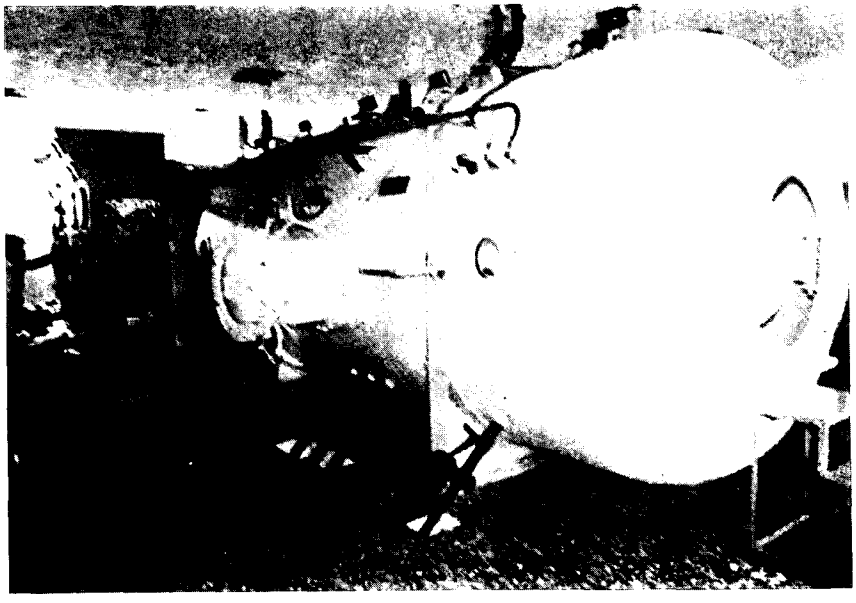

Fig. 2. The frontal view of hyperbaric chamber at NGH Taiwan R.O.C. Burn patients are treated in Igloo chamber connected with the main chamber at the other side.

An accurate evaluation of how much fluid was saved during the resuscitation phase using HBO therapy was not accurately determined in patients who were not primary referrals because of preexisting dehydration. Occasionally, lack of availability of hyperbaric medical personnel precluded optimal

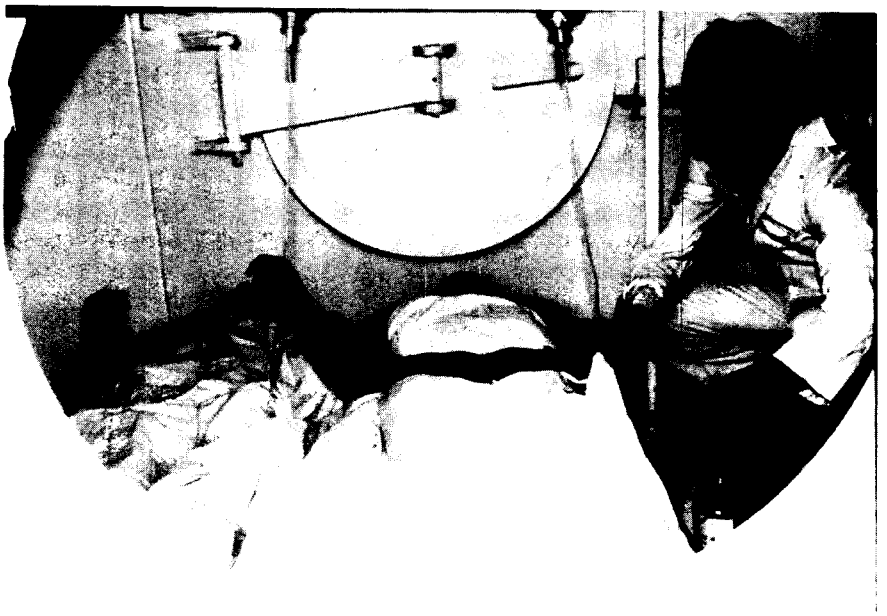

Fig. 3. Patients are being treated in the upper part of Igloo chamber; fluid resuscitation could be manipulated adequately.

3-times-a-day treatment in the first 24 h. However, having treated more than 400 burn patients with HBO, we recommend the following protocol for fluid resuscitation.

1. Initiate fluid resuscitation according to the Parkland formula (one is usually able to subtract 30% from the total amount of volume) or use hypertonic solutions (one can usually subtract an additional 20-30% from the total amount of volume) keeping the sodium concentration approximately 250 meq/liter. Serum sodium levels should be checked every 4 h to keep the sodium level between 145 and 160 meq/liter.
2. Start HBO therapy as soon as possible after emergency treatment is completed.
3. Using hypertonic fluid resuscitation, crystalloid may be replaced with colloid 12 to 16 h after injury. During this period fluid begins to shift back into the intravascular compartment, and probably there is reduced protein leakage because of the vasoconstrictive effect of HBO therapy.
4. The Parkland formula calls for no colloid to be given before the 16th h postinjury unless adequate urine flow cannot be induced with copious fluid infusion. Without HBO, we would suggest colloid not be given earlier than 18 h postinjury.
5. Start nasogastric hyperalimentation at the beginning of the second 24 h, or as early as the 20th h after injury when HBO is used in combination with hypertonic fluid resuscitation. The return of bowel sounds is an indication of initiation of tube feeding.

6. Start fresh blood transfusions on the 2nd d.

Vitamin C, 500 to 1000 mg give daily as an aid to wound healing and parenteral hydrocortisone in smoke inhalation and suspected preseptic patients, did not potentiate oxygen toxicity if blood CO_2 and bicarbonate levels were properly managed. We recommend that patients never be placed in the chamber without good planning or adequate conventional management. Some physicians labor under the misconception that patients benefit from only 1 or 2 HBO treatments. In this situation, success cannot be anticipated.

Only one complication occurred, an eardrum rupture caused by otitis media in a 2-yr-old male with a 52% TBSA scald burn. HBO therapy was interrupted and the problem was resolved after ENT consultation and treatment.

Reviewing our total experience to date, we are convinced that the results of hyperbaric oxygen therapy as an adjunct in the treatment of burns is encouraging. It tends to reduced the mortality and morbidity rates in serious burn injury.

CASE NOTES

Case 1

A 35-yr-old male with flame burns (TBSA 78% and full-thickness burns of 25% TBSA) had impending sepsis with serious local wound infection. He had abdominal distention and a spiking fever on arrival, 8 d after injury. Daily HBO therapy successfully reepithelialized most of the deep, partial-thickness burned areas which very easily could have converted to full-thickness burns due to infection. The rest of the full-thickness burned areas were excised using staged debridement and covered later with mesh graft (Fig. 4).

Case 2

A 53-yr-old British sailor with a 51% TBSA burn, of which 15% was full thickness, resulting from a shipboard fire. He was not expected to survive because of smoke inhalation, subsequent pulmonary compromise, and massive burns. Using aggressive respiratory therapy and HBO treatment, wound healing was accelerated and he was able to survive the critical period. For the split-thickness skin grafts on the patient's back and shoulders, we harvested skin from his legs. The meshed skin was refrigerated overnight and applied early the next morning on the granulating burn. The patient was maintained in a prone or sitting position for the following 24 h with HBO being given at 2.5 ATA twice a day. The graft had 100% take and he was discharged 3 mo. after admission (Figs. 5 and 6).

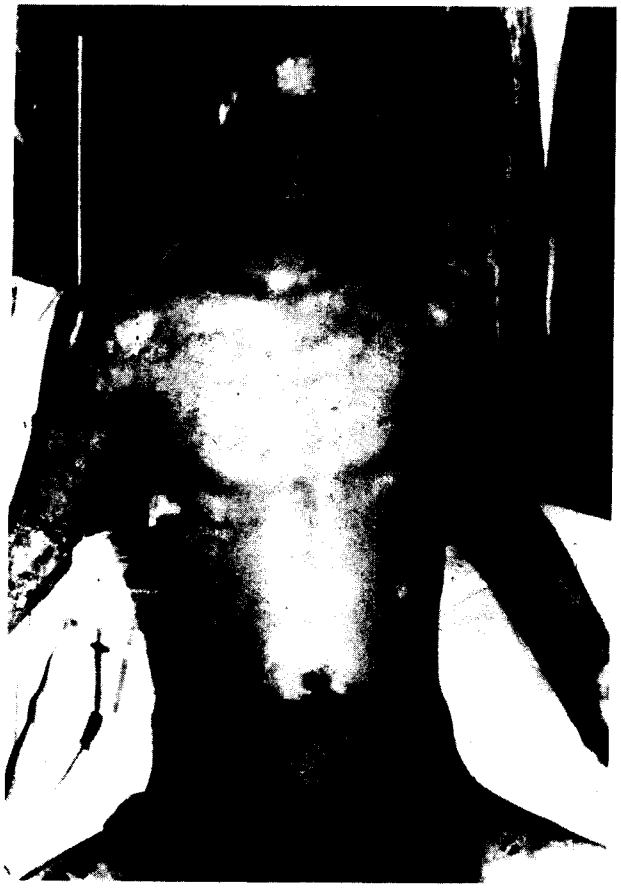

Fig. 4. A 35-yr-old male with TBSA 78%, injured in a dust explosion finally overcame the critical period and survived as the result of HBO adjunctive therapy.

The factors influencing oxygen transport and the local utilization of oxygen still need to be investigated. The effects of high pressure oxygen on cellular metabolism are not completely understood, and how HBO affects hemodynamics and oxygen toxicity at the cellular level during long-term treatment in the presence of "burn toxin" is still unknown. Further animal studies are needed.

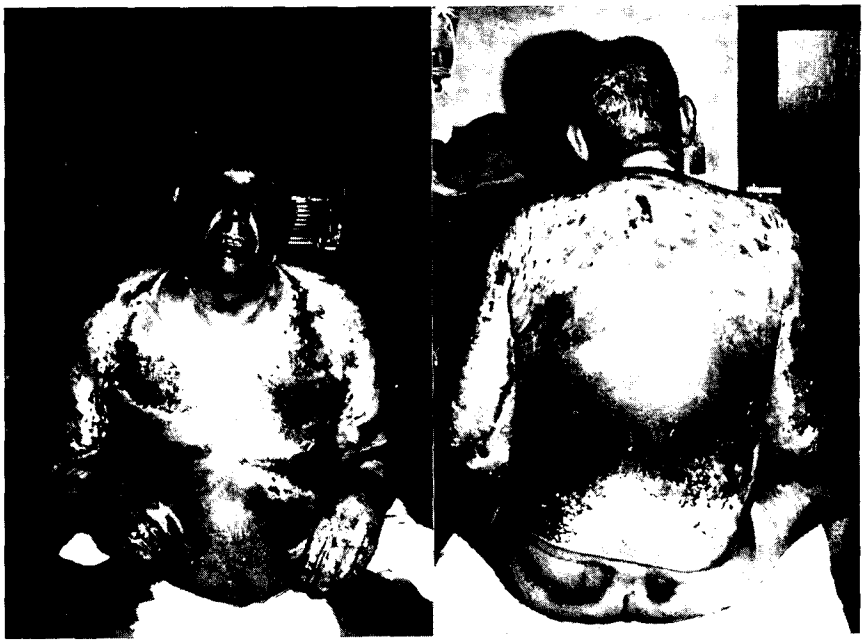

Fig. 5. A TBSA 51% burned case with 15% full-thickness burns over both forearms and back. This 53-yr-old sailor also had pulmonary problems.

Fig. 6. The patient was requested to be in prone or sitting position. The meshed skin was only immobilized by automatic staple. 2.5 ATA HBO twice a day was given.

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CELL INJURY AFTER ACUTE CARBON MONOXIDE EXPOSURE IN THE RAT

L. Marzella, S-H. Cho, and R. A. M. Myers

Carbon monoxide gas is a major environmental hazard in the United States and worldwide (1, 2). Carbon monoxide poisoning accounts for half of the poisoning fatalities occurring each year in the United States. Acute and chronic poisoning with carbon monoxide occurs in many settings such as homes, workplaces, motor vehicles, and during fires. The affinity of carbon monoxide for hemoglobin is much greater than that of oxygen. Carbon monoxide therefore accumulates in the body by binding to the hemoglobin of erythrocytes. A small amount of carbon monoxide also binds to cellular cytochromes primarily in heart and skeletal muscle.

The severity of carbon monoxide poisoning depends on the concentration of the gas in the air and on the duration of the exposure. Physiologic parameters such as cardiac output and respiratory rate affect the uptake as well as the elimination of carbon monoxide by the body. The half life of carbon monoxide varies from 4 to 5 h in a man at rest breathing air. The elimination of carbon monoxide from the body is enhanced by increasing the concentration of oxygen in the inspired air. At hyperbaric pressures of 3 atmospheres, breathing 100% oxygen shortens the half life of carboxyhemoglobin to approximately 20 min in man.

The presence of carboxyhemoglobin causes a corresponding reduction in the capacity of erythrocytes to carry oxygen. In addition carboxyhemoglobin impairs the ability of the hemoglobin to release oxygen to body tissues by shifting the hemoglobin dissociation curve to the left. Carbon monoxide therefore induces injury by decreasing the delivery of oxygen to cells. Virtually all body tissues are affected. In man the most important target tissues are the myocardium and the brain. Hemodynamic and neuropsychiatric manifestations may be seen (3-5). The behavioral performance of experimental

animals is affected by carbon monoxide; the impairment is however usually demonstrable only during the exposure (6, 7). Severe poisoning can cause arrhythmias and electrocardiographic abnormalities, particularly if coronary artery disease is present (8). No specific alterations of myocardium induced by carbon monoxide have been described in man or in experimental animals. The neuropsychiatric dysfunction caused by carbon monoxide has been more studied and characteristic lesions have been described in the cerebrum (9-15). Typically the lesions include necrosis of the globus pallidus and zones of demyelination of the white matter. The location and type of this structural tissue damage is not fully explainable by hypoxic injury. The delayed functional manifestations of cerebral injury induced by carbon monoxide are also not fully explainable by hypoxia. Hyperbaric oxygenation is used in the treatment of severe carbon monoxide poisoning; hyperbaric oxygenation promptly reverses the tissue hypoxia and may prevent the central nervous system sequelae induced by carbon monoxide (16, 17).

In addition to uncertainties about the pathogenesis, the means of quantitating the injury induced by carbon monoxide are lacking. We present here the results of enzymatic and histologic assessment of cell injury in a rat model of acute carbon monoxide exposure. In this model, histologic evidence of cell injury occurred soon after the exposure to carbon monoxide and regressed in a typical fashion. On the other hand enzymatic evidence of cell injury lagged behind the histologic alterations, and in several animals enzyme elevations were present long after the exposure to carbon monoxide.

MATERIALS AND METHODS

Male Sprague-Dawley rats supplied by Charles River, Waltham, MA, were used. The rats were exposed to 1500 ppm of carbon monoxide for varying intervals of time and were killed 1 d, 3 d, 1 wk, 2 wk, or 3 wk after exposure. After induction of ether anesthesia, blood specimens were obtained and the heart, liver, kidney, and brain were fixed in phosphate buffered formalin.

To determine organ damage, the following enzymes were measured in the serum: glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), lactate dehydrogenase (LDH), and creatine kinase (CK). Creatine kinase isoenzymes were determined in selected cases by electrophoretic separation and quantitation of BB-CK, MB-CK, and MM-CK.

Systematic sampling of the tissues was carried out for histopathologic evaluation. After paraffin embedding, sections were prepared and routinely stained with hematoxylin and eosin. Brain sections were also routinely stained with luxol fast blue. Cryosectioning of some brains followed by oil red-O staining was carried out. Evaluation of all tissues was performed in a blinded fashion on the basis of an arbitrary injury-severity score.

One group of animals received hyperbaric oxygenation 5 min after carbon monoxide exposure. The treatment consisted of 100% oxygen at 3 atmospheres

absolute for 1 h at 20°C with a suitable relative humidity.

RESULTS

The Animal Model

Animals of 4 or 11 wk of age were exposed to 1500 ppm of carbon monoxide for 2, 2.5, or 3 h. Carboxyhemoglobin levels between 60 and 65% were achieved. The mortality in the 11-wk-old rats was 13% for 2-h exposure and 50% for 2.5-h exposure. The mortality in the 4-wk-old rats exposed to carbon monoxide for 3 h was 10%. All deaths occurred during the exposure. The survivors in the group with the highest mortality did not show a corresponding enhancement in the severity of the injury. Behavioral testing did not show any abnormalities in the rats examined. The half life of carboxyhemoglobin was 32 min in the rats breathing ambient air. Hyperbaric oxygen decreased the half life of the carboxyhemoglobin to approximately 5 min.

Evaluation of Cell Injury by Measurement of Serum Enzymes

The extent of cellular injury induced by carbon monoxide was estimated by measuring serum levels of GOT, GPT, LDH, and CK at 1, 3, 7, 14, and 21 d after exposure. Glutamic oxaloacetic transaminase levels remained normal. Glutamic pyruvic transaminase levels were slightly (not significantly) elevated at Days 1 and 3 and returned to normal thereafter. Lactate dehydrogenase elevations were found in some animals; the peak elevation occurred at Day 7. Creatine kinase was elevated in some animals at Days 7, 14, and 21 after carbon monoxide exposure. The means of the LDH and CK values were not significantly different between groups by analysis of variance. Measurement of isoenzymes suggested that brain and skeletal muscle were the sources of the CK elevations.

Evaluation of Cell Injury by Light Microscopy

The histopathology in brain, heart, liver, and kidney was evaluated in parallel with the serum enzyme measurements. Systematic evaluation of the brain by routine as well as myelin and fat stains did not reveal any evidence of histopathology. The kidney was also normal. The liver showed a minimal amount of injury consisting of isolated hepatocyte necrosis, occasional accumulation of lipid, foci of inflammatory cells, and increased mitotic figures. These hepatic alterations had disappeared by Day 21.

The heart showed marked alterations 1 d after exposure to carbon monoxide. Foci of mononuclear inflammatory cells were present in the subendocardial region (Fig. 1). In severe cases these foci extended throughout the entire thickness of the myocardium. Isolated single cell necrosis was occasionally identifiable at the light microscopic level. The inflammatory reaction was disproportionately much greater than the amount of necrosis. The myocardial injury peaked at Day 1, and was substantially diminished by Day 7.

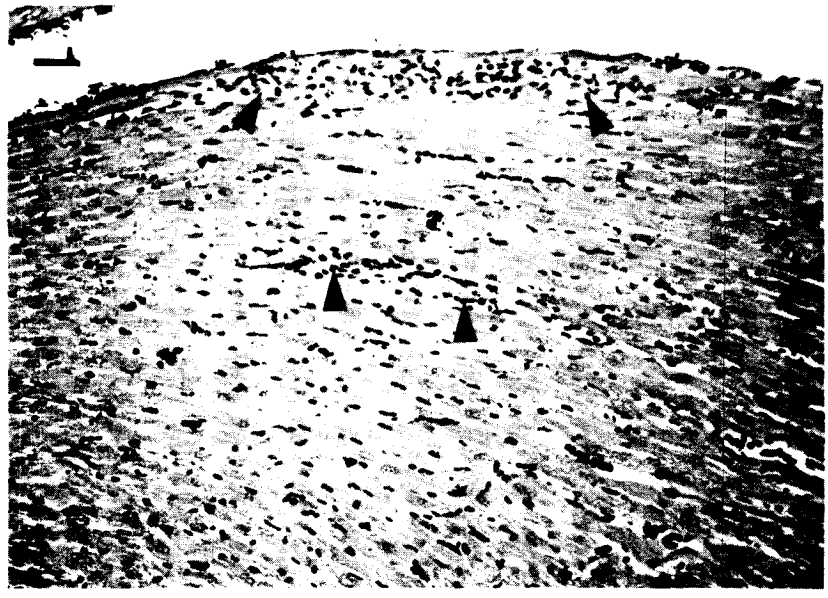

Fig. 1. Light microscopy of myocardium from a rat exposed to carbon monoxide. The animal was exposed to 1500 ppm of carbon monoxide for 2 h and was killed 22 h later. This is the typical appearance of the infiltrates of mononuclear cells (arrowheads) present in the subendocardial region. Occasionally, degenerative changes in isolated myocytes could be seen in these foci of inflammation. Hematoxylin and eosin stain. ×160.

At Day 21, complete healing of the lesions had occurred leaving almost no residual.

Hyperbaric Oxygenation

Treatment with hyperbaric oxygenation (100% O_2, 3 ATA, 1 h) was administered to 17 rats 5 min after exposure to carbon monoxide (1500 ppm, 2 h). The rats were evaluated for up to 21 d after exposure. There were no differences in enzyme levels (GOT, GPT, LDH, CK) or histologic appearance between treated and untreated animals.

DISCUSSION

Carbon monoxide poisoning induces a nonischemic hypoxia which in man affects primarily the cerebrum and the myocardium. The manifestations of this impairment are often subtle and difficult to quantitate. Moreover, the precise series of events, which leads from the poisoning to acute as well as delayed organ damage, is as yet not characterized. For these reasons the development of means to objectively assess injury and to characterize the pathogenesis of the injury is needed.

The injury induced by a single acute exposure to carbon monoxide in the rat differs from human and other reported animal models in that by histopathologic and behavioral assessment we did not detect any evidence of cerebral injury. However delayed elevations in CK and LDH were demonstrated in some of the rats for as long as 21 d after the exposure to carbon monoxide, indicating the presence of persistent cell injury. The other interesting and novel finding was the presence of a remarkable acute inflammatory response in the myocardium. The response at the first time interval examined (24 h) and all later time points involved only mononuclear leukocytes and was disproportionate to the amount of acute cell injury detected by light microscopy as well as by serum enzyme measurements. The myocardial lesions we have found are not typically seen in anoxia or ischemia but are found in injury caused by toxins and other harmful conditions such as hypokalemia (18).

In conclusion, the exposure to carbon monoxide in the model induces acutely little cell necrosis and little or no serum enzyme elevations. A marked inflammatory response occurs acutely in the myocardium. After 1 wk, when the histopathologic changes are subsiding or have returned to normal, elevations in CK and LDH are seen in some of the animals. Therefore, the cellular necrosis induced by the acute exposure in this model is minimal and inflammatory and/or immunologic reactions to the initial insult may be responsible for the persistent enzymatic manifestations of injury. These findings may be relevant to the pathogenesis of delayed manifestation of carbon monoxide injury.

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SESSION 16: CLINICAL DIVING MEDICINE II

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SEVERE REFRACTORY DECOMPRESSION SICKNESS RESULTING FROM COMBINED NO-DECOMPRESSION DIVES AND PULMONARY BAROTRAUMA: TYPE III DECOMPRESSION SICKNESS

T. S. Neuman and A. A. Bove

With the development of a large diving community, more clinical experience and observations have been possible on a variety of forms of decompression sickness. In the sport diving community, where diving practices may not be well supervised and the quality of training of individual divers is uncertain, many types of diving accidents have been observed. These generally follow the lines of decompression sickness (DCS) due to inadequate decompression from moderate depth air dives (1), and cerebral air embolism subsequent to pulmonary barotrauma due to poor diving techniques or to apparent air trapping in seemingly healthy individuals (2).

Among the clinical syndromes associated with diving, there seems to be a third clinical syndrome which we report here that involves pulmonary barotrauma but also demonstrates severe and unexpected symptoms compatible with the diagnosis of DCS. This syndrome appears in divers who complete a moderate-depth air dive within prescribed no-decompression limits from U.S. Navy air tables, and who during the same dive subsequently develop pulmonary barotrauma on ascent. These individuals acquire a severe and diffuse form of DCS that would not have been expected from the dive profile. This has been suggested in an animal model reported by Greenbaum et al. (3). The combined syndrome suggests that the presence of free air in the vascular system may initiate or augment a process of DCS in individuals who otherwise would not have sustained these lesions.

Decompression sickness is usually classified as Type I (predominantly joint pain) or Type II (predominantly spinal cord lesions). Although either variety can be a consequence of any diving activity, more severe DCS is generally associated with larger deviations from accepted decompression protocols or

greater amounts of omitted decompression. In general, prompt treatment of any type of DCS results in a good-to-excellent outcome (4, 5). We present here 5 cases that are all characterized by severe and relatively refractory DCS involving the spinal cord and occurring in conjunction with pulmonary barotrauma and arterial gas embolisms (AGE). We consider the AGE minor in 4 of the 5 subjects because in 2 cases there was no initial loss of consciousness, and in the other 2 cases the signs and symptoms of AGE had resolved spontaneously before recompression therapy began. The subsequent DCS was severe and resistant to recompression therapy even though treatment had begun relatively promptly after the accident. None of the divers had exceeded the U.S. Navy "no-decompression" limits for the depth at which they were diving, and to date none of the cases has had a good neurologic outcome.

Clinical observations from these 5 cases suggest that an alternative to standard treatments for DCS and arterial gas embolism may be needed, because these subjects may deteriorate during therapy and they seem to have a uniformly poor long-term outlook after therapy.

CASE REPORTS

Case 1

A 30-yr-old male sport diver made a scuba dive to 175 fsw. Total dive time was only the time of descent, estimated by the diver and his two partners to be less than 5 min. During ascent, the subject lost control of his buoyancy compensator and rose rapidly and directly to the surface without making a planned, but not required, safety stop for 2 min at 10 fsw. The subject described himself as "weak and dizzy," whereas his two companions described him as "incoherent" and "unable to swim." Calls for help prompted lifeguards to swim out to the trio and the subject was brought to shore. By that time, the above symptoms had resolved but the patient was nevertheless transported to the local U.S. Navy recompression chamber, where a complete neurologic examination by a Diving Medical Officer revealed no objective deficits. The patient complained only of not feeling "quite right."

Due to the patient's subjective complaint and the nature of his dive, a tentative diagnosis of DCS was made and he was recompressed to 60 fsw on 100% oxygen. Upon arrival at 60 fsw, the patient stated he felt entirely normal. The remainder of the U.S. Navy Table VI at 60 ft, the ascent to 30 ft, and the first oxygen period at 30 ft were uneventful; however, at the beginning of the second oxygen breathing period, the patient complained of numbness and inability to move his legs. Neurologic examination revealed marked sensory and motor deficits consistent with physiologic transection of the spinal cord at the level of T4 on the left and T1-2 on the right. No mental status abnormality or cranial nerve deficits were detected.

The patient was immediately returned to 60 fsw on 100% oxygen where no improvement was noted, and after 10 min a U.S. Navy Table IV was started. This provided no significant improvement. Additional adjunctive therapy

during the hyperbaric treatment consisted of steroids, fluids, and heparin. Although improved by physical therapy, 2 yr after the accident the patient remained impotent and required crutches and braces to walk.

Case 2

A 60-yr-old man made a sport scuba dive to a depth of 72 fsw. The total time of the dive measured by the dive master was 22 min. At the end of the dive, the subject did not stop at a 10-ft bar for a planned 3-min safety stop. Upon surfacing, the dive master noted that the subject's movements were "slightly slow" and that he was pale. He was alert and coherent. The dive master swam with the subject to the boat only a few yards away. The subject then climbed the ladder to the boat and immediately collapsed, losing consciousness. He was given oxygen and quickly regained consciousness; however, he appeared to be confused. Physical examination revealed normal vital signs but a right hemiplegia, decreased reflexes, and bilateral conjugate deviation of the eyes to the right. He continued to receive oxygen and was transported to shore (20-30 min) where by that time his mental status had returned to normal; a neurologic survey performed by a physician and consisting of testing reflexes, sensation, and strength was also normal.

He was transported to the nearest hyperbaric chamber, but just before commencing therapy had a seizure which was treated with intravenous diazepam. This markedly decreased his level of consciousness. At the end of recompression therapy using two U.S. Navy Table VI schedules, he was paralyzed below the level of T12. Further testing during subsequent hospitalization revealed a left parieto-occipital infarct on CT scan.

Five years postincident, the patient was impotent and markedly weak in both lower extremities, and psychometric testing revealed defects consistent with the parietal lobe infarct.

Case 3

A 32-yr-old female made a sport scuba dive for 7.5 min to 130 fsw. Just before beginning ascent, the patient had a spontaneous episode of severe coughing. Her ascent, verified by her partner, was at a "standard" rate. Approximately halfway to the surface, the patient had another even more severe paroxysm of coughing but continued her ascent. After this episode, on the surface she reported the onset of right pleuritic chest pain, chest tightness, dyspnea, and dizziness.

She was brought to the local dispensary where the previous symptoms of dizziness, chest tightness, and shortness of breath were no longer present. She was noted to have hyperflexia with clonus of the right patellar and Achilles' tendon and a positive Babinski sign. A chest radiograph revealed no pneumothorax. She was transported to a hyperbaric chamber and recompressed to 165 fsw. Total time from surfacing to the beginning of recompression was 40-45 min.

Repeat examination in the chamber revealed anesthesia bilaterally to the

level of T4 with progressing flaccid paralysis of both legs. While at depth, the sensory level improved to T10, but there was no other improvement in her neurologic status. She remained with bilateral absences of all reflexes in the lower extremities, loss of motor function in the lower extremities, and inability to appreciate pain or light touch. Table VIA progressing to Table IV with multiple repeat hyperbaric treatments produced some slight improvement.

One month after injury, she had sensation down to L1 with dysesthesias below that level. Although able to move her lower extremities slightly, she had profound weakness in all muscle groups. Bladder catheterization remained necessary. One year after the accident, she is able to ambulate but is still markedly weak. Bladder catheterization is still required.

Case 4

A male sport scuba diver made a 70-ft maximum depth dive (average depth 65 ft) for 20 min. He remained on the surface 1 h 45 min and made a second dive to a maximum depth of 60 fsw for 20 min against a heavy current, and descended to avoid it. At a depth of 6 to 8 fsw, he experienced difficulty and surfaced rapidly. By the report of the other divers, he became unresponsive for approximately 20 min. Upon regaining consciousness, the diver described himself as having blurred vision and being unable to move his legs or trunk. After 45 min, the diver was able to walk without assistance, and a short time afterward was able to walk normally. He noted at that time, however, that his thighs and legs were not quite as strong as usual.

Later that evening, the diver developed weakness and paresthesias in his legs and was unable to urinate spontaneously. After 30 h with these symptoms, the diver was able to void with difficulty but felt he had not completely emptied his bladder.

A day later he sought medical attention and received 2 hyperbaric oxygen treatments. The symptom of weakness had resolved with this treatment, but severe paresthesias have persisted 1 yr postincident.

Case 5

(We are indebted to Dr. R. L. Moon, Duke University Medical Center, for supplying details related to this case.) A 42-yr-old male diver made a 60-ft sport dive. After 40 min at depth, he developed nausea and vomited, prompting a rapid ascent to the surface. Upon reaching the surface and attempting to enter the dive boat, he appeared disoriented and rapidly became unconscious. He was evacuated to a local chamber and placed under treatment within 45 min of arriving on the surface. Treatment was to 165 ft using standard U.S. Navy Table VIA. Upon arrival at depth, he regained consciousness but demonstrated a severe and profound paralysis of both upper and lower extremities, slurred speech, and urinary retention. At the end of the treatment regimen, he was noted to have profound quadriplegia, diffuse sensory abnormalities, slurred speech, disorientation, and urinary retention. After two additional treatments with U.S. Navy Table VI locally, the patient was evacuated to a larger medical

facility and treated with repeated Table VI oxygen therapies. Minor improvement of neurologic symptoms was noted after multiple recompression treatments. On the 5th hospital d, the patient developed a cardiac arrest and could not be resuscitated. Postmortem evaluation demonstrated ischemic necrosis of the frontoparietal lobes of the brain and thoracic-lumbar and sacral spinal cord. Massive pulmonary embolism was demonstrated as well.

DISCUSSION

These cases all demonstrate the sequence of apparent pulmonary barotrauma in the course of air dives conducted within what are conventionally considered to be safe limits for no-decompression diving. This syndrome, which might be termed Type III DCS, resulted in the development of severe neurologic DCS which was generally refractory to treatment with either a 60-ft oxygen table or a 165-ft combined air-oxygen table.

An additional case was published in a recent Undersea Medical Society Workshop (6) and is recounted by the victim who is a biomedical scientist. In that case, a similar series of events occurred after a no-decompression air dive and apparent mild pulmonary barotrauma which resulted in a brief period of unconsciousness, followed by progressive and profound quadriplegia over a period of several hours. Although adequate treatment was provided, the subject sustained a severe neurologic deficit and recovered slowly. The combined syndrome resulted in a permanent neurologic deficit.

The development of severe diffuse spinal cord involvement suggests that either a large amount of inert gas moved from a soluble to a free phase or that some other mechanism compromised blood flow in the spinal cord. Additionally, the adherence to presumably safe no-decompression limits in all 5 cases suggests that this severe form of decompression sickness would not have developed but for the presence of pulmonary barotrauma and associated gas embolism.

These cases suggest that pulmonary barotrauma which allows even small amounts of free gas to enter the arterial circulation may result in several events that cause tissue injury: a) the direct occlusive effect of arterial gas embolism in the cerebral circulation; b) a possible seeding of gas nuclei throughout the vascular system with subsequent rapid growth of free gas bubbles in blood and tissue; and c) alterations in structure or function of proteins in the blood occurring at the blood-bubble interface at the site of air embolism. We feel it is unlikely that the entire clinical syndrome we have observed can be explained by the first mechanism alone. Overwhelming evidence (7) indicates that spinal cord damage in DCS is not caused by direct arterial embolization. We believe that the initial symptoms in these cases referable to cerebral dysfunction are best explained by arterial gas embolization; however, another mechanism must be found to explain the later findings of spinal cord dysfunction.

The second mechanism is one way to explain the secondary deterioration

focused in the spinal cord found in these cases. This mechanism could occur only in divers with some degree of gas supersaturation resulting from uptake of inert gas while diving.

The seeding of bubbles into the vascular system from air embolism in this situation could allow rapid growth of bubbles in the venous system as the arterial bubbles pass through the peripheral circulation and into the venous circulation (8). This rapid growth of bubbles in the venous circulation would occlude the drainage of the spinal cord, compromise spinal cord flow, and further augment growth of interstitial bubbles within the spinal cord. Previous studies have demonstrated that spinal cord injury from DCS is due in part to occlusion of the paravertebral vein and reduction of venous outflow from the spinal cord (7), and autopsy evidence from case 5 suggests that massive spinal cord damage occurs in these cases. This lesion is in keeping with the combined venous occlusion and interstitial bubble growth hypothesis. In general, cerebral air embolism that attacks the brain does not manifest symptoms of spinal cord injury (9). However, free gas in the arterial system will allow seeding of small bubbles through the peripheral circulation into the venous system (7) with the possibility of an explosive decompression process because of the introduction of gas nuclei into the vascular system.

The third process, either alone or in conjunction with the second, can also explain the secondary deterioration in the spinal cord found in these cases. Once alterations in the structure or function of proteins occur at the site of arterial gas embolization (10, 11), these altered proteins might, when distributed throughout the body by the vascular system, interact strongly at the site of gas phase separation induced by decompression. It has been demonstrated that gas phase separation does occur in asymptomatic divers (12), thus the altered proteins produced at a remote site, acting additively or synergistically with nucleated gas bubbles forming in the epidural plexus (due to the moderate air dive), might produce a generalized rheologic disturbance. If the major factor in this venoocclusive process were the altered proteins perhaps triggering the coagulation cascade rather than nucleated gas bubbles, the relative resistance to standard recompression therapy could be explained.

Unlike the secondary cerebral deterioration which can follow a lucid period in isolated arterial gas embolism, these subjects developed severe injury to the spinal cord after recovery from cerebral injury and, therefore, the purported mechanism of cerebral edema (13) to explain that process does not seem to be applicable.

Whatever the cause, the combination of arterial gas embolism and supersaturated tissue inert gas seems to produce a clinically distinct decompression syndrome for which we have suggested the term Type III DCS. This form of DCS may require more aggressive treatment than the standard 60 ft of oxygen (U.S. Navy Table VI) or 165 ft combined air and oxygen (Table IV or VIA), which are usually considered to be adequate therapy for DCS or AGE, since the final outcome in these cases has been uniformly poor.

In conclusion, we have observed a previously unreported clinical syndrome

characterized by severe DCS subsequent to AGE after pressure-depth exposures that would not be expected to produce DCS. We postulate that AGE may precipitate or predispose to this form of DCS involving the spinal cord. Treatment of these cases may require nonconventional approaches to achieve an acceptable neurologic outcome.

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NEUROLOGIC SEQUELAE OF DECOMPRESSION SICKNESS: A CLINICAL REPORT

D. F. Gorman, C. W. Edmonds, D. W. Parsons, R. G. Beran, T. A. Anderson, R. D. Green, M. J. Loxton, and T. A. Dillon

Although decompression sickness (DCS) and cerebral arterial gas embolism (CAGE) arising from decompression are known to affect the nervous system (1-6), the neurologic sequelae of these illnesses remain controversial (7-11).

In 1984, a neurologic review program was initiated to study divers treated for either DCS or CAGE by the Royal Australian Navy's School of Underwater Medicine (RAN SUM) at HMAS *Penguin*. The findings of this clinical review are presented.

CLINICAL REVIEW PERIOD 1984 to 1986

During the 18-mo. review (February 1984-August 1986), 88 divers suffering dysbaric illness were treated by recompression at RAN SUM. Only 1 of these divers is involved in litigatory action. All divers were treated in a multiplace recompression chamber and were intravenously rehydrated. Initial treatment was at 2.8 bar absolute pressure breathing 100% oxygen. If this regimen failed to relieve symptoms and signs, the diver was changed to an oxygen-nitrogen gas mixture and compressed further to either 4 or 6 bar. Recurrent or persistent symptoms and signs were treated with daily hyperbaric oxygen exposures, until either resolution had been achieved or the deficit was considered refractory to treatment.

CLINICAL REVIEW PROTOCOL

The neurologic review was divided into clinical neurologic, psychologic,

neurophysiologic, and radiologic categories.

Clinical Neurologic Review

All divers were asked to attend for clinical examinations 1 wk, 1 mo., and 1 yr after their discharge from RAN SUM. They were assessed by a RAN SUM Medical Officer and by a clinical neurologist.

Psychologic Review

A psychologic test battery was administered to the divers presenting for review, 1 wk and 1 yr after discharge. The battery had 3 components: a) the Australian Council of Education Research (ACER) WL test; b) the Benton Visual Retention Test (VRT); and c) a subtest of the Weschler Adult Intelligence Scale, the digit symbol test.

The ACER WL test is a language-based measurement of intelligence quotient, standardized on an Australian population. The Benton VRT is a test of visuospatial perception and memory for recently learned material. It is sensitive to organic brain damage, has high scorer reliability, can be applied quickly, and, most important, can be directly compared with the ACER WL test results. The digit symbol test is another measure of visuospatial perception. In contrast to the Benton VRT it includes an efficiency, rather than a memory component. The tests were scored by a clinical psychologist who was not aware of either the diver's history or the findings of other examinations.

Neurophysiologic Review

The clinical neurologic examination included a 19-lead EEG under 3 conditions: at rest, with photic stimulation, and with hyperventilation. The records were evaluated by 2 neurologists, 1 of whom was not aware of either the diver's history or the findings of other examinations. The EEG records were classified in 3 ways: normal, abnormal, or doubtful.

Radiologic Review

All divers were asked to present 1 wk, 1 mo., and 1 yr after their discharge for a computerized tomographic (CT) head scan, performed without use of intravenous contrast media. The scans were interpreted by a clinical neurologist and by a radiologist who were not aware of either the diver's history or the results of other investigations.

Where appropriate, data were tested by chi-squared analysis.

CLINICAL RESULTS

Eighty-eight divers were treated by recompression at RAN SUM during the 18-mo. period. All had developed DCS or CAGE after a compressed air or oxygen-nitrogen gas mixture dive. One of the 88 was considered to have had CAGE, and 87 to have had DCS. Before any treatment, a neurologic deficit could be detected in 68 (78%) of the 87 divers with DCS.

Resolution of the acute symptoms and signs was obtained before discharge from RAN SUM in 85 (96.6%) of the 88 divers, and in 84 (96.55%) of the 87 divers with DCS. Data from the clinical reviews are summarized in Table 1.

Table 1
Numbers of Divers Showing Abnormal Test Results
1 wk and 1 mo. After Treatment for DCS

Investigation	Total Number	Abnormal Results, 1 wk	Abnormal Results, 1 mo.
Clinical examination	46	10	2 ($P < 0.01$)
Psychologic assessment	61	20	
Neurophysiologic assessment	46	22	8 ($P < 0.001$)
Radiologic assessment	40	8	5

Clinical Neurologic Review

Only 46 divers returned for examination both 1 wk and 1 mo. after their discharge; a 47.7% loss of subjects.

At the 1 wk examination, 10 divers (21.7%) had detectable neurologic deficit on clinical examination. These 10 divers comprised 5 with pyramidal-pathway deficit, 2 with Brown-Sequard spinal cord syndromes, 2 with lower motorneuron weakness, and 1 with a brainstem lesion. The 3 divers who had detectable abnormality at the time of discharge from RAN SUM were still considered to be abnormal 1 wk later.

Although 10 divers had detectable neurologic deficit on clinical examination 1 wk after discharge, only 2 (4.3%) remained abnormal at the 1 mo. review. Both had Brown-Sequard spinal cord syndromes. The reduction in the percentage of divers with clinical neurologic abnormality from 21.7 to 4.3 % is significant ($\chi^2 = 8.18$, $df = 1$, $P < 0.01$).

Psychologic Review

Only 61 divers presented for initial psychologic testing, which represents a 31% loss of subjects. Twenty (32.8%) had a significant deficit, demonstrated by the Benton VRT results when they were compared to the scores expected from the diver's performance on the ACER WL test. This included 7 divers who were initially considered to have had isolated musculoskeletal DCS. Only 8 of these 61 divers have returned for their 1 yr psychologic assessment. All have had normal test results, including 3 who were considered abnormal 1 wk after discharge.

Neurophysiologic Review

Only 46 divers presented for EEG recording both 1 wk and 1 mo. after their discharge; a 47.7% loss of subjects. The 2 neurologists were in agreement on the classification of 87% of EEG records.

One week after their discharge, 22 divers (47.7%) had abnormal EEG records. Most of the abnormal records were characterized by excess slow wave activity. The 22 divers included 5 who were initially considered to have had isolated musculoskeletal DCS.

One month after their discharge, only 8 divers (17.4%) were still considered to have abnormal EEG records. The reduction in the percentage of abnormal EEGs from 47.8 to 17.4% is significant ($\chi^2 = 17.08$, $df = 1$, $P < 0.001$).

Radiologic Review

Only 40 divers had CT head scans performed both 1 wk and 1 mo. after their discharge; a 54.5% loss of subjects.

One week after their discharge, 8 divers (20%) had an abnormal CT head scan. One of these had an arachnoid cyst, 4 had atrophic brain changes, and 3 had areas of abnormal brain density. The 8 divers included 1 who was initially considered to have had isolated musculoskeletal DCS.

One month after their discharge, the CT scans of 5 of these 8 divers remained abnormal. The 3 divers whose CT scans returned to normal were those who showed areas of abnormal brain density on the initial scan. The 5 divers with persistent abnormality showed no progression of defect.

DISCUSSION

The results of a clinical neurologic review of patients with dysbaric illness, after treatment, have been presented. Any data from this series must be interpreted cautiously because of the progressive loss of subjects.

Despite reports that the majority of divers with DCS have isolated musculoskeletal disease (12, 13), 78% of divers with DCS in this series had overt neurologic symptoms and signs, attributable to DCS, before treatment. Also, over half of the 19 divers who were initially considered to have only musculoskeletal DCS demonstrated a neurologic deficit after discharge from RAN SUM (7 with abnormal psychologic tests, and 5 with an abnormal EEG record). These data are consistent with other reports suggesting that most DCS incidents involve the nervous system (2, 5, 6).

The apparent clinical morbidity among this group of divers when they were discharged from RAN SUM (3.4%) was considerably lower than the abnormality frequencies demonstrated 1 wk later. It follows that it is essential to standardize a method of measuring treatment success in any comparison of treatment regimens, particularly when more than one treatment center is involved.

A single, cross-sectional measurement of the prevalence of neurologic deficits in this or any group without control data, makes it difficult to relate

the prevalence to any acute event. However, the natural history of most of the abnormalities presented in this review was for complete remission. It can be argued from this observation that many of the abnormalities were related to the acute episode of DCS. This is particularly true for clinical neurologic deficits and EEG abnormalities, where the reduction in abnormality frequency was shown to be statistically highly significant.

Computer tomography head scans were the least sensitive of these investigations. The relationship of the atrophic and cystic changes reported in 5 of the divers to the acute episode of DCS could not be established by this review. Three divers had abnormal CT head scans 1 wk after discharge, but showed normal scans at 1 mo. These divers had areas of abnormal brain density on the initial scan. Because gas-endothelial interactions do induce disruption of the blood-brain barrier (14-16), it is likely that the initial scan abnormality represented an increase in brain-water content, secondary to DCS.

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AN EVALUATION OF DEXAMETHASONE IN THE TREATMENT OF ACUTE EXPERIMENTAL SPINAL DECOMPRESSION SICKNESS

T. J. R. Francis, A. J. Dutka, and J. B. Clark

Glucocorticoids in general, and dexamethasone in particular, have been used for some years in the treatment of decompression sickness (DCS) affecting the CNS (1). The rationale for the use of steroids in this condition is unclear, but seems to be based on the assumption that they would reduce any increase in intracranial pressure, acting in a similar manner to that described in the treatment of cerebral tumors and closed head trauma (2-7). To date, the use of steroids in CNS DCS has only been justified by anecdotal evidence of success, there being no report of a controlled clinical trial or experiment that has demonstrated their unequivocal value.

The role of glucocorticoids in the treatment of head and spinal cord injury is increasingly controversial. Following early reports of their beneficial effect, some more recent studies have demonstrated that glucocorticoids have no significant influence on the outcome of head injury (8-10), cerebral infarction (11), or spinal cord injury (12, 13). A further report suggests that glucocorticoids may potentiate ischemic injury to neurons (14). In view of this, a study was undertaken to assess the efficacy of dexamethasone in a conventional, anti-inflammatory dose (0.5 $mg \cdot kg^{-1}$) on the recovery of an experimental animal model of spinal cord decompression sickness.

METHODS AND MATERIALS

Animal Preparation

Conditioned adult male mongrel dogs (9.5 to 13 kg) were sedated with xylazine (2.2 $mg \cdot kg^{-1}$, s.c.) and atropine (0.05 $mg \cdot kg^{-1}$) before anesthesia was induced with i.v. administered pentobarbital (13.5 $mg \cdot kg^{-1}$). After 20 min, half the initial dose of pentobarbital was given, and subsequently anesthesia was maintained with a dose of 1.3 $mg \cdot kg^{-1}$ every 20 min. The dogs were then

ventilated, prepared, and their physiologic parameters monitored in the manner described by Leitch and Hallenbeck (15). The measured parameters included: aortic blood pressure (AoP), mean arterial pressure (MAP), right ventricular pressure (RVP), cerebrospinal fluid pressure (CSFP), ECG, end tidal P_{CO_2} ($ETco_2$), and core temperature (via rectal probe). In addition, arterial blood was sampled periodically for the estimation of blood gases and hematocrit. Spinal cord function was assessed by the use of somatosensory evoked potentials (SEPs). Core temperature was maintained between 37 and 38.5°C by varying the temperature of a heated pad upon which the dog rested.

Somatosensory Evoked Potentials

Experience with the model in this laboratory has shown that it is the SEPs recorded from the lumbar spinal cord that are most frequently reduced in amplitude in association with DCS (15-17). In consequence, only lumbar SEPs, generated by the stimulation of both peroneal nerves, were recorded. Eliminating measurement of the cortical evoked response meant that during preparation, surgical trauma to the dog was significantly reduced. The SEPs were recorded from a pair of insulated, 1-mm stainless steel electrodes introduced percutaneously, in a bipolar configuration, into the interspinous spaces at T13-L1 and L1-2 and advanced until their tips were firmly embedded in the spinal lamina of the adjacent vertebra. Impedance of the spinal electrodes was less than 4 kΩ. Stimulating electrodes were pairs of stainless steel needles mounted on double banana plugs and were introduced percutaneously into each hind leg, astride the neck of the fibula, where the peroneal nerve was palpable. The evoked potentials were collected using a Nicolet CA 1000 clinical averager. The stimulus was 100 V (about 3 times motor threshold) and 16 mA for 100 μs at a rate of 2.3/s for 128 epochs. After 150- to 3000-Hz bandpass filtering, the signal was analyzed for 30 ms with a sensitivity of ± 100 μV. This method enabled a maximum rate of interrogation of each side of the spinal cord of about once every other minute.

Analysis of the Evoked Potentials

Leitch and Hallenbeck (18) demonstrated that in this model SEPs could be adequately analyzed by the summation of the peak-to-trough amplitudes. The Nicolet CA 1000 was interfaced with a Digital PDP 11-70 computer. After the recording of each SEP, the data were transferred to the computer and graphically displayed, visually inspected, and compared with the display on the CA 1000 to confirm the accuracy of the data transfer. The record was then stored and divided into two so that the first half contained all the SEP data and the second half contained just the noise associated with the signal. The mean and standard deviation of the peaks and troughs in the second half of each record was then calculated. Any peak-to-trough amplitude in the 2- to 15-ms latency range that exceeded 3 standard deviations of the noise signal were recorded as "significant" and identified on the graphic display of the record. The sum of these peak-to-trough amplitudes was finally calculated. The

operator could include in the calculation any peaks or troughs that were of a lower amplitude but of the right form, or exclude obviously artifactual potentials. In practice, this override was rarely required. The program was run so that while each SEP was being collected the previous record was analyzed.

Collection of Control Data

After preparation, each dog was moved into a compression chamber (Bethlehem Corporation Size 66 x 30 in.) and control measurements of arterial blood gases, end tidal CO_2, CSFP, MAP, and SEPs for each side were recorded. Some variation in the SEPs occurred early in each experiment. It is likely that because the animals were not paralyzed, this was due to some movement of the stimulating electrodes relative to the peroneal nerves as the hind limb twitched in response to each stimulus. In consequence, records for each side were collected until the sum of the peak-to-trough amplitudes of four consecutive SEPs varied by no more than 8%. The mean of these summed amplitudes was then calculated and used as a reference for all subsequent SEPs recorded from that side, so that after analysis a value of each SEP was displayed that expressed the summed amplitudes as a percentage of the control value.

The Generation of Decompression Sickness

Immediately after collection of satisfactory control data, the dogs were submitted to the same dive profile as that used by Leitch and Hallenbeck (15): namely, compression to 300 fsw (10.0 bar) at 75 $ft \cdot min^{-1}$, 15 min spent on the bottom, followed by decompression at 60 $ft \cdot min^{-1}$ to 60 fsw and at 45 $ft \cdot min^{-1}$ to the surface. SEPs were recorded at least 3 times from each side while on the bottom and then continuously from alternating sides with the commencement of decompression. The onset of DCS was diagnosed when the sum of the SEP amplitudes reached 80% or less of control for two consecutive readings from either or both sides.

Treatment

Once DCS was diagnosed, the lesion was allowed to consolidate for 15 min before recompression. At the start of each experiment the dog was allocated a treatment on the basis of its weight (to keep the mean value of each group comparable). The controls were recompressed to 60 fsw on 100% O_2 with a 5-min air break each half hour for a total treatment time of 2 h. The test group received the same treatment with the addition of dexamethasone sodium phosphate 0.5 $mg \cdot kg^{-1}$ i.v. given at the start of recompression.

The hypotension frequently associated with DCS was treated with 50 ml i.v. boluses of lactated Ringer's solution. Additional Ringer's and 8.4% sodium bicarbonate were given i.v. as required to maintain the hematocrit and pH, respectively, as close as possible to control values. The experimental protocol of Leitch and Hallenbeck (15) provided for a 2nd dive if DCS did not develop within 30 to 40 min of surfacing. No such dive was found to be necessary in this series.

RESULTS

Discarded Data

Seventeen experiments were performed; however, data from 5 were excluded using the criteria of Leitch and Hallenbeck (15); 2 dogs for uncontrolled hypotension (1 control, 1 dexamethasone), and 3 for failure to show any recovery (2 control, 1 dexamethasone).

Spinal Evoked Potentials

As SEPs from the stimulation of both sides were collected, it was possible to follow the course of the DCS in each side of the cord. The onset in one side was within 2 min of the other in all but one case when the interval was a maximum of 4 min. The severity of the DCS in terms of minimum SEP amplitude was comparable between the two sides ($r = 0.843$, $P < 0.01$). Assessment of outcome for each animal was taken as the mean of the two sides at maximal recovery and after 2 h of treatment. A Student's t test for unpaired data showed no significant difference between the 2 groups in either case. Figures 1 and 2 show the mean and 95% confidence interval, based on the t distribution, of the SEPs of the test and control groups before and after the onset of DCS. In Fig. 1 the x axis represents the time elapsed after leaving the surface for the 300-fsw dive. The numbers in brackets adjacent to the mean values refer to the number of animals from which the data were collected. Once DCS was diagnosed in each animal it was removed from the group. Hence, only the pre-DCS data are shown in this graph and it demonstrates that before the diagnosis of DCS there was little variability in SEP amplitude in either group. Figure 2 shows the mean and 95% confidence intervals of the SEPs after diagnosis of DCS, $n = 6$ for all data points. In each graph, the *top curve* represents the upper 95% confidence interval of the higher mean value and the *bottom curve*, the lower 95% confidence interval of the lower mean value.

Other Physiological Data

Tables 1-3 show the mean and standard error of the physiologic parameters recorded once or intermittently for the two groups. There were no significant differences between the values at the 5% level using Student's t test for unpaired data. Figures 3-8 show the mean and 95% confidence interval, based on the t distribution, of the physiologic parameters that were monitored continuously throughout the experiments. The data are displayed in the same manner as the SEPs, with Figs. 3-5 showing data before and Figs. 6-8 data after the diagnosis of DCS. In these latter figures, $n = 6$ for all points except RVP in the dexamethasone group where $n = 5$ due to the failure of a transducer to function shortly after the diagnosis of DCS.

Figure 3 shows that a moderate decrease in MAP tended to precede the onset of DCS, particularly in the dexamethasone group, and Fig. 6 shows that it was satisfactorily treated by the time DCS was diagnosed. Figure 6 also

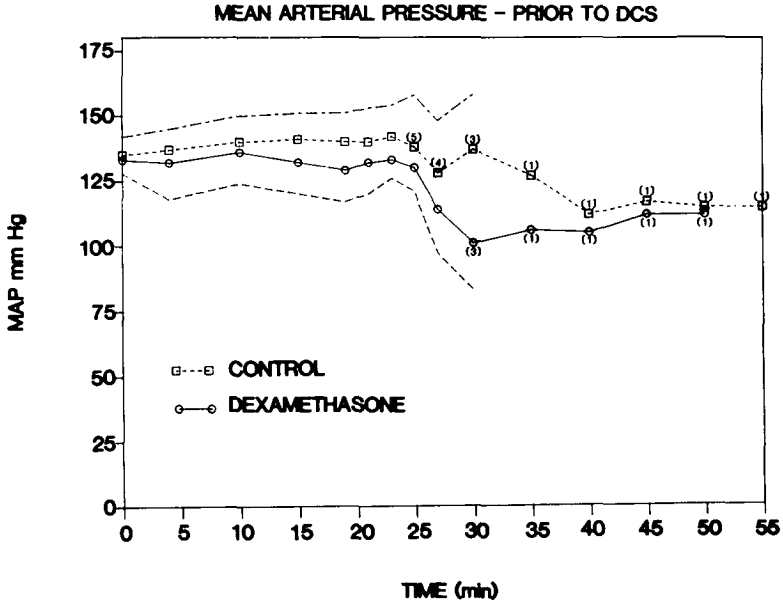

Fig. 1. Spinal evoked potentials—prior to DCS.

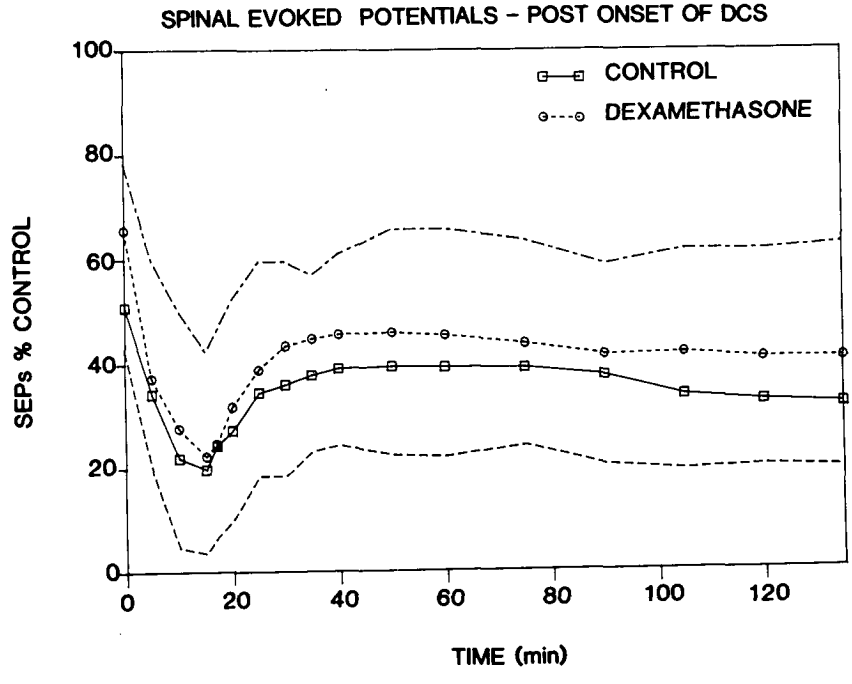

Fig. 2. Spinal evoked potentials—post onset of DCS.

Table 1
Physiological Variables, ± SEM

	Control	Dexamethasone
Dog weight, kg	10.3 ± 0.26	11.10 ± 0.72
Time from surfacing to onset of DCS, min	6.17 ± 1.82	10.67 ± 4.82
Volume of Ringer's lactate given, ml	456 ± 71.9	317.00 ± 45.9
Fluid balance during the experiment, ml	19.3 ± 39.2	-29.50 ± 59.6
Volume of 8.4% HCO_3 given, ml	8.3 ± 4.0	14.20 ± 9.5

Table 2
Physiological Variables of the Control Group, ± SEM

	Hematocrit, %	pH	PCO_2, Torr	ET CO_2, %	T_c, °C
Predive control	42.0 ± 1.8	7.38 ± 0.02	37.2 ± 0.87	3.97 ± 0.14	37.9 ± 0.26
On diagnosis of DCS	36.0 ± 3.1	7.33 ± 0.01	37.5 ± 0.88	3.71 ± 0.30	38.1 ± 0.30
15 min into treatment	38.1 ± 1.9	7.36 ± 0.01	37.8 ± 1.96	1.41 ± 0.04	37.6 ± 0.23
End of treatment	40.3 ± 2.8	7.36 ± 0.01	38.7 ± 1.71	1.46 ± 0.04	38.4 ± 0.18

Table 3
Physiological Variables of the Treated Group, ± SEM

	Hematocrit, %	pH	PCO_2, Torr	ET CO_2, %	T_c, °C
Predive control	42.2 ± 1.1	7.39 ± 0.01	36.7 ± 1.2	3.90 ± 0.10	37.9 ± 0.26
On diagnosis of DCS	41.2 ± 2.2	7.36 ± 0.01	38.2 ± 1.5	3.95 ± 0.12	37.6 ± 0.23
15 min into treatment	40.7 ± 2.9	7.36 ± 0.01	37.5 ± 1.6	1.37 ± 0.01	37.9 ± 0.42
End of treatment	41.2 ± 2.3	7.36 ± 0.02	37.3 ± 1.9	1.47 ± 0.05	38.1 ± 0.34

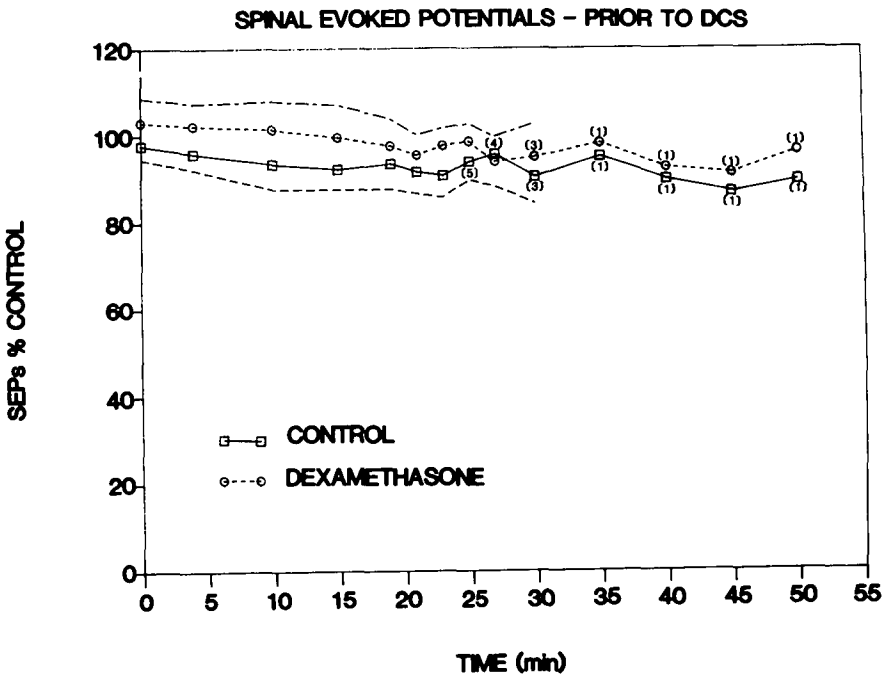

Fig. 3. Mean arterial pressure—prior to DCS.

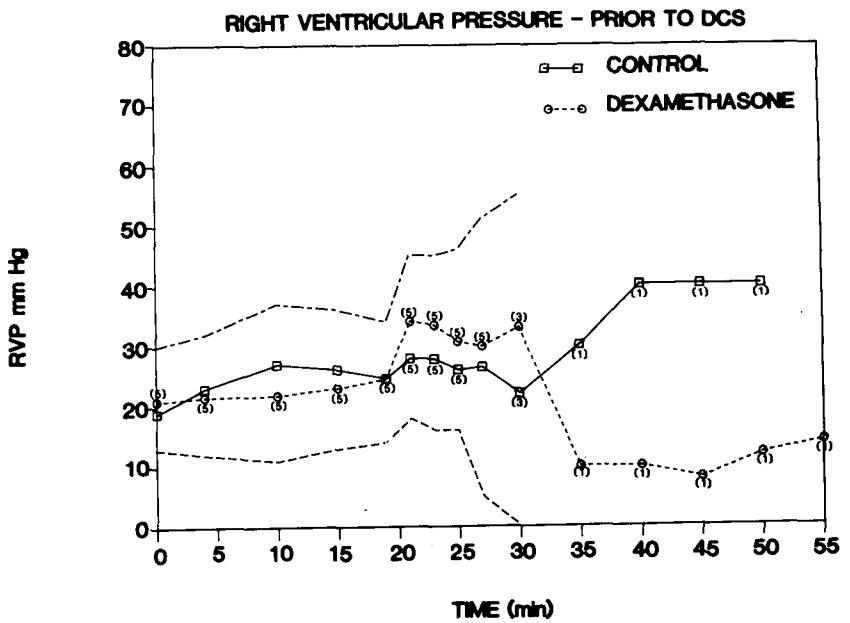

Fig. 4. Right ventricular pressure—prior to DCS.

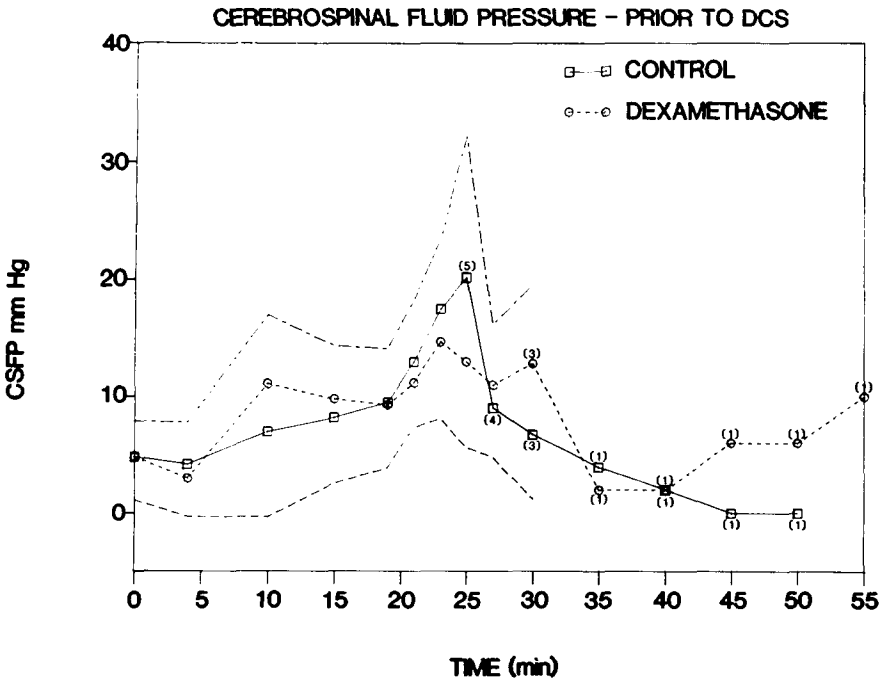

Fig. 5. Cerebrospinal fluid pressure—prior to DCS.

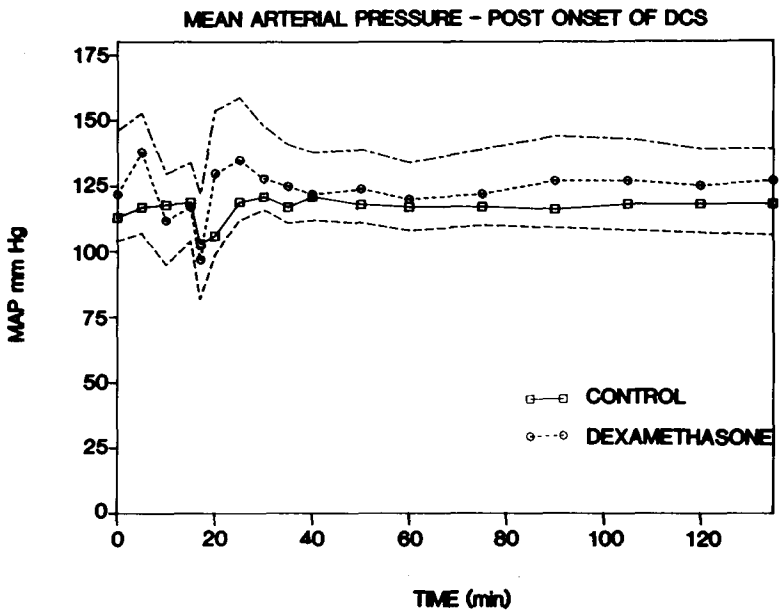

Fig. 6. Mean arterial pressure—post onset of DCS.

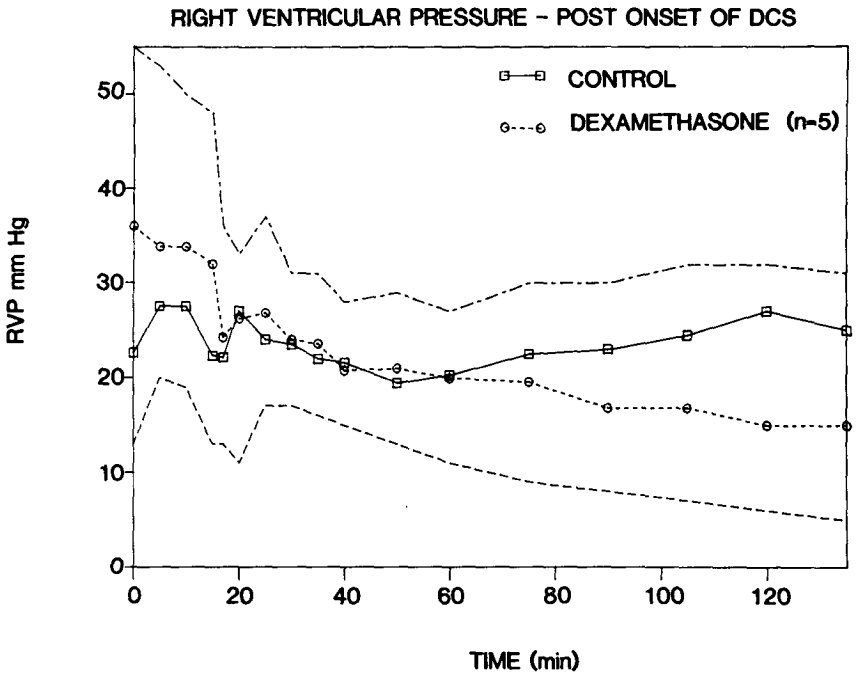

Fig. 7. Right ventricular pressure—post onset of DCS.

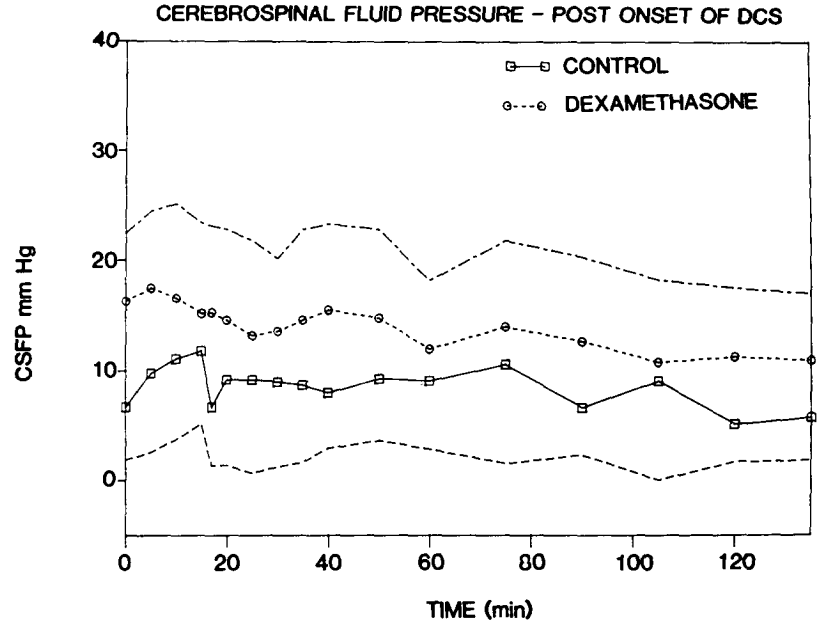

Fig. 8. Cerebrospinal fluid pressure—post onset of DCS.

shows that a brief decrease in MAP accompanied therapeutic recompression in both groups. This often resolved without therapeutic intervention. Figure 4 shows that the RVP tends to increase before the onset of DCS, although the confidence intervals indicate that this change was not significant. A greater decrease in RVP during treatment was seen in the dexamethasone than in the control group (Fig. 7). CSFP increased in both groups before the onset of DCS and tended to decrease during treatment (Figs. 5, 8). There was no significant difference between the groups in this respect.

The results demonstrate that the 2 groups were comparable in terms of the collected physiologic data and the severity of spinal cord injury. There was no significant difference in the outcome of treatment between the control and dexamethasone-treated animals. It is also evident that after an initial recovery, a considerable secondary deterioration in SEP amplitude occurred during treatment in both groups.

DISCUSSION

In discussing the finding that dexamethasone, in an anti-inflammatory dose, seems not to influence the outcome of DCS of the spinal cord in this experimental model, two aspects need to be considered. First is the nature of the injury in spinal cord DCS and second is the mode of action of dexamethasone.

It has been recognized for many years that the manifestations of DCS are a consequence of the liberation of gas bubbles within the body. In the case of the spinal cord however, it remains unclear how these bubbles generate an injury. Tissue ischemia may result from the obstruction by bubbles of either the arterial supply (19) or venous drainage (20) of the cord. Equally, ischemia may result from an increased local tissue pressure caused by the autochthonous liberation of gas bubbles so that arteriolar closing pressure is exceeded (21). Another mechanism by which gas bubbles may cause injury is by their interaction with blood. They have been shown to activate complement (22), blood coagulation (23), and in a rat model of DCS, aggregate and activate platelets and leukocytes (24). The activation of these blood components may result in an inflammatory tissue reaction as a consequence of increased arachidonic acid metabolism (25) and the liberation of oxygen-derived free radicals (26). Finally, gas bubbles may cause direct injury to tissue. Such injury may be to the vascular endothelium, causing the breakdown of the blood-brain barrier (BBB) and CNS edema. It seems however that gas bubbles are not as injurious in this respect as some other emboli, and that breakdown of the BBB from gaseous emboli is limited in extent and duration (27). Alternatively, bubbles may be liberated within the myelin and cause sufficient mechanical disruption to interfere with axonal conduction. At present the only evidence for this mechanism of spinal cord DCS is the finding of disrupted myelin figures in an animal model of spinal cord DCS (28).

Glucocorticoids have a number of actions that might modify the outcome

of some of the above pathogenic mechanisms of spinal cord DCS. Their action as anti-inflammatory agents would be of potential benefit in the event of a significant bubble-induced inflammatory reaction. This, however, may be of limited value in DCS due to the significant delay between administration and the onset of their anti-inflammatory action. Glucocorticoids react with receptor proteins in the cytoplasm of sensitive cells to form a steroid-receptor complex. This complex then moves into the nucleus where it binds to chromatin and stimulates the synthesis of mRNA for specific proteins (29). One or more of these proteins inhibit phospholipase A2 (30, 31). The result of this action is to reduce arachidonic acid release and consequently limit prostaglandin, hydroxy- and hydroperoxyicosatetranoic acid synthesis (32). Some evidence exists that a small amount of phospholipase inhibitor is stored in cells and its release is stimulated by glucocorticoids (33). It is generally recognized, however, that the anti-inflammatory action of glucocorticoids takes a matter of hours to become maximal. Thus, if the anti-inflammatory action of corticosteroids were the means by which they exerted a therapeutic effect in DCS, they are unlikely to be as effective as more rapidly acting, nonsteroidal anti-inflammatory agents. it is also improbable that any such therapeutic effect would have been detected in the time frame of this experimental model. The role of inflammation in spinal cord DCS is far from proven and no clinical or experimental trial has demonstrated that anti-inflammatory agents, given therapeutically, have a beneficial effect on the outcome of spinal cord DCS. Thus it is unlikely that this particular action of corticosteroids would be of benefit in treating DCS.

Corticosteroids have been ascribed an anti-edema role in cerebral edema, but the mechanism for this action is far from clear and it is thought to be least effective when the cause is ischemia (34). Although this action may be important in other etiologies of CNS injury, these results suggest that it is of equivocal value in DCS, because over the course of the experiment, no greater reduction of a raised intracranial pressure was observed in the dexamethasone-treated group than in the controls. Furthermore, there was no apparent correlation between intracranial pressure and outcome in this series.

Although the matter remains controversial, there is growing evidence that "megadose" steroids (dexamethasone 15 $mg \cdot kg^{-1}$ or methyl prednisolone 30 $mg \cdot kg^{-1}$) may be beneficial in the treatment of CNS trauma (35). The mechanism of action of steroids in such huge doses is not fully understood. In the spinal cord, there is evidence that they may improve blood flow (36, 37) and microvascular perfusion (38). This effect is thought to account for the improved metabolism (39) and reduced neurofilament degradation (40) observed in animal models of spinal cord injury treated with megadoses of methylprednisolone compared with untreated controls. Another proposed mechanism for megadose steroid action in traumatic spinal cord injury is a reduction of superoxide-induced damage (41). It has been shown that glucocorticoids may inhibit superoxide generation by leukocytes (42), and methyl prednisolone in particular possesses antioxidant properties (41). There is, as

yet, no evidence that lipid peroxidation plays an important role in spinal cord DCS; however, ischemia is thought to be an important mechanism in the generation of the injury. Finally, there is evidence that glucocorticoids may have a direct action on the conduction of impulses by the spinal cord. They are reported to increase the excitability of spinal motor neurons as evidenced by a decrease in the threshold depolarizing current and an increase in their susceptibility to repetitive discharges (43). Such an action, combined with possible improvements in synaptic transmission (44), may improve the performance of an injured spinal cord without directly influencing the injury. Many of the above actions of glucocorticoids may prove to be beneficial in the treatment of spinal cord DCS, but to be effective they will have to be given in considerably larger doses than those currently used.

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FUNCTIONAL IMAGING OF THE CENTRAL NERVOUS SYSTEM (CT, MRI, XENON-BLOOD FLOW) AND USE OF EVOKED POTENTIALS DURING THERAPY OF DECOMPRESSION SICKNESS AND ARTERIAL GAS EMBOLISM

R. E. Moon, E. M. Camporesi, E. W. Massey, C. W. Erwin, and W. G. Djang

Diagnosis and response to treatment of arterial gas embolism (AGE) and decompression sickness (DCS) are made largely on clinical grounds. There has been little application of sophisticated techniques of CNS imaging or electrophysiologic monitoring in these conditions. Computerized tomographic (CT) scanning has been described in AGE (1). In that report, 2 cases, 1 of which was thought initially to be spinal cord decompression sickness and the other AGE, had abnormal brain CT scans, which were felt compatible with AGE, within 12 to 24 h after the diving accident. The sensitivity of this radiographic technique is unknown. One of its drawbacks in the analysis of diving injuries is that it images the spinal cord poorly. Magnetic resonance imaging (MRI) holds some promise for the analysis of these types of injuries because of its exquisite sensitivity in detecting regional changes in brain water, which may result from ischemia, and the fact that it can image the spinal cord (2). Xenon-enhanced CT scanning is a technique in which brain CT is obtained before and after breathing xenon, which is radiodense. Subtraction of the baseline image from the xenon-enhanced one reveals the pattern of cerebral blood flow. This technique may provide high resolution images of brain blood-flow patterns (3). Short-latency evoked potential (SSEP) monitoring has been described in animal models of AGE and DCS (4, 5). Indeed Leitch and Hallenbeck (6) have correlated loss of SSEP amplitude with neuronal ischemia in dogs with central nervous system DCS. The use of SSEP monitoring of human diving casualties has not yet been reported, however. In this report we present 7 diving casualties treated at Duke University Medical Center in whom one or more of these diagnostic modalities were used.

MATERIALS AND METHODS

Computerized tomography scans were performed on either GE 9800 or 8800 scanners. Xenon regional blood flow (rCBF) images were obtained from CT scans before and after breathing 30% nonradioactive xenon. These scans were all performed on a GE 9800 scanner using sequences from 30 s to 3 min after the start of xenon inhalation. MRI scans were obtained using a GE 1.5T prototype SIGNA system. T1 weighted (PS 500/25) and T2 weighted (SE 2000/40-80) pulse sequences were used routinely. Surface coils were used for imaging the spinal cord. These imaging procedures were performed in the Radiology Department during intervals between hyperbaric treatments. SSEP monitoring responses to lower extremity (tibial nerve) electrical stimulation was performed in all patients, and median nerve responses were obtained in 4 of 7 patients using a Nicolet Pathfinder II connected to the patient by a through-hull penetrator. The technique was basically that recommended by the American Electroencephalographic Society (7).

CASE SUMMARIES

TK was a 44-yr-old male with a past history of pulmonary sarcoidosis. The first time he dived was to a depth of 13 msw for 30 min. Immediately upon surfacing he experienced paraesthesias and weakness in both legs. His symptoms resolved after a few minutes with no specific treatment. The following day he dived to a depth of 18 msw for 30 min. A few minutes after surfacing he noted the same symptoms, along with interscapular pain. He was given oxygen at the surface with some improvement in leg strength. About an hour after the onset of symptoms he was given a U.S. Navy Treatment Table 6 with full extensions. He initially improved and then worsened during recompression to the surface. He was transferred to Duke Medical Center and on examination had a sensory level of T_{12} bilaterally, mild left arm weakness, and bilateral leg weakness, left greater than the right. There were no cranial nerve or cortical signs. MRI studies of brain and thoracic spinal cord were normal. SSEP studies were obtained 4 d after his last scuba dive, both before and during his 6th therapeutic recompression treatment. At this time he still had a profoundly abnormal neurologic exam. Latencies and amplitudes were within normal limits. There was no significant change during the course of the USN Table 6.

GB was a 30-yr-old male who had made multiple dives over a 4-d period to depths ranging from 12 to 50 msw. After the last dive during a 5 msw decompression stop, he had a major seizure. Pertinent past medical history included the use of an inhaled bronchodilator for asthma. He was treated at the local chamber for presumed AGE with a USN Table 6A and then transferred to Duke Medical Center. On exam he had a T_8 sensory level. He had profound lower extremity weakness with only a flicker of movement on the right. He also had mild confusion. Upper extremity SSEPs were normal. Lower

extremity stimulation demonstrated mild bilateral slowing (> 3 SD) of transmission in spinal segments compatible with a diffuse myelopathy. Both CT and MRI demonstrated an area of abnormality in the subcortex of the left parietal-occipital region. The lesion appeared hypodense on CT and slightly more extensive on MRI with hyperintensity (prolonged T_2) on the T_2 weighted sequences, compatible with infarct. An additional abnormal subcortical focus missed on CT scan was identified in the right temporal region by MRI. His spinal cord MRI images were normal.

MP was a 28-yr-old male diver who had noticed several episodes of transient dizziness immediately on surfacing after diving 5 yr before this accident. These episodes lasted only a few seconds and had been attributed to alternobaric vertigo. On the day of his accident he made a dive to 24 msw for 40 min. He experienced his "typical vertigo" and descended to about 5 msw with no improvement. He then developed lower extremity weakness. He was treated locally with a USN Table 6 treatment, at the end of which he still had marked lower extremity weakness, absent anal sphincter tone, and urinary retention. He was treated again on the same day with marginal improvement. On transfer to Duke Medical Center he had mild upper extremity weakness and profound lower extremity weakness with a sensory level at C_7. SSEP 9 d after his accident (neurologic exam still abnormal) showed normal tibial and median nerve stimulation amplitudes and latencies. Brain MRI and CT were normal.

LD (a 49-yr-old male), 2 yr before this accident, had experienced pain and weakness in his right leg after a long dive. He was hospitalized but not recompressed and recovered with no known residual symptoms. His most recent decompression accident resulted from a series of dives spanning 1.5 h at a depth of 35 to 50 msw using a total of 6 scuba tanks. When he surfaced he noticed heaviness in his chest followed by paralysis of his lower extremities. Twelve hours after the accident he received a USN Table 6 treatment at the local recompression chamber and experienced some improvement. The following day he was given a USN Table 5 treatment with some further improvement. He was transferred to Duke Medical Center 10 d later. On exam he had normal upper extremity strength but only 0 to 1/5 strength in his lower extremities. He had an L_2 sensory level. SSEP using median nerve stimulation was normal. Tibial nerve stimulation showed no definable wave forms above the level of the lumbar recording site. MRI of the spinal cord was normal.

SS was a 41-yr-old female who made a 27 msw, 27-min dive and noted abdominal pain and mild transient lower extremity numbness. These symptoms resolved and she made a 2nd dive on the same day to 23 msw for 29 min. After this dive, numbness recurred along with right-foot weakness and jerking movements of the right leg. On exam she had decreased sensation in the right thigh. She had weakness of the right triceps, hamstrings, psoas, and everters of the right foot. She also had right-sided hyperreflexia and a right Babinski response. SSEP were normal. Brain and spinal cord MRI showed small abnormalities.

RJ was a 42-yr-old experienced male diver. On the day of the accident he

made a dive to 18 msw for 40 min. It was believed that he became nauseated during the dive and began vomiting. Shortly after surfacing he indicated that he was in distress. After being pulled into the boat he immediately became unconscious and had a major motor seizure. He was compressed within 45 min using a USN Table 6A. After this treatment the patient was confused, quadriplegic, and had a C_4 sensory level. The following day he received 2 further USN Table 6 treatments with minimal improvement. On arrival at Duke Medical Center he was noted to be lethargic. He was also dysphasic with anomia. He was flaccid in all of his extremities, with intermittent reflexic movement of his left foot.

Short-latency evoked potentials were performed immediately on arrival at Duke and during treatment on Days 1, 3, and 5. Figure 1 shows responses to left and right tibial nerve stimulation before HBO treatment. Peripheral (PF) and spinal (L_3S-$T_{12}S$) responses are present bilaterally. An abnormally delayed cortical response [Cz-Fpz] at 47.84 ms is present to left tibial nerve stimulation. The response is totally absent to right tibial nerve stimulation.

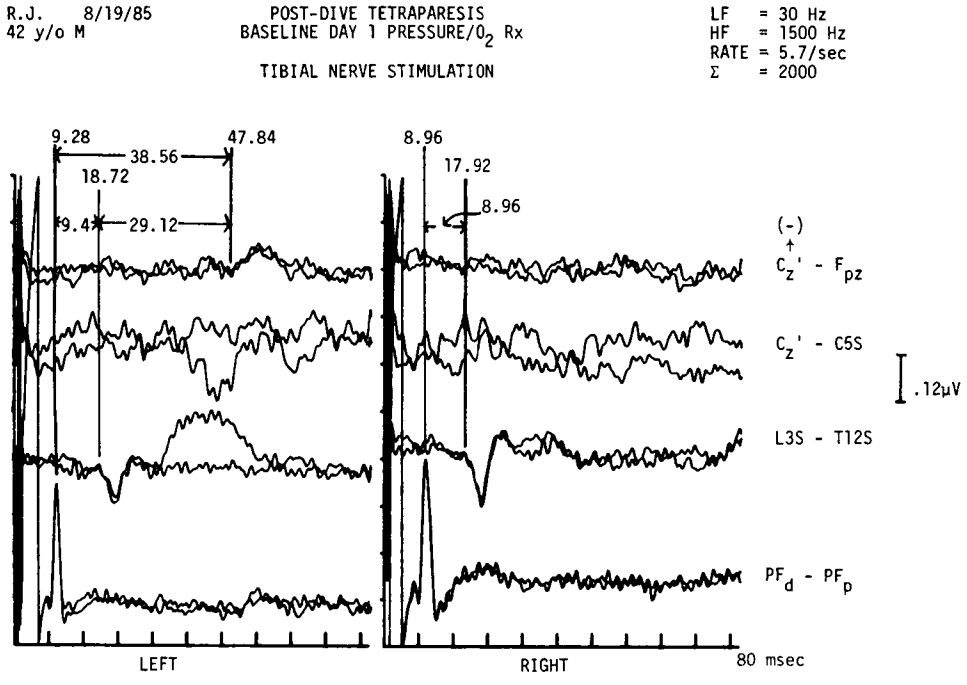

Fig. 1. SSEP of patient RJ before hyperbaric treatment at Duke Medical Center (he had previously received 3 treatments before transfer). Two replications are shown for each of the 4 recording sites (cortical, subcortical, spinal, and popliteal fossa, from top to bottom of figure). Latency measurements (ms) are shown. Peripheral and spinal responses are present. Cortical response to left tibial nerve stimulation is delayed. No cortical response is seen to right tibial nerve stimulation.

Figure 2 demonstrates SSEP documentation of physiologic response on treatment Day 1 during a USN Table 6. The P38 component showed an unequivocal shortening of latency of over 10 ms to achieve a normal value. On Day 3 there was SSEP regression detected before hyperbaric treatment, but a more rapid subsequent improvement during treatment than on Day 1. The improvement remained stable by Day 5.

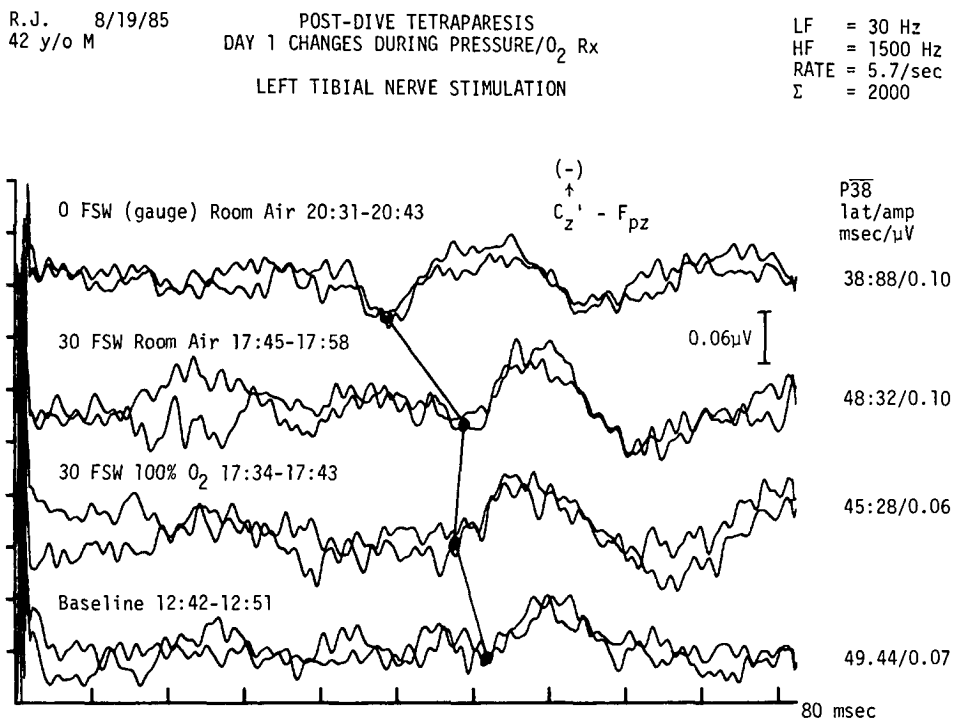

Fig. 2. Four sets of cortical SSEP responses (subject RJ) shown during the course of a USN Table 6. Each pair of 2 superimposed waveforms represents consecutive replications. Bottom, baseline (pretreatment) SSEP, top, posttreatment SSEP. The intermediate waveforms were measured during the 9.14 msw (30 fsw) phase of the table, while breathing air or O_2, respectively. Times of acquisition are indicated. Connected dots demonstrate reduction of latency of the P_{38} (P_1) component with treatment.

Two day after the injury, a CT scan was normal. MRI examination of the brain subsequently showed extensive areas of long T_2 involving the parietal white matter and the cortical portions of the parietal lobes. Figure 3 shows the MRI abnormality. Subsequent xenon-enhanced CT scan showed mild decrease in rCBF in the areas of MRI abnormalities. Figure 4 shows the Xe-CT along with a corresponding conventional CT cut, which is normal. MRI also showed a suspicious focus of increased T_2 in the dorsal columns of the cervical cord.

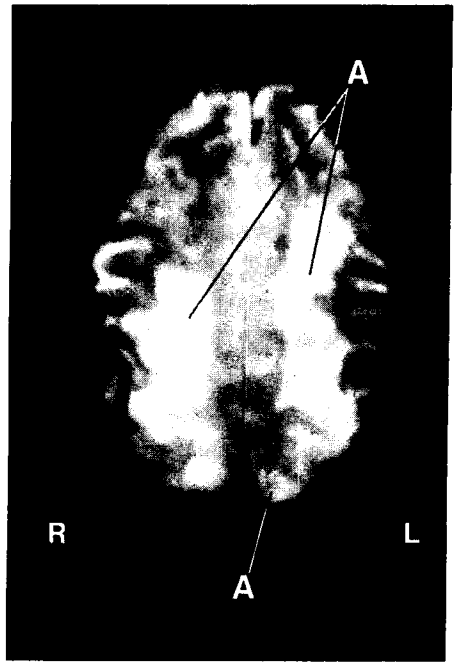

Fig. 3. MRI (T_2 weighted) cut of the brain of RJ 2 d post air embolism. Areas of long T_2 appear lighter (A), indicating localized increased brain water content.

Six days after his accident this patient suffered an acute cardiopulmonary arrest, from which he could not be resuscitated. Postmortem examination revealed a large saddle pulmonary embolus. Gross inspection of the brain was unremarkable but histologic examination showed cytopathologic changes of anoxic injury within and confined to the areas of MRI abnormality. Figure 5 shows a transverse section of the freshly cut brain, which is grossly normal. Figure 6 shows a corresponding stained, mounted, thin section. Subcortical pallor is demonstrated, indicating intracellular edema, confirming the exquisite sensitivity of MRI in detecting these changes which were not visible by gross inspection of the cut brain. Additionally, there were marked changes in the spinal cord at multiple levels, in dorsal and lateral tracts with greater involvement on the right. These histologic changes were compatible with DCS.

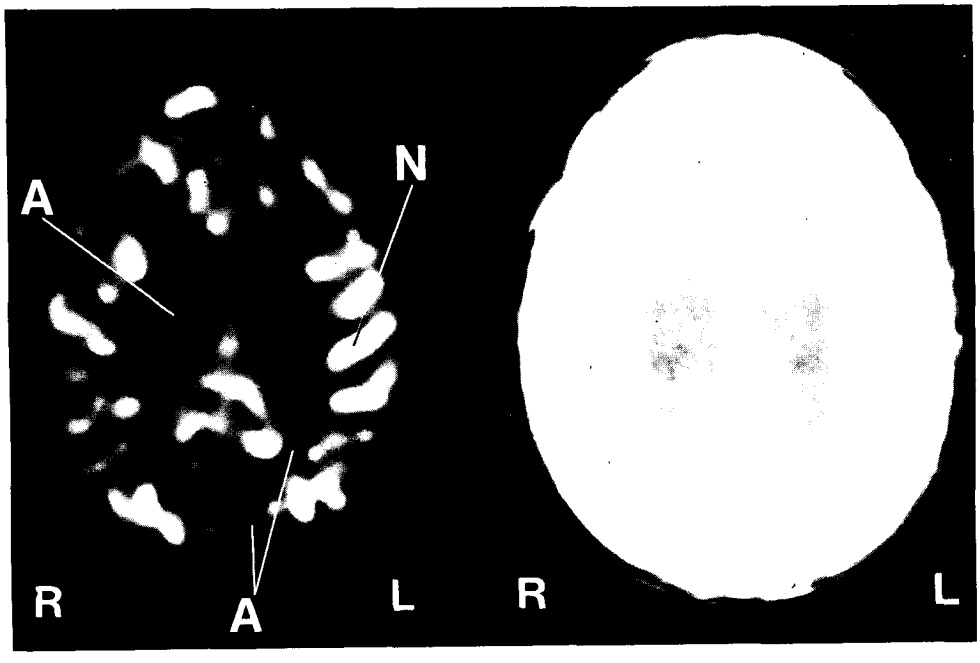

Fig. 4. A standard CT cut of the brain of RJ is shown on the right, with a corresponding xenon-enhanced image on the left (Day 5 after the accident). The standard CT image shows no abnormality, whereas areas of abnormally low rCBF (A) are clearly seen on the xenon-enhanced image. These areas correspond to regions of edema seen in the MRI scan (Fig. 3). An area of normal blood flow in indicated (N).

LN, a 22-yr-old male, made 2 dives to a depth of 18 msw, each for about 15 min on the day before the accident. On the day of the accident he made 2 dives to 21 msw, each for about 30 min. About 3 h after surfacing from the 2nd dive he noted numbness in his right forearm and hand which disappeared overnight. The following day he had mid- and low-back pain. When he went for medical evaluation 3 d after his last dive he had normal muscle strength but decreased pinprick sensation over the left side of his torso posteriorly, as well as patchy areas of decreased pinprick sensation in his left leg. Position and vibration sensation were normal. Reflexes were symmetrical. SSEPs with tibial nerve stimulation were normal bilaterally with no further change during a USN Table 6 treatment.

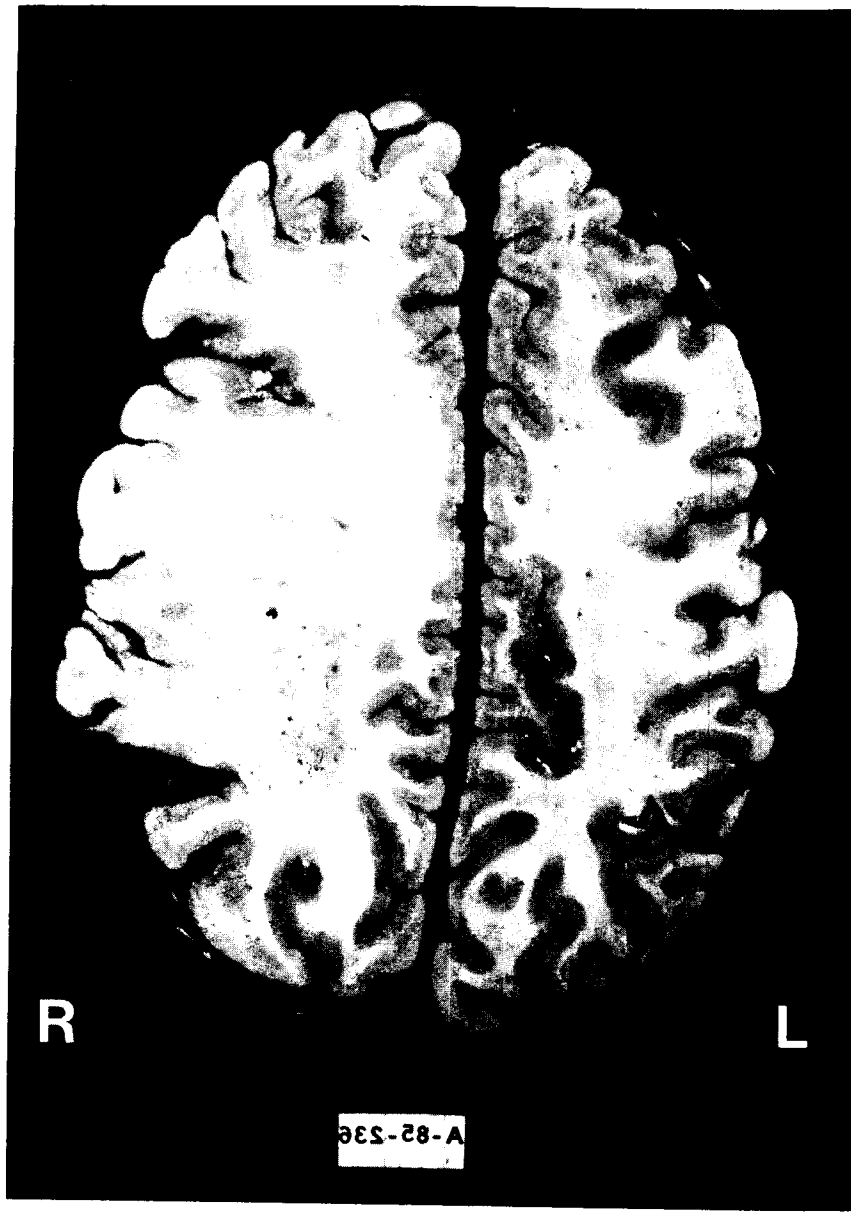

Fig. 5. A freshly cut transverse section of the fixed brain of RJ. No abnormalities are visible.

DISCUSSION

GB and RJ had clinical evidence of AGE. Six of the 7 patients had clinical evidence of spinal cord DCS. The 7th (RJ) had histopathologic evidence of it. Clinical severity of DCS varied from very mild (LN) to severe (RJ, LD,

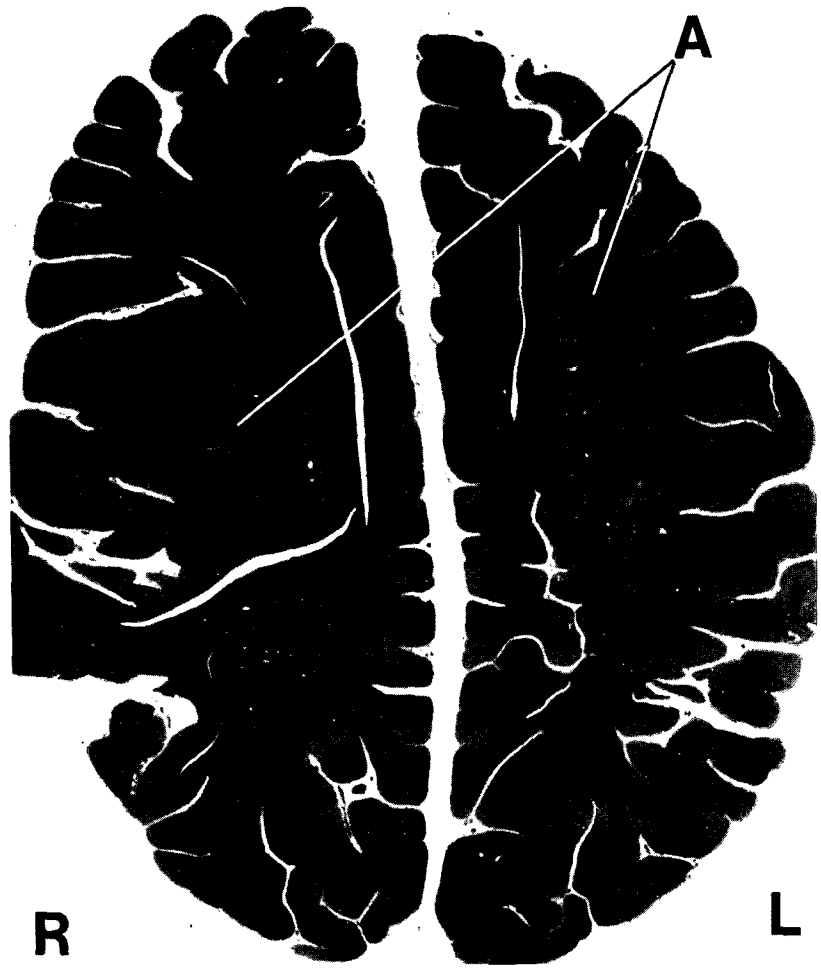

Fig. 6. A stained, thin, brain section of RJ. Subcortical areas indicated (A) demonstrate poor staining of myelin. Microscopic examination of these areas showed increased cytoplasm, compatible with edema.

and GB). The finding of profound histopathologic changes of spinal cord DCS in patient RJ after a dive which should have had a low probability for DCS provides further confirmation of the observation of Leitch and Hallenbeck (8) that the presence of AGE can predispose to neurologic DCS.

In only 1 of the 2 individuals with clinical AGE was the CT scan abnormal. However, MRI detected areas of abnormality in both patients. MRI was able to show an area of abnormality in GB in addition to the one demonstrated by CT. Furthermore, in RJ, rCBF measured by xenon-enhanced CT scanning was decreased in the areas of MRI abnormality. MRI was able to

detect a spinal cord abnormality, in 2 of 5 patients.

All 7 patients had abnormalities on neurologic examination at the time of SSEP testing. SSEPs were abnormal in 3 of the 7 patients. All patients with abnormal SSEP had severe DCS. We further demonstrated that SSEP may show a marked sensitivity to physiologic improvement to hyperbaric oxygen treatment of DCS, providing objective support for continuation of therapy beyond initial hyperbaric treatment.

In summary, based on this limited series of patients with gas bubble disease due to scuba diving, we offer the following observations. In the examination of the brain MRI seems to be more sensitive than CT scanning. MRI as used in these imaging studies was relatively insensitive for the detection of spinal cord DCS. However, image resolution is being continually improved and it is possible that newer systems may be more sensitive. Xenon-enhanced CT scanning may be an alternative to MRI in the detection of AGE when this latter modality is unavailable. Finally, SSEP seems to be abnormal in severe spinal cord DCS. We have seen improvement in SSEP latencies during therapeutic recompression, and suggest that this technique may be of particular value during chamber treatment where it may be difficult to obtain frequent neurologic examinations.

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LATENCY AND AMPLITUDE OF SOMATOSENSORY EVOKED POTENTIALS IN NORMAL SUBJECTS BREATHING AIR AND OXYGEN AT 1 AND 2.8 ATA

P. Mitchell, C. W. Erwin, E. M. Camporesi, R. E. Moon, R. Goad, Y. Mebane, B. Stolp, and P. B. Bennett

The neurologic evaluation of the patient with spinal cord decompression sickness (SCDS) undergoing recompression treatment is fraught with difficulty. Communication is suboptimal, conditions cramped, and evaluation of changes in neurologic function are notoriously subjective. Despite this, the presence or absence of neurologic signs of recovery are used to determine further therapy such as extension of O_2 breathing periods at pressure or the decision to enter saturation therapy. Somatosensory evoked potentials (SSEP) provide a safe, noninvasive means of evaluating spinal cord integrity (1-3). They may also provide a means of quantitating dysfunction (4), as well as possibly offering prognostic indications of recovery in patients with spinal cord injury (5).

Despite the potential applicability of this technique to patients with SCDS there are at present no data describing the effect of the varying inspired gas concentrations or the effect of increased ambient pressure on short latency SSEP in normal humans.

After obtaining institutional approval we subjected 5 normal human volunteers to the depths and inspired gas concentrations experienced while undergoing conventional U.S. Navy Table 6 (18.3 msw, 100% O_2) and measured concurrently the changes in the short latency tibial evoked potentials.

METHODS

Five healthy male volunteers ranging in age from 29 to 57 yr were selected. After Institutional Review Board approval for the experiment, individual informed consent was obtained. The volunteers were prepared for the

experiment in the following manner. Initially, the subjects underwent standard EEG electrode placement over the scalp, Fz and Fpz, with recording electrodes over the popliteal fossa (PF), the second thoracic vertebral spine, and over the third lumbar spine (LIII) with electrode impedances of < 2 K Ω. Approximately 30 minutes before baseline measurements, each subject received diazepam, 5 mg i.v. This was done to decrease interference from myogenic scalp potentials. A left radial arterial cannula was placed in a conventional manner, and baseline atmospheric blood gas analysis performed with the subject reclining at 45° in an armchair. Two stimulating needle electrodes, separated by 1 cm, were placed at the right tibial nerve (cathode proximal), and baseline atmospheric SSEP obtained. Potentials were recorded with a Nicolet Pathfinder with high and low pass filters set at 30 and 1500 Hz, respectively. Stimuli consisted of constant current square wave pulses of 5 to 20 mA, 0.1 to 0.2 ms in duration, stimulus rate 5.4/s which were averaged over 2000 stimulus presentations. All responses were replicated twice at each period of data collection (Fig. 1).

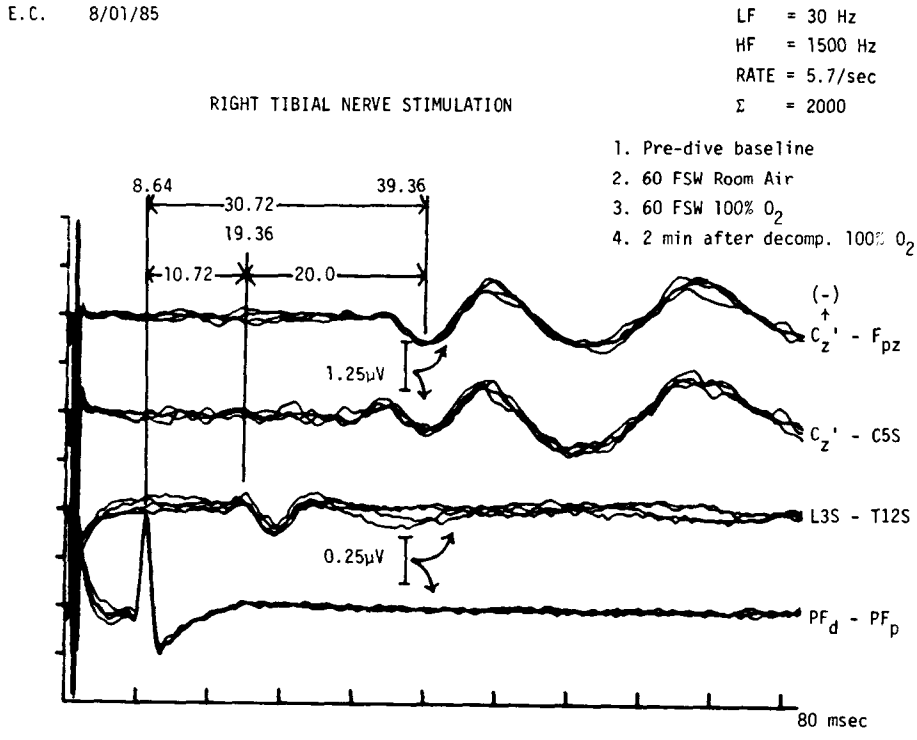

Fig. 1. Evoked responses from a typical subject. Each group of 4 superimposed tracings obtained under the 4 conditions of the study represent, from top to bottom: cortical, subcortical, spinal, and PF waveforms. There is no obvious systematic effect of ambient pressure or breathing gas. Top row, predive baseline; second row, 60 fsw room air; third row, 60 fsw 100% O_2; bottom row, 2 min after decompression, 100% O_2.

After baseline measurements were complete, the subjects underwent compression at 25 ft/min on air to a depth of 60 fsw where repeat blood gas analysis was performed and two SSEP series completed. The subject's head was then placed in a Duke oxygen hood (a plastic hood encasing the entire head and sealed over the shoulder girdle with adhesive tape). The oxygen flow rate was adjusted to maintain a measured inspired O_2 greater than 0.98 and 10 min allowed for a respiratory steady state to be achieved, with normal breathing. Repeat blood gas analysis at depth and 2 trials of SSEP were performed. The subjects were then decompressed to the surface at 60 ft/min where repeat measurements of arterial blood gases and 2 additional trials of SSEP were performed, still breathing oxygen in the hood.

RESULTS

The numerical results of the experiments are detailed in Table 1. A typical series of SSEP waveforms is shown in Fig. 1. One subject had no discernible L_3 potential while breathing O_2 at 1 ATA. Therefore some of the table entries represent means of only 4 values. Statistical analysis of the latencies and amplitudes was performed using repeated measures analysis of variance with two classification factors (inspired gas and ambient pressure).

Latency

There were no significant changes in latency of the L_3, P_1, N_2, P_F-L_3, L_3-P_1, or P_F-P_1 intervals that occurred in relation to changes in inspired O_2, ambient pressure, or their interaction. PF latencies were statistically longer while breathing O_2 ($P < 0.05$). This change (approximately 0.25 ms) is not clinically significant. Moreover, both O_2 breathing periods were performed later in each study session. Therefore, PF latency, representing peripheral nerve conduction velocity, may have been prolonged by cooling of the exposed experimental limb.

Amplitude

The response to pressure of the amplitude of the P_1-N_2 complex showed considerable variation among subjects. Baseline amplitude ranged from 0.58 to 1.49 μV. In 4 individuals, a small decrease in amplitude occurred on compression with a return toward baseline during administration of 100% O_2 at 2.82 ATA. These changes, however, did not reach statistical significance.

Blood Gases

Pa_{O_2} values measured confirmed that 100% O_2 was being breathed for an adequate period to allow equilibration, and that the subjects had normal respiratory capabilities (Table 1).

Table 1
Variation of Short Latency Tibial Evoked Potentials and Arterial Blood Gas and Changes in FI_{O_2} and Ambient Pressure
($n = 5$, mean ± SD). See text for Statistics

	Latency, ms							Amplitude, μV	Blood Gas Analysis		
	P_F	L_3	P_1	N_2	P_F-L_3	L_3-P_1	P_F-P_1	P_1N_2	pH	PO_2 mmHg	PCO_2 mmHg
1 ATA air	9.28	20.06	39.52	45.76	10.78	19.46	30.24	1.05	7.39	80	37
SD	1.08	1.48	2.84	2.32	0.73	2.26	2.10	0.43	0.03	6	2
2.82 ATA air	9.31	20.26	39.17	45.38	10.94	18.91	29.86	0.93	7.42	283	33
SD	1.10	1.89	2.43	3.07	0.92	1.54	1.55	0.54	0.02	33	3
2.82 ATA O_2	9.41	20.45	39.39	45.44	11.04	18.94	29.98	0.97	7.45	1439	30
SD	1.14	1.47	3.24	2.61	0.49	2.31	2.38	0.52	0.03	99	3
1 ATA O_2	9.57	20.68*	39.26	45.38	11.24*	18.76*	29.70	0.90	7.43	504	35
SD	1.24	2.33	2.80	2.38	0.97	1.45	1.80	0.44	0.02	27	4

* $n = 4$ (see text).

DISCUSSION

Somatosensory evoked potentials are used extensively to define the integrity of sensory pathways within the spinal cord (6). They are particularly sensitive to changes in neurologic function within the dorsal columns. This would suggest that SSEP could be used to monitor changes in neurologic function that occurs in SCDS since the dorsal columns are frequently involved. SSEP have proved to be clinically useful in acute situations where they can detect alterations in neurologic function of the spinal cord caused by manipulation of the spinal cord during spinal surgery (7) or interference with the blood supply during aortic surgery.

However, to date, there have been no previous reports of the use of SSEP during recompression therapy for SCDS in humans. In addition, there are no data in the literature describing the effects on short latency evoked potentials of the variations of pressure and inspired gas that occur with conventional Navy treatment protocols in normal humans. Hence, our data are useful as a baseline for SSEP use during recompression therapy. Changes in SSEP latencies during recompression treatment therefore cannot be ascribed to artifacts of the treatment or to physiologic effects of O_2 or N_2 on normal neural tissue.

In 4 of the 5 individuals we observed a slight reduction in *amplitude* of the P_1-N_2 complex with compression in air to 2.82 ATA. This finding parallels that of other workers who examined changes that occurred in visual evoked potentials (VEP) and brainstem auditory evoked potentials (BAER) (8). This decrease in amplitude may be attributed to the depressant central anesthetic effect of nitrogen at 2.82 ATA. The recovery of amplitude that occurs when the breathing mixture is changed from air at 2.82 ATA to 100% O_2 supports this concept. However, amplitude changes of the P_1-N_2 complex of the SSEP are notoriously unreliable indicators of changes in neurologic function because they are affected by sleep, depressant drugs, temperature, and state of awareness. In fact, it has been stated that the most reliable alteration in signal amplitude is the complete loss of the P_1-N_2 complex (9). A change of more than 50% from baseline amplitude is usually required to substantiate some change in neurologic function.

In contrast, *latency* intervals P_1-N_2, P_F-L_3, L_3-P_1, or P_F-P_1 are very reproducible among the different individuals, with no variation occurring with changes in ambient pressure or inspired gas. All our subjects maintained normocapnia throughout this study, but we noted a tendency toward lower $Paco_2$ values during O_2 breathing at 2.8 ATA. This factor deserves discussion in light of a recent study (10) that analyzed SSEP alterations during acute hypocapnia in anesthetized individuals. In that study end-tidal Pco_2 was acutely altered from 40 to 21 mmHg resulting in a small (1 ms) but statistically significant reduction in the latency of median nerve SSEP. The smaller change in Pco_2 we observed (37-30 mmHg mean values before and during HBO) would probably have resulted in even smaller alterations in latency. The latency

interval is used clinically at present to detect changes in conduction velocity, particularly associated with demyelinating conditions such as multiple sclerosis. These diseases show a general prolongation of the latency interval. The latency changes of SSEP may be peculiarly sensitive in detecting minor changes in neurologic function particularly in the dorsal columns that occur during recompression of individuals with SCDS.

Changes such as decreasing latency interval of the SSEP on recompression of injured divers, or a prolongation of the interval on decompression to the surface after treatment, may provide valuable additional information concerning neurologic function. At present, the decision to enter saturation recompression therapy or to continue multiple recompression treatments of injured divers is guided by relatively "soft" neurologic signs and symptoms. We believe that, used in conjunction with the clinical exam, short latency SSEP will provide more exact, quantitative neurologic evaluation of the spinal cord in the injured diver and help guide the conduct of further therapy.

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REDISTRIBUTION OF CEREBRAL ARTERIAL GAS EMBOLI: A COMPARISON OF TREATMENT REGIMENS

D. F. Gorman, D. M. Browning, and D. W. Parsons

Compression of patients with cerebral arterial gas embolism (CAGE) in a recompression chamber (RCC) is the definitive treatment (1-8), but significant human data only exist for compression on air to 6 bar absolute pressure (1, 3, 4, 8). Animal-model studies of CAGE have reported clearance of emboli from pial arterioles during compression to 6 bar (6, 7). These studies did not allow for spontaneous embolus redistribution, and consequently may have overestimated the efficacy of this regimen. We have demonstrated that most gas emboli entering the pial arteries distribute spontaneously to the venous circulation (9, 10). Spontaneous redistribution of gas emboli from cerebral and pial arteries to the venous circulation has also been reported by other researchers (6, 11-16).

The mechanisms of gas embolus redistribution have not been thoroughly studied. We have established that trapping of gas emboli in pial arterioles, and subsequent embolus redistribution to the venous circulation, depends on the relative balance of embolus length and cerebral perfusion pressure (CPP) (9, 10). Embolus length is a function of both embolus volume and vessel diameter. One of our studies also established that vasoreactivity of pial arterioles can persist, despite local gas embolus entrapment and loss of cerebrovascular autoregulation (9).

Comparison of different CAGE treatment regimens must allow for spontaneous embolus redistribution, as well as measure changes in embolus volume, cerebral arteriole diameter, and CPP. CPP will passively follow mean arterial blood pressure (MABP) because cerebrovascular autoregulation to increases in blood pressure is lost following CAGE (9, 10, 14, 15, 17).

This study compared the success of conventional treatment regimens in redistributing arrested gas emboli from pial arterioles.

MATERIALS AND METHODS

Rabbit pial arterioles were selected for this study. Normal vasoreactivity to changes in the partial pressure of carbon dioxide (P_{CO_2}) and normal cerebrovascular autoregulation to alterations in blood pressure have been demonstrated in exposed rabbit pial arterioles (18). Rabbit pial arterioles also show similar cerebrovascular reactivity and autoregulatory responses to intraparenchymal vessels of the same size (18). New Zealand white rabbits, of either sex, weighing 5 to 6 kg were used in all experiments.

Preliminary Studies

Preliminary studies were performed on 20 rabbits without craniotomies to obtain both jugular venous partial pressure of oxygen (P_{O_2}) and carbon dioxide (P_{CO_2}), and MABP data for the following conditions: a) breathing oxygen at atmospheric pressure (5 rabbits); b) breathing 95% oxygen:5% carbon dioxide at atmospheric pressure (5 rabbits); c) breathing oxygen at 2.8 bar (5 rabbits); and d) breathing air at 6 bar (5 rabbits).

These data allowed us to determine gas mixtures for bathing the open brains of rabbits in subsequent studies. The mixtures ensured that the brain surface was exposed to P_{O_2} and P_{CO_2} levels similar to the jugular venous gas tensions measured in the above conditions, and avoided alkalosis of the brain surface (19, 20). The gas mixtures were humidified to reduce open-brain desiccation.

Surgical Preparation

A further 76 rabbits were anesthetized with air and halothane. Halothane was the only anesthetic tested that enabled a steady state of anesthesia and survival of rabbits after pial circulatory arrest due to CAGE. Halothane anesthesia does not significantly alter the behavior of rabbit pial arterioles (21).

A tracheostomy allowed intubation with a cuffed (saline filled) endotracheal tube. The tube was connected to a respiratory circuit that included gas cylinders, pressure regulators, Rotameter flow meters, Fluotec vaporizer, and an Ulco Campbell ventilator. Fresh gas was delivered to the circuit at 6 liter/min to prevent rebreathing. A pressure cycle ventilator was mounted in the RCC door to ventilate rabbits compressed in the small animal RCC (Fig. 1).

The left jugular vein draining the cranial contents was isolated by dissection, cannulated, and connected to a 3-way tap to enable blood sample collection. Mechanical ventilation parameters were adjusted to maintain femoral arterial P_{O_2} between 90 and 110 mmHg, and P_{CO_2} between 30 and 35 mmHg. The delivered halothane vapor pressure was maintained between 11.62 and 11.7 mmHg at the different ambient pressures by adjustment of the vaporizer settings. Halothane vapor pressure was measured with an Interferometer, with vapor pressure measurements corrected for temperature.

Body (rectal) temperature was maintained between 37.5 and 37.8°C with a

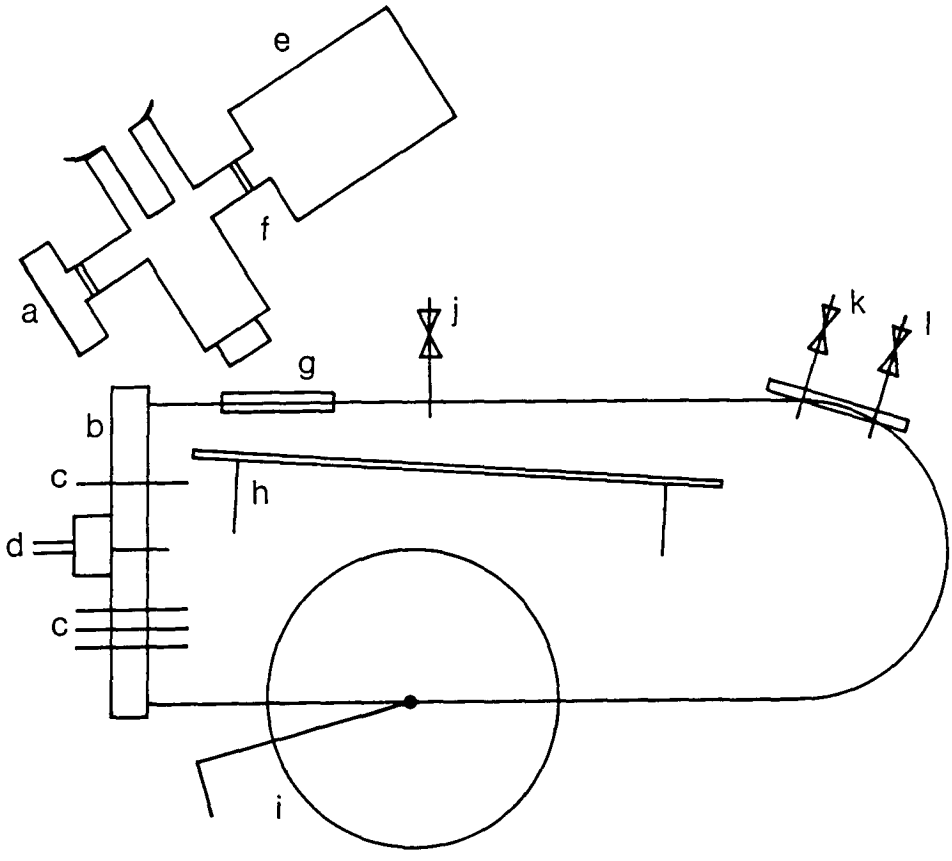


Fig. 1. Small animal RCC, lateral view. a. 35mm camera. b. Hinged door. c. Chamber penetrations. d. Ventilator. e. Video camera. f. Microscope. g. Viewing port. h. Animal immobilization tray. i. Tilt axle. j. Exhaust. k. Pressure gauge. l. Inlet valve.

variable output heat pad. Three electrodes were implanted in the rabbit's chest to provide a continuous ECG record on a Neotrace 8-channel chart recorder.

The right femoral artery was isolated by dissection, cannulated, and connected via a 3-way tap to a Bell and Howell pressure transducer (with pressure displayed on the chart recorder) and to an infusion line. Infusate was warmed to 37.5°C. Venous drainage from the right leg was occluded by ligature.

Arterial and cerebral venous blood samples were collected for Po_2 and Pco_2 estimates. A Radiometer ABL 30 blood gas analyzer was used with appropriate temperature corrections.

A parieto-occipital craniotomy of approximately 2 × 3 cm was created with a high-speed drill. Removal of the dura enabled direct observation of the brain and pial vessels with a Zeiss dissecting microscope. The microscope had a magnification range of 125 to 800, and had both video (Sony DXC-150P) and still camera (Contax 35 mm) attachments. The open brain was bathed with the warmed, humidified gas mixtures described previously, applied as a 1.5 liter/min diffuse jet into the craniotomy.

Small Animal RCC Compression

Rabbits were compressed in a specially built, small-animal RCC (Fig. 1). All compression phases lasted 2 min regardless of origin and target pressure.

Pial Arteriole Diameter Studies

The effect of different treatment regimens on pial arteriole diameter was measured using 20 of the 76 rabbits described in Surgical Preparation. A pial arteriole with an external diameter between 50 and 200 μm was selected for measurement. We have demonstrated that pial arterioles of this size trap gas emboli (9, 10). The segment of the arteriole where the measurements were performed was fixed at the intersection of the microscope ocular cross hairs. The microscope was locked in position except for the vertical focus control. Measurements were only performed at optimal focus, so that the distance from the segment being studied to the lens remained constant. Measurements were made at ×500.

The external arteriole diameter was calculated as the mean of 6 measurements performed on 3 successive photographs and 3 successive still frames of the video sequence. The diameter was measured with calibrated metal calipers (measurement error < 1%). Because of the diameter range of arterioles studied (50-200 μm), changes in diameter were recorded as fractional changes referenced to the original diameter.

The diameter of the selected arteriole was measured while the rabbit was breathing air. Each rabbit was then subjected to 1 of the 4 experimental conditions described in Preliminary Studies. The diameter was measured at 15-min intervals during the 30-min exposure.

Redistribution Studies

The success of different treatment regimens in redistributing entrapped gas emboli from pial arterioles was measured using the remaining 56 rabbits.

Each rabbit was fixed to a frame in a head-up posture at 10° to the horizontal (Fig. 1) and mechanically ventilated. Five-milliliter aliquots of air was infused into the femoral artery as microbubbles of less than 200 μm diameter, by using a RCC inlet of internal diameter 0.025 ml and a gas infusion rate of 0.2 ml/s (22). Three milliliter of normal saline was infused at 0.1 ml/s to clear all gas from the arterial line. Infusions were repeated at 2-min intervals until a gas embolus became trapped in a pial arteriole under observation on the brain surface.

Criterion for Pial Circulatory Arrest

We have demonstrated that trapped gas emboli can redistribute spontaneously from pial arterioles to the venous circulation at any time during the period of hypertension that follows CAGE (9, 10). Accordingly, each rabbit was ventilated at atmospheric pressure with air for 5 min after emboli were trapped. This delay ensured that MABP returned to preinfusion levels before treatment commenced. If the embolus redistributed during this period, further gas infusions into the femoral artery were performed. Infusions were repeated until another embolus became trapped. Only when an embolus remained trapped after MABP had returned to preinfusion levels was circulatory arrest assumed.

Electrocardiography recordings were used to confirm that animals had not suffered cardiac arrest. Cardiac arrest followed massive cerebral arterial hemorrhage in 1 rabbit; data from this rabbit were not included in any of the studies described here.

When circulatory arrest of a pial arteriole by a gas embolus had been confirmed, rabbits were subjected to one of the experimental conditions listed in Table 1. The volume of gas infused into the femoral artery to cause circulatory arrest for each rabbit was recorded. The ECG and the clearance of gas emboli from the pial arterioles were monitored for each animal. Rabbits ventilated only at atmospheric pressure were monitored without gas mixture change for 30 min.

Rabbits that were compressed and ventilated with air were monitored for 5 min at the target pressure. If emboli had cleared from the pial arterioles and the associated cardiac bradyarrhythmias had resolved the experiment was deemed successful, and terminated. If any emboli remained in the arterioles or if a bradyarrhythmia persisted in any form the rabbits initially compressed to 1.9, 2.8, or 4 bar were taken to 6 bar, and if necessary to 11 bar. The rabbits initially compressed to 6 bar without success were taken to 11 bar. All exposures at these increased pressures were also for 5 min.

Rabbits compressed to 2.8 bar and ventilated with oxygen were monitored for 5 min at the target pressure. If emboli had cleared from the pial arterioles and associated cardiac bradyarrhythmias had resolved the experiment was

Table 1
Experimental Conditions of Redistribution Studies

Experimental Group	Number of Rabbits	Ambient Pressure, bar	Ventilation Gas
1	5	1.01	air
2	5	1.01	oxygen
3	5	1.01	95% O_2:5% CO_2
4	5	1.9	air
5	5	2.8	air
6	15	2.8	oxygen
7	5	4.0	air
8	10	6.0	air

Note: 1.01 bar = atmospheric pressure

deemed successful, and terminated. If any emboli remained in the arterioles or if a bradyarrhythmia persisted in any form, rabbits were alternatively left at 2.8 bar without change of regimen for a further 25 min, or were compressed with air ventilation to 6 bar and if necessary to 11 bar. Subsequent exposures at 6 bar were for 5 min.

Statistical Analysis

Data were tested, as appropriate, by analyses of variance (ANOVA), chi-squared analyses, and paired and unpaired *t* tests.

RESULTS

Preliminary Studies

While breathing air, no significant differences occurred in cerebral venous P_{O_2} ($F = 0.4$), P_{CO_2} ($F = 0.43$), or MABP ($F = 0.65$) data between any of the groups (one-way ANOVA). The plateau cerebral venous P_{O_2}, P_{CO_2}, and MABP data are listed in Table 2. The data for air breathing at atmospheric pressure (1.01 bar) in this table are combined group data.

Arteriole Diameter Studies

The mean (± SD) fractional diameters of pial arterioles for each experimental condition are recorded in Table 3 and are displayed in Fig. 2. Testing the data with two-way ANOVAs showed no significant effects due to time alone ($F = 0.99$), whereas significant effects were produced by both the different experimental conditions alone ($F = 57$, $P < 0.001$) and the interaction of the different experimental conditions with time ($F = 2.49$, $P < 0.05$). Arteriole dilatation was produced by breathing either a 95% oxygen:5% carbon dioxide gas mixture at atmospheric pressure or oxygen at 2.8 bar. Arteriole constriction was produced by breathing either oxygen at atmospheric pressure or air at 6 bar.

Table 2
Mean (± SD) Cerebral Venous Partial Pressures of Oxygen and Carbon Dioxide, and Mean Arterial Blood Pressure Data Under Varied Experimental Conditions

Experimental Condition	Parameter, mmHg		
	PV_{O_2}	PV_{CO_2}	MABP
Air 1.01 bar, $n = 20$	69 ± 2.6	32.5 ± 0.6	79.6 ± 1.9
Oxygen 1.01 bar, $n = 5$	216 ± 24 ($P < 0.005$)	31.6 ± 1.5 ($P = 0.25$)	78.3 ± 7.1 ($P = 0.17$)
95% oxygen:5% carbon dioxide 1.01 bar, $n = 5$	235 ± 15 ($P < 0.001$)	45.4 ± 1.4 ($P < 0.001$)	81.2 ± 0.9 ($P = 0.21$)
Oxygen 2.8 bar, $n = 5$	516 ± 46 ($P < 0.001$)	32.4 ± .01 ($P = 0.51$)	102.0 ± 7.2 ($P < 0.05$)
Air 6 bar, $n = 5$	287 ± 32 ($P < 0.005$)	31.0 ± 0.9 ($P = 0.38$)	90.4 ± 4.5 ($P = 0.54$)

All data were tested by paired t tests.

Redistribution Studies

The effects of intraarterial gas infusion included significant but transient hypertension, loss of cerebrovascular autoregulation, dilatation of pial arterioles, and depression of respiration. Most gas emboli distributed spontaneously from the pial arterioles to the venous circulation. The mean volume of gas necessary to effect circulatory arrest in each experimental group is recorded in Table 4. The gas volumes did not differ significantly between groups (one-way ANOVA, $F = 1.36$).

Cerebral arterial gas embolism was always associated with cardiac bradyarrhythmias (Fig. 3). The effect of the selected treatments on the cardiac bradyarrhythmias is summarized in Table 5. Rabbits ventilated in atmospheric pressure with air, or oxygen, or 95% oxygen:5% carbon dioxide never demonstrated bradyarrhythmia resolution; all died. Survival times (mean ± SD) were 26 min ± 6.9 (air), 20.4 min ± 3.5 (oxygen), and 24.4 min ± 3.1 (95% O_2:5% CO_2). These survival times were not significantly different (one-way ANOVA; $F = 0.23$).

Compression produced correction of cardiac bradyarrhythmias for 30 of the 40 rabbits. A typical example is displayed in Fig. 3. Arrhythmia resolution occurred during the 2-min compression phase. The frequency of arrhythmia correction did not differ significantly between groups of rabbits compressed in the RCC, regardless of ambient pressure or ventilation gas ($\chi^2 = 0.1$, $df = 4$).

Table 3
Mean (± SD) Fractional Pial Arteriole Diameter Data
Under Varied Experimental Conditions

Experimental Condition	Fractional Arteriole Diameter		
	10 min	20 min	30 min
Oxygen 1.01 bar, $n = 5$	0.96 ± 0.02	0.92 ± 0.01	0.86 ± 0.03
95% Oxygen:5% carbon dioxide 1.01 bar, $n = 5$	1.06 ± 0.02	1.02 ± 0.01	1.01 ± 0.01
Oxygen 2.8 bar, $n = 5$	1.00	1.03 ± 0.02	1.04 ± 0.01
Air 6 bar, $n = 5$	0.88 ± 0.01	0.87 ± 0.02	0.84 ± 0.02

In contrast, the frequency of arrhythmia correction was significantly greater for rabbits compressed in the RCC than for rabbits kept at atmospheric pressure ($\chi^2 = 10.4$, $df = 1$, $P < 0.01$).

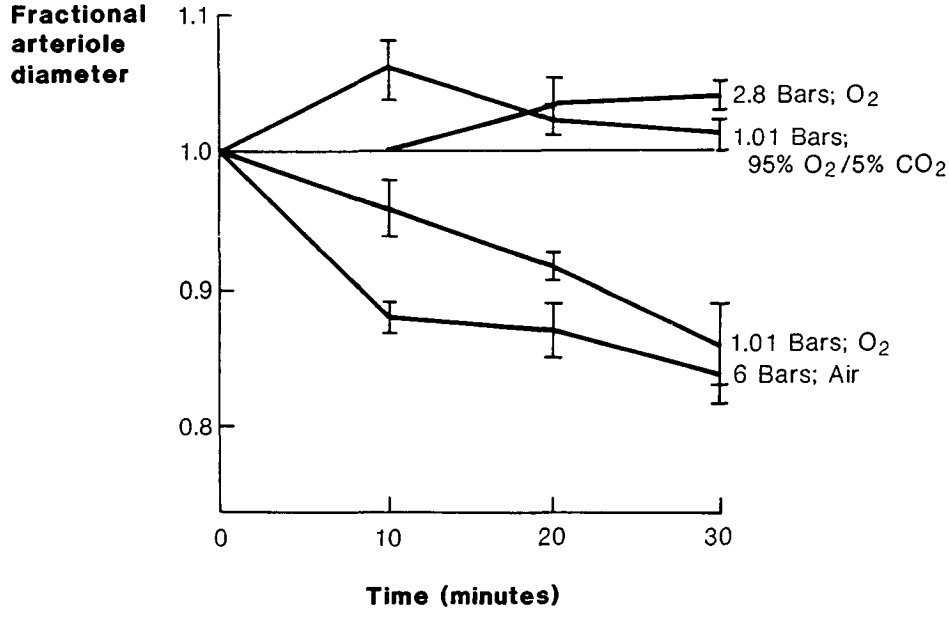


Fig. 2. Mean (± SD) fractional pial arteriole diameters in rabbits under varied experimental conditions.

Table 4
Mean (± SD) Volumes of Gas Infused into the Femoral Artery of Rabbits to Cause Circulatory Arrest of an Observed Pial Arteriole

Experimental Condition	Mean Volume of Gas to Effect Arrest, ml
Air 1.01 bar, $n = 5$	10.0 ± 1.6
Oxygen 1.01 bar, $n = 5$	10.8 ± 0.4
95% oxygen:5% carbon dioxide 1.01 bar, $n = 5$	14.0 ± 1.1
Air 1.9 bar, $n = 5$	12.0 ± 1.1
Air 2.8 bar, $n = 5$	11.0 ± 0.9
Oxygen 2.8 bar, $n = 15$	13.3 ± 0.9
Air 4 bar, $n = 5$	12.0 ± 1.1
Air 6 bar, $n = 10$	11.8 ± 1.2

Eight of the 10 rabbits whose arrhythmia was not corrected with initial compression were subsequently compressed to a greater pressure (Materials and Methods). Bradyarrhythmias were never corrected by these additional compressions. The remaining 2 rabbits without bradyarrhythmia correction were compressed initially to 2.8 bar with oxygen ventilation. These 2 animals were maintained at this pressure for a further 25 min without success.

In rabbits receiving compression, the volume of gas needed to cause circulatory arrest of a pial arteriole did not differ significantly between those rabbits with 12.7 ml ± 3.2 [SD]), and those without (13.2 ml ± 3.2 [SD]) subsequent arrhythmia correction ($t_{38} = 0.05$).

The effect of the different treatments on the clearance of gas emboli from pial arterioles is summarized in Table 6. Rabbits ventilated at atmospheric pressure with air, or oxygen, or with a 95% oxygen:5% carbon dioxide gas mixture did not demonstrate embolus clearance.

Compression produced total or partial clearance of gas emboli in 28 of the 40 rabbits. Embolus clearance occurred either during or within 2 min of compression and was accompanied by the return of normal cardiac rhythm (Fig. 3). The frequency of complete embolus clearance did not differ significantly between groups of rabbits compressed in the RCC, regardless of ambient pressure and ventilation gas or gas mixture ($\chi^2 = 0.3$, $df = 4$). In contrast, the frequency of complete embolus clearance was significantly greater for

Table 5

The Effect of Treatment Regimens on Cardiac Bradyarryhthmias Induced in Rabbits by Experimental CAGE

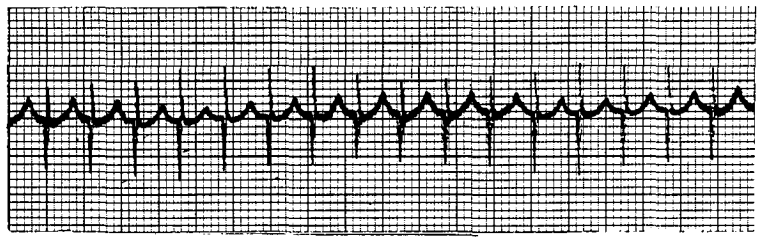
Experimental Condition	Arrhythmia Corrected	Arrhythmia not Corrected
Air 1.01 bar, $n = 5$	0	5
Oxygen 1.01 bar, $n = 5$	0	5
95% oxygen:5% carbon dioxide 1.01 bar, $n = 5$	0	5
Air 1.9 bar, $n = 5$	4	1
Air 2.8 bar, $n = 5$	4	1
Oxygen 2.8 bar, $n = 15$	11	4
Air 4 bar, $n = 5$	4	1
Air 6 bar, $n = 10$	7	3

rabbits compressed in the RCC than for rabbits kept at atmospheric pressure ($\chi^2 = 9.11$, $df = 1$, $P < 0.01$).

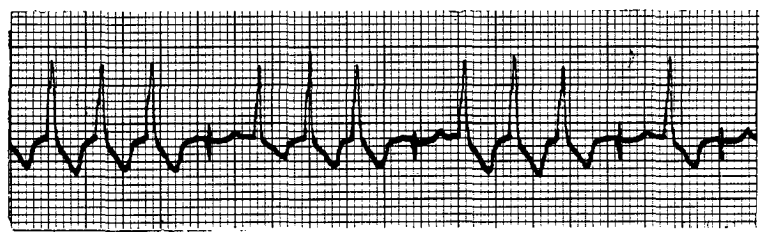
Twenty-one rabbits compressed in the RCC did not demonstrate complete gas embolus clearance with initial compression. Seventeen of the 21 were subsequently compressed to a greater pressure (Materials and Methods). Additional compression caused further embolus clearance for only 2 of these 17 rabbits. One rabbit initially compressed to 4 bar without success, showed complete embolus clearance during further compression to 6 bar. Another rabbit was initially compressed to 1.9 bar and then to 6 bar without success, but demonstrated complete embolus clearance with further compression to 11 bar. In this rabbit, embolus clearance was accompanied by pial arterial hemorrhage and cardiac arrest.

The other 4 rabbits with incomplete embolus clearance were initially compressed to 2.8 bar with oxygen ventilation. These rabbits were maintained at this pressure for another 25 min without success.

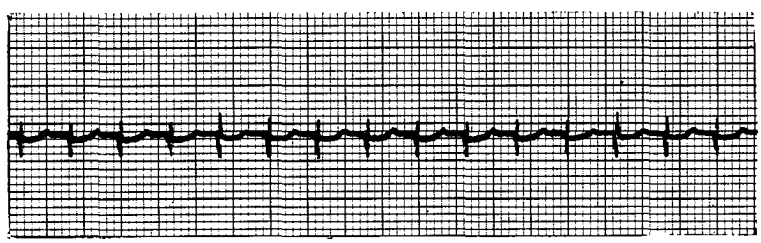
In rabbits receiving compression, the volume of gas needed to cause circulatory arrest of a pial arteriole did not differ significantly between those rabbits with (13.3 ml ± 3.5 [SD]) and those without (12.1 ml ± 3.2 [SD]) subsequent embolus clearance ($t_{38} = 1.15$).



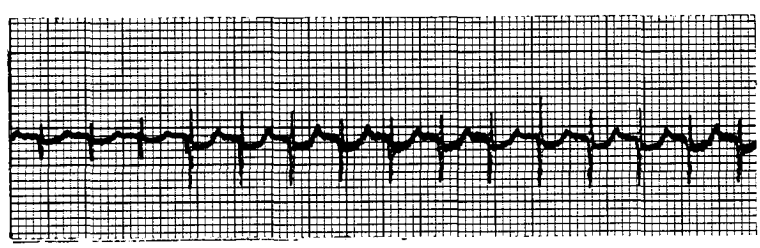
PRE-EMBOLISM



2 MINS POST-EMBOLISM



DURING COMPRESSION



1 MIN POST-COMPRESSION

Fig. 3. The effect of compression on a cardial bradyarrhythmia induced in a rabbit by experimental CAGE.

Table 6
The Number of Rabbits with Embolus Clearance from Observed Pial Arterioles under Varied Experimental Conditions

Experimental Condition	Complete Embolus Clearance	Incomplete Embolus Clearance	No Embolus Clearance
Air 1.01 bar, $n = 5$	0	0	5
Oxygen 1.01 bar, $n = 5$	0	0	5
95% oxygen:5% carbon dioxide 1.01 bar, $n = 5$	0	0	5
Air 1.9 bar, $n = 5$	2	2	1
Air 2.8 bar, $n = 5$	3	0	2
Oxygen 2.8 bar, $n = 15$	7	2	6
Air 4 bar, $n = 5$	2	2	1
Air 6 bar, $n = 10$	5	3	2

DISCUSSION

Preliminary Studies

With the exception of rabbits breathing oxygen at 2.8 bar, the values of MABP were consistent with previous studies that reported that significant changes in blood pressure do not occur under hyperbaric conditions (23, 24). The reason for the increase in MABP in rabbits breathing oxygen at 2.8 bar was not addressed. The values of MABP at atmospheric pressure were within the range that produces normal cerebrovascular autoregulation and cerebral vasoreactivity in rabbits subject to halothane anesthesia (18, 20). The cerebral venous P_{CO_2} values did not change when breathing oxygen at 2.8 bar, which agreed with one researcher's findings (24); in contrast, other authors report increased cerebral venous P_{CO_2} values with this regimen (25, 26).

Arteriole Diameter Studies

Breathing oxygen at atmospheric pressure (P_{O_2} = 1.01 bar) and breathing air at 6 bar (P_{O_2} = 1.26 bar) caused immediate constriction of pial arterioles. Vasoconstriction of cerebral arterioles in response to similar P_{O_2} exposures has been shown by other workers (27-32). Because spontaneous embolus redistri-

bution usually occurs within several minutes of embolus entrapment, and pial arterioles remain reactive despite CAGE (9), both regimens would increase embolus length during the critical time of spontaneous redistribution. This would oppose, at least in part, the benefits of any gas volume reduction with these regimens.

Breathing a 95% oxygen:5% carbon dioxide mixture at atmospheric pressure (Po_2 = 0.96 bar) caused immediate dilatation of cerebral arterioles, without changing MABP. This increase in vessel diameter would reduce embolus length, enhancing redistribution (9, 10). With cerebrovascular autoregulation being disrupted by CAGE (14, 15, 17), this regimen would also increase cerebral blood flow (CBF). Increases in CBF are important in the treatment of CAGE, because restoration of flow above neuron-disabling levels was shown to be critical to outcome in experimental animals (33-37). The probable mechanism underlying the dilatation of pial arterioles was the increase in cerebral venous Pco_2 (29, 32, 38-40). Reservations have been expressed about the clinical use of carbon dioxide admixtures (41), but the benefit of breathing oxygen with a 5% carbon dioxide admixture has been demonstrated by other researchers in an experimental CAGE model (6).

Breathing oxygen at 2.8 bar caused dilatation of cerebral arterioles that was detectable after 20 min. Although the immediate gas volume reduction with compression on oxygen to 2.8 bar would be less than that with compression on air to 6 bar, the effects of the latter would be opposed by the associated vasoconstriction. Conversely, a 2.8 bar oxygen regimen would increase both cerebral arteriole diameter and MABP, and hence would increase CBF following CAGE (14, 15, 17). This argument is supported by the significantly greater CBF seen in dogs with CAGE at 2.8 bar breathing oxygen than at 6 bar breathing air (42).

Cerebral vasodilatation has been reported with 2.8 bar oxygen exposures (27-29, 31, 32). The mechanism proposed by the authors of these reports , one of increased cerebral tissue Pco_2 as a consequence of saturation of hemoglobin with oxygen (27, 28, 32), was not supported by the data from this study (Results, Preliminary Studies).

Redistribution Studies

Multiple infusions of gas into the femoral artery, as well as large volumes of gas relative to previous studies (6, 7, 12-15, 33-37, 42-45), were necessary to cause circulatory arrest of a pial arteriole. The significant number of gas emboli spontaneously redistributing to the venous circulation was in agreement with the findings of other workers (6, 11-16). The reasons for the relatively large gas volumes needed in this study include the method of defining an endpoint of circulatory arrest and the site of gas infusion.

Previous animal-model studies of CAGE treatment have not measured or allowed for spontaneous embolus redistribution (6, 7), and it follows that the embolus redistribution seen in these experiments may have occurred independently of the treatment. Emboli from carotid artery gas infusions (6, 7, 12-15,

33-37, 42-45) may bypass the brainstem circulation and so avoid triggering an increase in blood pressure (1, 6, 9-11, 14, 44-46). We have demonstrated that the transient and significant increases in blood pressure that accompany CAGE are critical to gas embolus distribution (9, 10). Carotid artery cannulation itself may alter cerebral perfusion and hence further distort the balance of forces that determine embolus distribution.

The gas volumes needed to produce pial circulatory arrest in our rabbit studies were much greater than those used in other animal-model studies (12, 42-45, 49). Those studies have reported significant brain pathology. It is likely that such pathology can be associated with CAGE even though cerebral circulatory arrest, due to gas emboli, may not have occurred.

Cerebral arterial gas embolism was always associated with cardiac bradyarrhythmias and with significant but transient hypertension. Since treatment regimens were not introduced until the MABP had returned to preinfusion levels, the effect of the regimen on the blood-pressure changes that accompany CAGE was not measured.

No rabbits ventilated at atmospheric pressure demonstrated either embolus clearance or correction of the associated cardiac bradyarrhythmias. Although no significant differences occurred between any of the atmospheric pressure groups, theoretic arguments do support the use of oxygen with a carbon dioxide admixture rather than oxygen during the first-aid treatment of CAGE (6).

Compression should remain an important part of CAGE treatment because rabbits compressed in the RCC fared significantly better than rabbits kept at atmospheric pressure, both for gas embolus clearance and correction of cardiac bradyarrhythmias.

No significant differences occurred among any of the compression regimens tested in this study; consequently the ideal compression regimen for CAGE treatment was not identified. The negligible benefit derived from further compression to 6 and 11 bar, in those animals whose initial compression was unsuccessful, suggests that this practice may be of limited therapeutic value.

Some authors have proposed the substitution of the conventional 6 bar air treatment of CAGE with a 2.8 bar oxygen treatment (42-45). This proposal is supported by the argument that additional gas volume reduction at 6 bar, whether breathing air or a 32.5% oxygen:67.5% nitrogen gas mixture as advocated by one author (50-52), will be opposed by cerebral vasoconstriction due to the partial pressure of oxygen (27-32, 38, 53). Compression to 6 bar may be more effective if such vasoconstriction could be opposed by breathing either a 50% oxygen:50% nitrogen gas mixture (Po_2 = 3 bar) (24-28, 30, 39) or air with a carbon dioxide admixture (27, 29, 32, 38-40).

In rabbits compressed to 2.8 bar the clearance of emboli from pial arterioles, and the correction of bradyarrhythmias, did not differ significantly between those ventilated with oxygen and those ventilated with air. One explanation is that 2.8 bar of oxygen did not produce a detectable increase in

pial arteriole diameter until 20 min after its introduction. Four rabbits initially compressed to 2.8 bar with oxygen ventilation did not demonstrate embolus clearance even when maintained on this regimen for a further 25 min. This lack of success was perhaps predictable from our earlier studies, which demonstrated that gas emboli could only be redistributed from pial arterioles within 15 min of circulatory arrest (10).

This study demonstrates that clearance of gas emboli trapped in pial arterioles and correction of associated cardiac bradyarrhythmias can be produced as readily with minimal compression (1.9 bar) as with compression to the conventional treatment pressure of 6 bar. The study also demonstrates that further compression has negligible benefit if the initial treatment pressure is unsuccessful. It follows that the selection of the optimal compression regimen, from among those tested, should be determined by its effect on CBF and gas embolus resolution (33-37).

Of the regimens tested, compression to 2.8 bar breathing oxygen is favored for many reasons. This regimen was as effective as any tested at redistributing emboli from pial arterioles and correcting associated cardiac bradyarrhythmias. Studies of the recovery of somatosensory cortical evoked response in dogs with CAGE displayed no advantage for 6 bar air compression over 2.8 bar oxygen compression (42-45). Recovery from CAGE correlates with restoration of CBF (33-37), which will be greater at 2.8 bar breathing oxygen than at 6 bar breathing air (45). In addition, breathing oxygen at 2.8 bar has been demonstrated to be an effective method of gas embolus resolution (54, 55); this practice also provides access to decompression regimens that are of short duration, cause negligible inert-gas narcosis, and rarely cause decompression sickness in medical attendants (54, 55).

Most authors advocate an initial 30 min breathing air or an oxygen-nitrogen mixture at 6 bar, before decompression to 2.8 bar and a change to oxygen, as the ideal treatment of CAGE (1, 3, 7, 8, 50-52, 54, 55). This practice conflicts with the observation that decompression of CAGE patients from 6 bar after only 30 min is associated with a significantly greater relapse rate than if decompression is delayed for 1 or 2 h (3, 4).

Despite compression to 6 bar 5 min after gas emboli were trapped in pial arterioles, 30% of rabbits in this study did not demonstrate correction of cardiac bradyarrhythmias and 20% did not experience any embolus clearance. These results do not agree with those from other animal-model studies of CAGE, where compression to 6 bar effected rapid clearance of gas emboli from pial arterioles (6, 7). However, spontaneous redistribution of emboli, shown by our previous studies to occur with more than 75% of emboli (9, 10) was not measured in those studies.

Most CAGE patients compressed to 6 bar within 5 min of onset of symptoms and signs have obtained complete relief within 5 min (3, 4, 56). In contrast, 20% of the rabbits in this study compressed to 6 bar, 5 min after gas emboli were trapped in a pial arteriole, did not demonstrate embolus clearance. Embolus clearance under the same regimen was incomplete in a further 30% of

rabbits. It can be argued from these data that the recovery of some CAGE patients is, for the most part, the result of spontaneous redistribution of emboli to the venous circulation. The argument is supported by many other findings. A number of researchers have also demonstrated significant levels of spontaneous redistribution of gas emboli from cerebral arteries to the venous circulation (6, 11-16). Repeated intraarterial gas infusions have been needed in other studies to maintain a constant decrement in brain function in experimental animals (33-37). Many CAGE patients have experienced partial or complete recovery without compression in a RCC (50, 57).

SUMMARY AND CONCLUSIONS

Anesthetized rabbits breathing oxygen at atmospheric pressure or air at 6 bar demonstrated immediate cerebral arteriole constriction, but MABP did not change significantly. Breathing 95% oxygen:5% carbon dioxide at atmospheric pressure caused immediate pial arteriole dilatation without producing any significant change in MABP. In contrast, breathing oxygen at 2.8 bar caused both a delayed pial arteriole dilatation, and an increase in MABP. Inasmuch as cerebral arterial gas embolism disrupts cerebrovascular autoregulation, both regimens would increase cerebral blood flow. The pial vasodilatation due to breathing oxygen at 2.8 bar was not the result of an increase in cerebral venous P_{CO_2}.

Multiple, large-volume, intraarterial gas infusions were necessary to produce gas emboli that caused prolonged circulatory arrest in pial arterioles, because most emboli redistributed spontaneously to the venous circulation. Cerebral arterial gas embolism was always associated with cardiac bradyarrhythmias and with significant but transient hypertension.

Treatment at atmospheric pressure never produced clearance of gas emboli from observed pial arterioles or corrected the associated cardiac bradyarrhythmias. Conversely, compression resulted in some embolus clearance for 70% of rabbits and cardiac bradyarrhythmia correction for 75% of rabbits. The frequency of success between compression groups and atmospheric groups was significant, but the difference between specific compression regimens was not significant. If initial compression was unsuccessful, further compression was of negligible benefit.

Of the regimens tested, compression to 2.8 bar on oxygen is favored for CAGE treatment because it will both increase CBF and promote resolution of gas emboli.

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SESSION 17: POSTER PRESENTATIONS
HYPERBARIC OXYGEN THERAPY AND DIVING MEDICINE

9th International Symposium on Underwater and Hyperbaric Physiology
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COMPREHENSIVE APPROACH TO CARBON MONOXIDE POISONING

R. A. M. Myers and S. K. Snyder-White

Carbon monoxide (CO) poisoning remains the most important single poisoning agent in the United States from the point of view of morbidity and mortality. At the present time it accounts for roughly 50% of all poisoning deaths in the country. In spite of this, there is no generally accepted method of assuring the degree of neurologic impairment in the CO victim, and there is almost a total lack of information on the long-term follow-up of CO victims (1). We believe a new approach in determining the neurologic status of the CO victim is essential and should take into account the wide range of neuropsychiatric abnormalities that have been described in CO poisoning. [Almost every known neurologic syndrome can occur following CO poisoning (2-4)].

In our work (5) we were able to document a 12% recurrence of sequelae following CO exposure and an apparent adequate treatment with normobaric oxygen. Having noted a very wide range of symptomatology as compared to levels of carboxyhemoglobin, we believe that the traditional methodology for assessing CO poisoning using carboxyhemoglobin levels is inadequate. We have observed patients with significant CO poisoning sequelae with zero levels of CO (6). We have also seen patients with minimal symptomatology from CO poisoning with high levels of carboxyhemoglobin. For this reason, we have determined that the carboxyhemoglobin level alone is an inadequate indicator of the severity of CO poisoning.

MATERIALS AND METHODS

Between 1973 and 1985, 315 CO-poisoned patients were treated at the Maryland Institute for Emergency Medical Services Systems (MIEMSS). The primary sources of CO exposure were smoke inhalation and fires, incomplete combustion from automobiles, faulty home-heating systems, and job-related environmental problems. Our group includes those patients with accidental

exposure as well as those with suicidal intent. In our early years, factors dictating treatment were the carboxyhemoglobin levels and unconsciousness, but after years of experience we now use the result of a psychometric screening test (7, 8) and the carboxyhemoglobin level as indicators for the type of treatment necessary.

A smoke inhalation detection program has been implemented for severely involved patients throughout Maryland, particularly through the central region of the state. The protocol for prehospital care providers dictates that all patients who are unconscious or who show neurologic impairment, with either excessive aggressiveness, confusion, lethargy, or unresponsiveness, must have a field blood sample taken at the time the intravenous line is inserted. Oxygen treatment by a face mask with a reservoir oxygen bag in-line is commenced, and the patient is transferred directly to MIEMSS Shock Trauma Center, bypassing the local hospitals. This complies with the Maryland echelons of care protocol for triage and treatment of seriously injured or ill patients.

An intensive educational program for prehospital care providers, including those who are part of the fire departments, was undertaken to assist them in determining which patients should be referred to specialty referral centers. Since the implementation of the program, the number of patients referred annually to MIEMSS for CO and smoke inhalation assessment has risen from 2 to 65. Taking carboxyhemoglobin field samples enables us to follow the half-life of CO in these patients, and it is apparent that traditionally accepted values do not hold (8).

RESULTS

There have been 315 patients assessed. Of these, only 247 have passed through the program since the incorporation of the psychometric test as the major clinical determinate of type of oxygen treatment to be followed. The age range of the patients has been from 8 mo. to 102 yr. (There were 25 pediatric patients who could not be assessed by the psychometric test, because the tests have been specifically designed for an adult population that has achieved at least a grade 7 education.) Table 1 reflects the complete age range.

There were 138 male and 109 female patients, with peak age group involvement between 21 and 30 yr. In general, there was a fairly even distribution among all ages up to 60, with the bulk of cases occurring between age 21 and 50.

Table 2 shows the cause of the CO exposure. Of the 243 cases documented, 103 were a consequence of a fire with smoke or toxic gas inhalation; 59 occurred as a result of automobile emissions, and of these, 22 were attempted suicides. The other three attempted suicides were by fire. Only 25 of the 243 cases documented were definite suicide attempts; it was not possible to determine whether others were failed suicide attempts, because patients denied this. Thus, only 10% of cases represented suicide. Seventy-six cases were due to faulty furnaces in homes where it is possible that the patient received

Table 1
Age Range of Patients With Acute CO Poisoning

Age Range	Number of Patients	
	Male	Female
0 - 10	11	14
11 - 20	18	10
21 - 30	40	18
31 - 40	27	23
41 - 50	20	19
51 - 60	13	16
60+	9	9
Totals	138	109

repeated exposures, although their condition at admission reflected acute exposure. Several of our patients were admitted after chronic exposure but with acute symptomatology. Once the diagnosis was made, the furnace fault was corrected.

Table 2
Causes of CO Poisoning

	Accident	Suicide	Total
Fire/smoke	100	3	103
Automobile	37	22	59
Boat	5		5
Furnace	76		76
Unknown			1
Totals	218	25	244

In Maryland, our field protocol allows us to take a blood sample at the time of insertion of the i.v. line for resuscitation. Thus we can obtain carboxyhemoglobin levels at the scene when resuscitation begins. This blood sample accompanies the patient into the hospital, so we can compare the initial highest carboxyhemoglobin level with the level at the time of admission, usually between 15 and 40 min later. The exact time is determined by the distance traveled and the continued need for field resuscitation vs. direct hospitalization. Because of this protocol, ambulances are able to bypass hospitals to come to our facility for assessment of serious poisoning.

Consequently, we are now able to observe the differences in reported signs and symptoms and carboxyhemoglobin levels at the various locations, such as the scene or hospital.

The only patient presenting with carboxyhemoglobin levels of more than 70% was a suicide via automobile emission. The patient was found comatose, with poor breathing and cardiac function. Blistering was present on the hands although there was no evidence of thermal burns. Resuscitation was undertaken, and the patient had a cardiac arrest that necessitated on-the-scene therapy, including defibrillation, before transfer. On admission, he was noted to have a normal rhythm, but with fixed dilated pupils, and was not breathing. In accordance with our protocol, he was given a single hyperbaric treatment followed by a detailed neurologic assessment. It was determined that the patient had no functional reflexes, further resuscitation was stopped, and the patient was declared dead.

Of the 38 unconscious or comatose patients (Table 3), 18 had carboxyhemoglobin levels of more than 40%. The other 20 had levels of less than 40% and in some situations between 0 and 10%, and yet the patient presented in an unconscious or comatose state. This is contrary to the traditionally presented views of Sayer and Davenport (9), in which the comatose patients have levels of more than 60% and decreasing levels of symptomatology below that. At the other extreme are the 2 patients presenting with no symptoms, awake, and alert, yet with carboxyhemoglobin levels of more than 50%. Five patients presented with carboxyhemoglobin levels of more than 30% but were totally asymptomatic and awake. This contrasts strongly with the 14 patients who were unconscious but with levels less than 30%. The most common symptoms observed in our patients were headache and lightheadedness, followed by sleepiness, lethargy, or confusion, then dizziness and nausea. The least common symptoms were brief loss of consciousness, tiredness, irritability, agitation, and combativeness. It is obvious that symptomatology can occur at any level of carboxyhemoglobin. The same symptoms are seen from the highest and lowest levels of carboxyhemoglobin. We believe that this shows conclusively that, with today's enhanced retrieval mechanisms, earlier treatment and resuscitation with oxygen, and fewer patients who are exposed to continuous, long-term CO poisoning, a new set of symptoms compared to carboxyhemoglobin levels should be developed. Sayer's work (9) represents the types of cases seen in the 1930s when there was long exposure, a slow journey to the hospital, no application of oxygen as the treatment modality, and only room air to breath.

Table 4 shows the symptoms experienced by the patient at the time of admission. Generally, 15 to 45 min elapses between the initial recording and sampling of blood at the time of resuscitation and the time of admission. The originally high level of comatose patients has dropped from 38 to 10. Those presenting with symptomatology of irritability, agitation, or combativeness increased from 7 to 21. There were more sleepy and lethargic patients reflecting an improvement in symptomatology, but poisoning involvement from CO still existed. There were 112 patients awake and asymptomatic as compared

Table 3
Symptoms vs. Scene HbCO Levels

HbCO level	Uncons. Coma	Irritable Agitation	Sleepy Lethargic	L.O.C. briefly	Nausea	Dizziness	Headache Lightheadness	Asymp. Awake	Total
> 71	1								1
61 - 70	1								1
51 - 60	6	1	2					1	10
41 - 50	10	1		1	1	4	14	1	19
31 - 40	6		3		2	2	5	3	21
21 - 30	5	2	1	1		3	4	8	24
11 - 20	3	2	6	2	3	4	7	11	38
0 - 10	6	1	14	5	12	12	13	14	77
Totals	38	7	26	9	18	25	30	38	191

Table 4
Symptoms vs. Admission, HbCO Levels

HbCO level	Uncons. Coma	Irritable Agitation	Sleepy Lethargic	L.O.C. briefly	Nausea	Dizziness	Headache Lightheadness	Asymp. Awake	Total
> 71	1								1
61 - 70									
51 - 60	2								2
41 - 50	1		1						2
31 - 40	1	4	4				2	3	14
21 - 30	1	4	14		2	3	8	16	48
11 - 20		2	8			3	8	18	39
0 - 10	4	11	30	2	10	16	33	75	181
Totals	10	21	57	2	12	22	51	112	287

to the 38 initially seen at the scene, and the carboxyhemoglobin levels had dropped considerably. It is still important to note that at least 3 patients presented asymptomatic and awake, with carboxyhemoglobin levels of more than 31%. Twenty out of 28 patients presented with coma or unconsciousness and had carboxyhemoglobin levels of less than 40%. Nine of these patients actually had carboxyhemoglobin levels of less than 20% and were still unconscious. From Table 4 it is apparent that neurologic symptomatology predominates, and very few patients complained of nausea and dizziness. The majority of patients presented with the symptoms of irritability, agitation, sleepiness, lethargy, headache, or lightheadedness. The commonest body system to be involved and to require full assessment is the central nervous system.

Table 5 reflects the cardiac rhythm as compared to the psychometric test, which is undertaken after the initial resuscitation has been completed. Of the 54 patients assessed, 45 had normal cardiac rhythm on ECG. Two patients presented with asystole. Both were initially revived and resuscitated, developed a normal heart beat, and were then treated with hyperbaric oxygen. One of the patients was unconscious and had lost all reflex function. He was declared brain dead after hyperbaric oxygen therapy. The other asystolic patient, on resuscitation with CPR, regained his normal rhythm and recovered. Other ECG changes were flattening of the ECG complex, evidence of old ischemia, and unifocal PVCs. In general, those patients with arrhythmias did not experience major complications, and in our experience cardiac problems have not caused morbidity. Those patients considered dead on arrival at the hospital generally had a cardiac insult which was the cause of death. Of the 8 patients who died as a result of CO poisoning and smoke inhalation, 1 died in the admitting area as a consequence of cardiac arrhythmias and did not respond to normal medical resuscitation. Two of the patients presented in coma with absent reflexes, and after a single hyperbaric treatment they were declared brain dead. The remainder of the patients died in the critical care or intensive care units from smoke inhalation and acute respiratory failure.

We also reviewed recurrent symptomatology posttreatment with no further exposure to CO poisoning and found that of 247 patients assessed, symptoms recurred in 16. All of these patients were treated with surface oxygen because they were mild cases of CO poisoning not requiring hyperbaric oxygen therapy. The CO level in 3 of the patients was 25%; however, a 4th patient's level of CO was 49% at the initial receiving hospital, and by the time we received the patient her level was 17%. Psychometric testing on these patients was in the normal range, which is why they were treated with surface oxygen. None of the hyperbaric-treated patients developed symptoms of recurrence. The time lag for presentation of recurrent symptoms was from 24 h to 30 d, although the majority of cases presented in the first 8 d. Only 2 patients developed recurrent symptoms beyond 8 d; 1 at 21 d and the other at 30 d. The major recurrent symptom in these patients was severe headache, and they needed follow-up treatment. All 16 patients with recurrent symptoms (Table 6) were subsequently treated with hyperbaric oxygen and showed a complete resolution

Table 5
Cardiac Rhythm vs. Psychometric Test

	Psychometric Test				
Rhythm	Abnormal	Borderline	Normal	Unable to Do	Total
Asystole			1	1	2
Flat waves			1		1
Old ischemia	1		2	1	4
Unifocal PVC		1	1		2
Normal	6	3	36		45
Totals	7	4	41	2	54

Table 6
Numbers of Patients with Recurrent Symptoms*

	Day of Recurrence												
Capboxyhemoglobin Level, g%	1	2	3	4	5	6	7	8	9	10	11	21	30
0	3		1										
5		1	4		1			1					1
25	2							1					
50												1	

* All patients with recurrent symptoms had been treated initially with surface oxygen via face mask. None of the patients treated initially with HBO developed recurrent symptoms.

of symptoms. Further follow-up of these patients showed no other recurrence of symptoms when observed at 6 mo. to 1 yr.

CONCLUSIONS

This study was designed to ascertain the effectiveness of carboxyhemoglobin level in the determination of clinical signs and symptoms of CO poisoning. From our results it is apparent that CO levels alone are a poor indicator of symptoms. Many of our patients presented with low levels of CO poisoning and yet were comatose, whereas more than 40% presented with high levels and were totally asymptomatic. It may be that the major difference between today's patient and those previously described by Sayer and Davenport (9) is a reflection of a longer exposure, a poorer resuscitation, and the lack of oxygen therapy in the prehospital phase. The further need to review the

classic descriptions of CO poisoning is reflected in our paper (6) reporting zero levels of CO poisoning after an aggressive 4 h of surface oxygen in intubated and ventilated patients, with no improvement. This study demonstrated the need for a more sensitive technique to assess the neurologic involvement of the patient. Consequently, the psychometric test was developed (7, 8). This test provides greater reliability than CO levels for determining CNS involvement. Our results demonstrate that the patient's neurologic condition dictated his ability to do the psychometric test. Those patients unable to do the test because of mental impairment from CO poisoning were given hyperbaric oxygen as their primary mode of therapy. We now use the psychometric test as our major determinator for hyperbaric therapy. Treated patients who are not yet recovered to normal status, as shown by the psychometric test, are given repetitive hyperbaric treatments until their psychometric tests are normal. The value of the psychometric test has been further demonstrated in our more seriously involved patients with levels of CO more than 40% or with abnormal psychometric test who have all been treated with hyperbaric oxygen. There have been no recurrences of symptoms. Less serious cases, with levels of CO below 30% and with a normal psychometric test, have been treated with surface oxygen until their carboxyhemoglobin levels have been less than 5 g% in 2 successive h. In this group there has been an 8 to 10% recurrence of symptoms with no further exposure to CO poisoning. Those persons presenting with recurrence of symptoms are generally abnormal on their repeated psychometric test, and after therapy become normal and asymptomatic.

The only patients we have seen with the "classic" description of cherry red mucosa, face, and extremities have been those with a long exposure to CO whose tissues are soaked with CO. These patients often have levels in the 40 to 50% range. They are comatose and require repetitive hyperbaric treatments to effect recovery. In this group it is difficult to know whether the recovery has been complete and the patient is back to his normal status or whether the deficiencies determined by detailed psychometric testing reflect CO brain injury. The major work in this situation was that of Smith and Brandon (1). This is an area requiring future study as well as an elucidation of the pathophysiology. It is possible that the secondary deterioration may be an autoimmune response.

We strongly advocate the use of the psychometric test to help determine the extent and diffuseness of the encephalopathy that has ensued after CO poisoning. The results will be the prime determinator of whether the mode of therapy should be surface or hyperbaric oxygen. Using this technique to assess CO poisoning in patients, we believe that those showing the potential for secondary deterioration or recurrence of sequelae should be treated primarily with hyperbaric oxygen. Should symptomatology recur, later treatment with hyperbaric oxygen will be effective in returning the patient to normal physiology and an asymptomatic condition.

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SYMBOL DIGIT MODALITY TEST AND CARBON MONOXIDE ENCEPHALOPATHY

I. B. Gensemer and G. H. Cohn

Patients who come to the emergency room with carbon monoxide encephalopathy show varying degrees of cognitive competency. Frequently it is easy to identify mental impairment in these patients because they are so clearly confused, but on other occasions it is less clear, even to the patient, that some degree of mental change has occurred. Brief neuropsychologic testing (1) can detect subtle changes and provide a useful tool for assessing cognitive competence and improvement, which may become valuable in making treatment decisions (2-5).

Comprehensive neuropsychologic testing is time-consuming and requires laboratory conditions if detailed results are desired. Unfortunately, the emergency room does not often provide the type of controlled environment that is best for detailed testing, and usually decisions must be made fairly quickly without adequate time for extensive testing.

MATERIALS AND METHODS

The Symbol Digit Modality Test (SDMT) is a standardized examination that requires 90 s of administration time and can be completed within 2 to 3 min. This is a brain-sensitive test that measures the speed with which an individual can process information. It is easy to administer, oral or written, and provides a quick measure of cognitive function. The SDMT has been shown to be sensitive as a measure of recovery of cerebral function. If it is used infrequently, it is not seriously influenced by practice effect. Essentially equivalent forms can be constructed if practice effect becomes a concern. In this study, oral responses were used, numbers were written by the examiner, and the score is the number of correct responses.

Ten patients who experienced carbon monoxide poisoning were selected for study. They ranged from 16 to 70 yr of age. Eight of the patients were alert enough to complete the SDMT before hyperbaric oxygen therapy. All 10 were able to complete the test after 1 treatment.

RESULTS

Table 1 shows the results of the examination of these patients and their carboxyhemoglobin level before treatment.

Table 1
Test Results

Patients,					
Age, yr	Sex	COHb, %	Before Treatment	After Treatment	Normal
16	M	36.0	29	41	49
17	M	33.4	70	92	50
21	F	29.0	0	13	61
22	F	18.2	43	48	61
29	M	60.0	0	45	61
36	M	6.2	29	41	60
38	M*	31.3	61	66	61
42	M*	30.4	60	68	61
69	F	14.8	4	19	42
70	F	18.6	25	48	42

* Attended college. $t = 4.25$, $P < 0.005$.

DISCUSSION

Every patient improved on the test after hyperbaric oxygen therapy. They were able to correctly respond to more items, indicating that their information processing capacity had improved, although their levels of performance varied widely both before and after treatment, as did degree of improvement. It seems clear that some of the patients had more ability than others before they experienced carbon monoxide poisoning, and probably some patients were seriously impaired in their information processing capacity before their initial treatment. Practice effect may have been to some extent a factor in the improved scores, but it seems unlikely that this played a significant role in most instances because the test was only repeated once and most of the patients were not fully alert the first time. Repeated administrations of the same test, however, can be expected to result in practice effect, especially in

patients who are functioning at a reasonably high level. It is possible to rearrange the order of the symbols and numbers in such a way that alternate forms are created. The normative data provided in Table 1 may be slightly high for those individuals 21 yr of age and over because the norms were established on the basis of an oral administration after a written administration, thus there would have been some practice effect. In our group, only the oral administration was used.

In our experience with patients who have carbon monoxide encephalopathy, this brief test has proved useful. Its advantages are that it is sensitive to the condition of the patient's brain, it can be administered quickly and easily even if the patients has an i.v. running, and there is some normative information available. Its disadvantages involve the limited sample of behavior and the possibility of practice effect. A similar test (6) has been used in anesthesia as a measure of "street fitness." Patients can be referred for more complete neuropsychologic testing should problems be identified that require a more detailed examination.

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HYPERBARIC OXYGEN THERAPY IN THE TREATMENT OF OSTEOMYELITIS

M. Kawashima, H. Tamura, and K. Takao

Osteomyelitis is common and despite modern advances in antibiotic therapy may still cause disastrous disability. Problems and disappointments associated with the treatment of osteomyelitis are well known to orthopedic surgeons. Closed irrigation suction is one of the most successful therapies. If such therapy does not arrest the infection and prevent recurrence, the disease may eventually become chronic.

The lack of vascularity in the tissue and sclerosis of the bone prevent oxygen, antibiotics, and nutrients from reaching diseased areas in appropriate concentrations. Bingham and Hart (1) described hyperbaric oxygen at a pressure of 2 or 3 ATA to be bacteriostatic and in some instances bacteriocidal. Hypoxia is corrected through saturation of the plasma and tissues in areas where some degree of diffusion is possible. Hyperbaric oxygen stimulates osteoblasts and osteoclasts to increase activity. Vascular proliferation is stimulated by repeated exposure to hyperbaric oxygen for periods of 10 d or more. Bone resorption with later osteoid tissue formation has been demonstrated by exposing cultures of calvaria of young mice to hyperbaric oxygen. That maximum osteogenesis and formation of collagen fiber take place under increased oxygen tension has also been demonstrated in tissue culture.

From 1980 to 1982, a total of 232 patients with osteomyelitis were treated by closed irrigation suction only. From 1982 to 1985, we treated 70 patients with osteomyelitis with hyperbaric oxygenation (HBO).

METHODS AND MATERIALS

Hyperbaric oxygen therapy was at an equivalent of 2 ATA or 29.4 psi, with patients breathing 100% oxygen by face mask or closed-loop hood

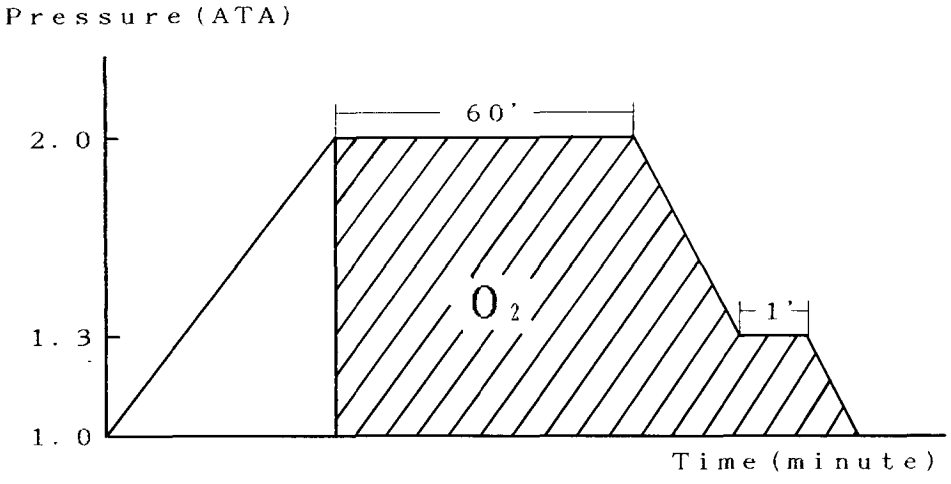

Fig. 1. Hyperbaric oxygen table for the treatment of osteomyelitis.

assembly. Patients breathed oxygen for a total of 1 h (Fig. 1). Each patient was treated once daily for at least 4 wk before and after surgery.

The steel, multiplace hyperbaric chamber accommodates 8 ambulatory patients (Fig. 2). The amount of oxygen in the chamber was continuously monitored to assure that it never exceeded 25%, and a flow-mixing system ensured that oxygen could not "puddle" in any part of the chamber. A pressurized water-sprinkler system throughout the chamber, with specially designed nozzles, can be activated either inside the chamber or from the outside. Two high-pressure, hand-held water hoses are inside the chamber. Pressure is controlled automatically by a computer system (Fig. 3).

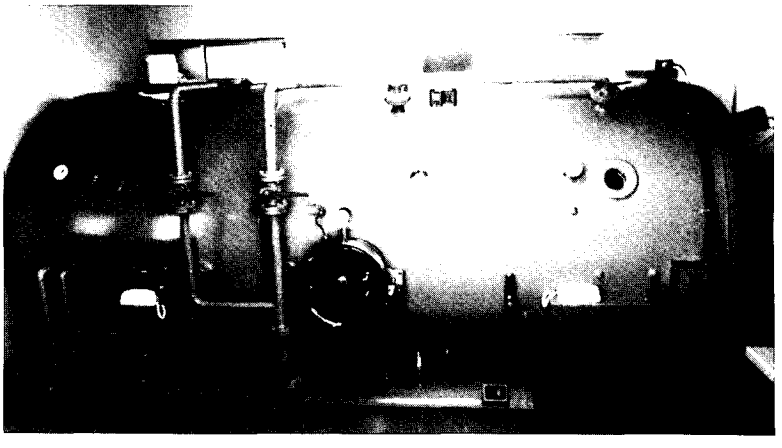

Fig. 2. Multiplace chamber.

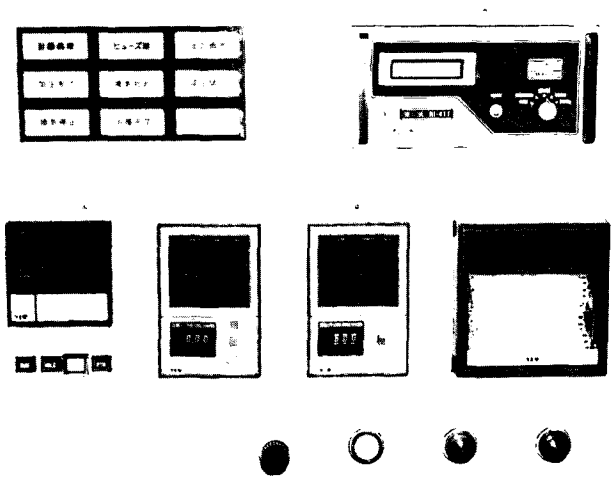

Fig. 3. Computer system for control of treatment table and ventilation.

Transcutaneous oxygen pressure TP_{O_2} was measured during HBO treatment to estimate the effect of oxygenation. The oxygen sensor was applied to the skin surface and held in place by an adhesive ring. The heating control element around the sensor was adjusted to 45°C. After checking the zero reading, a single-point calibration was made in atmospheric air and corrected for ambient barometric pressure. All measurements were carried out at room temperature of 23.2°C. Stable readings were always achieved after 20 min, at which time measurements were recorded from the anteromedial side of the ankle joint. Table 1 shows the TP_{O_2} before and during HBO; average TP_{O_2} before HBO at 1 ATA was 74 mmHg and during HBO was 659 mmHg.

From 1982 through 1985, 70 cases of osteomyelitis were treated; 36 patients by closed irrigation associated with HBO therapy, and 34 by HBO therapy only, with a minimum follow-up of 1 yr. Thirty-eight patients had hematogenous infection, and 32 had traumatic or postoperative infection. Table 2 shows age distribution of the treated patients; infection was localized in the sites noted in Table 3; bacteriologic analysis was made on materials obtained from 70 patients (Table 4).

RESULTS

Excellent results with complete objective and subjective healing of disease (success) were noted in all 36 patients treated by irrigation suction associated with HBO therapy (Table 5). Excellent results were noted in 23 of 34 patients (67.7%, Table 6) given HBO therapy only; in this group 7 patients improved, with either objective and subjective healing with later breakdown or with de-

creased drainage and lessening of pain, and 4 showed recurrence. The average number of treatment hours was 58.2, with a range from 4 to 240 (Table 7).

Table 1
Transcutaneous Oxygen Pressure of Patients with Osteomyelitis

Case No.	1 ATA, mmHg	Before HBO at 2 ATA, mmHg	After HBO at 2 ATA, mmHg
1	60	79	480
2	87	155	790
3	74	140	674
4	76	120	690
Average	74	124	659

Table 2
Age Distribution of the Treated Patients

Age	Male	Female	Total
0 - 9	0	0	0
10 - 19	3	1	4
20 - 29	4	5	9
30 - 39	11	1	12
40 - 49	8	0	8
50 - 59	10	7	17
60 - 69	9	5	14
70 and Over	5	1	6
Total	50	20	70

Table 3
Sites of Osteomyelitis

	Male	Female	Total
Mandible	3	6	9
Sternum	0	2	2
Radium	2	0	2
Fingers	1	0	1
Pelvis	2	0	2
Femur	14	5	19
Tibia	27	2	29
Foot	4	2	6
Total	53	17	70

Table 4
Microbiology

Microorganisms	Hematogenous	Traumatic	Total
Staphylococcus aureus	8	6	14
Staphylococcus epidermidis	1	2	3
Pseudomonas aeruginosa	4	6	10
Serratia, M	0	1	1
Tuberculosis	4	0	4
Streptococcus	1	0	1
Bacteroides	0	1	1
Klebsiella	0	1	1
Unclear	13	7	20
Negative	6	9	15
Total	37	33	70

Table 5
Results of Irrigation-Suction Associated with HBO Therapy

Results	Hematogenous Infection		Traumatic Infection		Total Infections	
	Number	%	Number	%	Number	%
Success	21	100	15	100	36	100
Improvement	0		0		0	
Failure	0		0		0	
Total	21		15		36	

Table 6
Results of HBO Therapy Only

Results	Hematogenous Infection		Traumatic Infection		Total Infections	
	Number	%	Number	%	Number	%
Success	15	88.2	8	47.1	23	67.7
Improvement	1	5.9	6	35.3	7	20.6
Failure	1	5.9	3	17.6	4	11.8
Total	17		17		34	

Table 7
The Treatment Hours and Cases

Hours	Cases
0 - 19	10
20 - 39	24
40 - 59	10
60 - 79	10
80 - 99	4
More than 100 h	12
Total	70

DISCUSSION

When basic treatment of the infection fails, osteomyelitis may be considered refractory. Bingham and Hart (1) described small sequestra noted to resorb under hyperbaric oxygen treatment; however, persistent sequestra should be removed surgically. Direct prolonged lavage with solutions containing antibiotics was used and reported first by Smith-Petersen (2) in 1945. In 1956, Mitra and Grace (3) reported 95 cases of chronic osteomyelitis treated by instillation of penicillin and a detergent through catheters into the wound for 10 d after surgery. In 1962, Compere (4) recommended the addition of Alevaire to the irrigation fluid. Kawashima and Tamura (5) reported a series of 256 closed irrigation suction procedures on 232 patients. Of these, 226 (88.3%) were treated successfully, 7 (2.7%) were improved, and 23 (9.0%) were failures. Closed irrigation suction therapy is generally thought to be one of the most successful treatments for osteomyelitis.

Hamblem (6) reported that the tibia of rats treated with hyperbaric oxygen showed improved healing of established *Staphylococcus aureus* infections. Goldhaber (7) noted that when cultures of calvaria of young mice were exposed to hyperbaric oxygen, bone resorption took place with later osteoid tissue formation. Shaw and Basset (8) demonstrated in tissue culture that maximum osteogenesis and collagen-fiber formation take place under increased oxygen tensions. Hunt et al. (9) demonstrated that HBO increases fibroblastic activity, probably by increasing the rate of synthesis of adenosine triphosphate in hyperoxic tissue. This results in mitosis and migration of fibroblasts, with concomitant increases in the formation of collagen matrix necessary for capillary ingrowth. Mader et al. (10) demonstrated that HBO alone was as effective as cephalosporins in controlling experimentally induced staphylococcus osteomyelitis in rabbits. The investigators concluded that the value of HBO was its enhancement of leukocyte function rather than its suppression of microorganisms. These laboratory studies support our encouraging clinical results.

Sippel et al. (11) reported a patient with osteomyelitis of the mandible treated with HBO therapy. Two years after completion of the treatment, the patient had no exacerbation and wore complete dentures without difficulty. Depenbusch et al. (12) noted excellent results with complete objective and subjective healing of refractory osteomyelitis in 35 of 50 patients (70%). Bingham and Hart (1) reported that of 70 patients with refractory osteomyelitis who received treatment with HBO all improved and 63% have remained free of disease.

As our treated cases showed, it is apparent that HBO is very effective in the treatment of osteomyelitis. We are convinced that HBO is of value as supplemental therapy, and closed irrigation suction associated with HBO will give superior results.

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ECONOMIC CONSIDERATIONS ON THE IMPACT OF ADJUNCTIVE HYPERBARIC OXYGEN IN POTENTIAL AMPUTEES

P. E. Cianci, G. J. Petrone, R. L. Shapiro, J. Ross, H. W. Lueders, H. Lee, and S. Drager

Hyperbaric oxygen therapy is currently applied to a variety of disorders which, in the past, have been refractory to standard medical and surgical care. Its utilization should be considered in selected cases where chronic infections, indolent wounds, or radiation injury require prolonged treatment and/or produce disability. The efficacy of hyperbaric oxygen in selected cases is well established and recognized by third-party carriers based on the recommendations of the Hyperbaric Oxygen Committee of the Undersea and Hyperbaric Medical Society. A well-managed hyperbaric unit utilizing a comprehensive and aggressive team approach, with careful coordination of subspecialties, can not only reduce morbidity and disability but can also effect a significant reduction in cost of care. In this era of constraints on medical reimbursement and hospital funding, it is necessary to carefully monitor the application of hyperbaric oxygen therapy to ensure maximum utilization of limited resources. The advent of diagnosis-related groups militates a careful analysis of the cost:benefit ratio of adjunctive hyperbaric oxygen on a case-by-case basis as hyperbaric oxygen therapy is treated as any other technology. Although the value of hyperbaric oxygen therapy as a medical adjunct is well established, its cost effectiveness is not generally appreciated. The following discussion serves to illustrate this fact.

There have been several reports of increased salvage of potential amputations of the lower extremity when hyperbaric oxygen therapy is used as an adjunct in an aggressive team approach to wound healing (1, 2). The decision to utilize hyperbaric oxygen therapy must be based on the rehabilitation potential of the prospective amputee and should take into account the following: Every year there are more than 60,000 amputations performed in the

United States (3). The risk increases 12-fold from the 5th to the 8th decade of life (0.3-3.6/1000) (4). The mortality associated with amputation is 16% (5). Ipsilateral reamputation will occur in 22% of cases (6). Contralateral amputation occurs at a rate of approximately 10%/yr. Eighty percent of elderly amputees will be alive at 4 yr (5). Only 40 to 50% of elderly amputees will be successfully rehabilitated (4). The length of stay for acute hospitalization secondary to primary amputation varies from 20 to 86 d, averaging 49 d for acute care (7, 8). Six to nine months may be needed to maximize walking ability.

The cost of acute hospitalization for primary amputation averaged $26,000 in Boston in 1984 (8). The total cost in the United States for primary amputation of the lower extremity may approach $1.5 billion. Readmissions for reamputation or other causes may cost an additional $750 million for any 2-yr period. Primary amputation is far from an expeditious solution to the problem of limb-threatening ischemia. The nonhealing of amputations and the need for late revision or contralateral amputation are always risks that can result in multiple, prolonged hospitalizations, just as graft failures are continuing risks in patients who undergo vascular reconstruction (8). Most surgeons recommend that every effort be made to salvage a functional limb and to preserve joints (4).

These grim statistics dictate an aggressive approach to limb salvage. Hyperbaric oxygen therapy as a part of a comprehensive program of care can be a cost-effective method of treatment. In selected cases at our institution, salvage has been accomplished for a cost of $16,000 to $20,000 (9). This compares favorably with the average cost of amputation (8, 9). If the additional costs of reoperation, prostheses, rehabilitation, and repeated or protracted admissions are considered, the potential savings are even more significant. An additional consideration of hyperbaric oxygen therapy can be to function as a "stop-loss" entity, facilitating discharge to home or a skilled nursing facility while preventing disfigurement or disability. A properly managed hyperbaric oxygen program can effect increased limb salvage economically, even within the framework of the present-day hospital reimbursement system.

We examined the records of 16 patients referred to us with problem wounds that were potentially destined for amputation. Our rate of salvage in these 16 patients was 88%. Fourteen of 16 patients had healing of their lesions. These patients included diabetic gangrene, diabetic ulcers, ulcers secondary to peripheral vascular disease, and large indolent wounds. The following cases will serve to illustrate these results.

Case 1

A 70-yr-old female was admitted to our vascular service with a painful, deep ulcer of her right foot sustained when she placed a poultice of aspirin and methyl salicylate on the dorsal surface to alleviate intractable pain. An angiogram demonstrated significant peripheral vascular disease, and successful revascularization was accomplished. Despite the operation's success, the

patient's lesion failed to heal for 6 wk after vascular repair. She was rapidly becoming addicted to narcotics, and hyperbaric oxygen therapy consultation was obtained. The patient was treated on a once-a-day basis for 2 h at 2 atm, and vigorous wound care was instituted constituting whirlpool and debridement. Over a period of 5 wk her wound healed completely without surgery, and she has remained healed for 3 yr without recurrence or readmission. Her treatment was accomplished as an outpatient for a total cost of $18,836, including whirlpool treatment and wound care. Result: limb salvage.

Case 2

A 65-yr-old female diabetic was referred to us for consideration of hyperbaric oxygen therapy because of a large, draining ulcer of her below-the-knee-amputation stump. The patient had been treated conservatively with antibiotics and wound care without success. We initiated adjunctive hyperbaric oxygen therapy as part of a comprehensive program of wound care, nutritional support, diabetic control, and appropriate antibiotic coverage. The patient was treated entirely as an outpatient, and after approximately 5 wk her wound was healed. The total cost of her hyperbaric oxygen therapy and wound care was $16,000. Result: limb salvage.

Case 3

A 55-yr-old male diabetic, status posttransmetatarsal amputation for diabetic gangrene and successful revascularization, was referred to our department because of a nonhealing amputation site. This wound had been present, essentially unchanged, for 6 wk before consultation. We initiated therapy as an inpatient with 90-min oxygen exposure at 2 atm twice daily. On this regimen his wound rapidly developed a fine capillary bed which was successfully grafted. After 4 yr, no recurrence or breakdown has occurred; no further hospitalizations for amputation site revision or contralateral amputation has been necessary. His hospital costs were $54,000. Hyperbaric oxygen represented $5,200 of this bill, or roughly 10%. Result: Stop loss in that this patient was able to be discharged to home.

Case 4

A 70-yr-old male diabetic amputee developed a lesion of his left remaining foot. Revascularization was successful, but his wound showed no signs of healing. Hyperbaric oxygen was initiated; after a suitable period of treatment, the wound accepted a graft, and the patient was discharged. One year later he is ambulatory on the transmetatarsal amputation. His hospital costs were $74,000. His hyperbaric oxygen cost was $14,000. Result: Stop loss and discharge to home.

SUMMARY

A carefully coordinated hyperbaric oxygen program can effect increased

salvage economically, even within the framework of the present hospital reimbursement. An aggressive team approach, however, is necessary to ensure success. Hyperbaric oxygen programs in the future will need skillful management to maximize outpatient therapy, coordinate in-hospital treatment and surgery, and to balance efficient utilization against the need for excellence of care.

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PROPHYLACTIC HYPERBARIC OXYGEN FOR THE PREVENTION OF OSTEORADIONECROSIS

R. W. Emery and R. A. M. Myers

The treatment of osteoradionecrosis (ORN) of the oral and maxillofacial structure has been a perplexing dilemma despite extensive investigation. The literature involving the prevention of ORN has been directed toward recognizing the risk factors (e.g., tumor site, absorbed radiation dose, area irradiated, post- vs. preradiation extraction, dental status, the use of dental appliances, and patient compliance) and avoiding them (1-6, 8). In a recent study, Marx (7) reported a series of 74 patients in the high-risk category for developing ORN after tooth extraction. He compared the prophylactic use of hyperbaric oxygen (HBO) with that of penicillin. The results demonstrated a 5.4% incidence in the HBO group vs. a 29.9% incidence in the penicillin-treated patients. No other published data have addressed the prophylactic administration of HBO before tooth extraction.

This study reviewed the incidence of ORN in patients prophylactically treated with HBO. The treatment protocol included both pre- and postextraction HBO treatments. The incidence of ORN in the literature is reviewed and discussed in light of the present data.

MATERIALS AND METHODS

Thirty-five patients were reviewed from 1979 to 1985. Patients were referred to the Department of Hyperbaric Medicine at the University of Maryland Hospital, Maryland Institute for Emergency Medical Services Systems from both academic and private sources. Dental, radiation oncology, inpatient, and hyperbaric medicine department records were reviewed by a single reviewer to collect data. All surgical procedures were conducted by the referring surgeon, therefore surgical techniques varied. Of the 35 patients

referred for the treatment of ORN, 25 were acceptable for inclusion in the study. Inclusion criteria included:

1. No absolute contraindication to HBO treatments.
2. No evidence of ORN.
3. Receiving an absorbed tumoricidal dose of radiation.
4. The presence of one or more teeth that required extraction in the mandible within the field of radiation.

Patients were excluded only if they had active ORN. Patients were to receive 15 pre- and 10 postextraction dives at 2.4 ATA for 90 min in oxygen. Dives were conducted on a daily basis, excluding weekends. The patients were followed until no evidence of ORN was evident based on clinical observations of both the Department of Hyperbaric Medicine and the referring surgeon. For the purpose of this review, ORN was defined as the presence of exposed bone with or without pain for at least 3 mo. During the period of record review an attempt was made at patient follow-up.

RESULTS

The overall results are summarized in Table 1. Of the 35 patients referred for treatment, 25 were referred for prophylactic treatment before tooth extractions. The other 10 patients presented with active ORN. Of the 25 applicable patients, 19 had complete records available, including rads received and the number of extraction sites. Six females and 19 males, with ages ranging from 21 to 70 yr (with an average age of 53) were treated. Table 2 shows that the primary sites of malignancy varied, the highest incidence being from the pharynx and larynx.

A total of 170 sockets were studied from the applicable group. The overall incidence of ORN from the applicable group of 25 was 2 (8%). There were 6 (3.5%) involved sockets. Both patients had primary lesions in the floor of the mouth that were fully resolved with further HBO treatments and sequestrectomy.

Radiation received by the 25 patients ranged from 4000 to 7000 rads (Table 2), with an average of approximately 6000 rads. Two patients received implants. One of these patients presented with active ORN.

Patients were to receive 15 pre- and 10 postextraction dives at 2.4 ATA for 90 min in oxygen. The range of preextraction dives was 5 to 16, with an average of 11.6 (Table 2). Preextraction dives varied with the patient's symptoms and the surgical scheduling. Postextraction dives ranged from 9 to 22 with an average of 13.76. Postextraction dives varied with patient response. All patients were discharged from treatment with full resolution. Follow-up from the applicable group ranged from 6 mo. to 5 yr; 12 patients were contacted at the time of record review.

Table 1
Overall Result for Period 1979 - 1985

Patients treated	35
Patients applicable	25
Patients with record of both rads received and tooth numbers	19
Follow-ups	12
Patients that died in follow-up group	3
Sockets studied	170
Incidence of ORN in group of 25	2 (8%)
Sockets with ORN	6 (3.5)

DISCUSSION

The rationale for the use of HBO in the treatment of ORN has been well documented in the literature. In 1973 Greenwood and Gilchrist (9) and Mainous et al. (10) introduced the concept for treatment of ORN. With daily elevation of partially ischemic and hypoxic bone, soft tissue oxygen tension near regions of functioning capillaries increases (11-13), while fibroblastic proliferation, collagen synthesis (14), and capillary angiogenesis proceed (15, 16). Impaired bactericidal activity of hypoxic leukocytes is restored to normal with the elevation of bone oxygen tension to normal or above normal (12).

These concepts have led to the use of HBO for the treatment of active osteoradionecrosis (17-27) and the reconstruction of these debilitated patients after resection (10, 18, 24, 27). The use of HBO in a prophylactic manner for the prevention of ORN after tooth extraction has only recently been reported. The rationale for use in a preventive fashion is based on the same concepts previously described and leads to the protocol presented in this study in 1979.

The results presented here support the role of hyperbaric oxygen in the prevention of osteoradionecrosis. The study was retrospective, however, and there were no controls. To draw definitive conclusions regarding the significance of the data, the incidence of ORN must first be known. The incidence of ORN in the literature varies considerably (Table 3), ranging from 0 to 44%. This wide range can be explained in part by variables in documentation.

The definition of osteoradionecrosis is important. Marx defines ORN as the presence of exposed bone for a period of 6 mo. Grant and Fletcher (28) report their incidence of ORN as 66 of 176 treated patients (37.5%) but note that in 12 of the 66 patients delayed healing persisted for 1 mo. or less. Coffin (29) notes only "major types" of ORN, or those that result in resection. In his series, this was 25 of 2853 patients.

Follow-up is frequently not mentioned, but is an important factor. ORN is related to the damage of the terminal vascular bed and bone, essentially a fixed postmitotic cell population. Chronic effects can be seen 16 yr after the original injury, although the incidence does decrease with time (28, 30).

Table 2
Patient Data from 1979 to 1986

Patient	Age	Number Pre-extraction Treatments	Number Post-extraction HBO Treatment	Rads Received	ORN	No. Tooth Sockets	1° Lesion
FB	65	12	11	5000	No	13	larynx
OB	66	12	15	5000	Yes	1	FOM
JB	41	12	13	7000	No	—	larynx
MC	43	10	22	6500	No	5	pharynx
GC	51	9	21	6800	No	12	pharynx
RD	56	10	16	5000	No	8	larynx
ED	53	16	11	6500	No	8	pharynx
EG	64	16	12	6000	No	—	nosoph
HG	43	15	10	6600	No	5	pharynx
FG	42	13	21	—	No	4	tongue
EG	48	14	18	6050	No	10	tongue/pharynx
AH	59	12	12	6000	No	—	larynx
AL	70	16	11	7200	No	11	pharynx
CL	51	10	11	6500	No	7	soft palate/ pharynx
PL	65	5	12	6000	No	14	larynx
JL	26	12	9	4000	No	—	mandible
CM	58	15	21	6900	No	16	tongue
EM	68	10	10	6700	No	15	maxilla
OM	69	15	10	4500	No	5	mandible
MM	57	15	14	6600	Yes	5	FOM
FT	74	5	10	—	No	5	tongue
LV	49	15	19	5000	No	2	parotid
WM	56	32	10	6040	No	22	larynx
RM	24	6	10	4500	No	4	base of skull
EM	21	7	15	5000	No	7	tongue

A number of investigators have altered the risk factors to determine their significance clinically. Beumer et al. (31, 32) varied pre- vs. postradiation extractions. Daly et al. (6) vary clinical modalities of dental therapy, soft vs. hard dentures, and fluoride vs. no fluoride, to determine their significance on the incidence of ORN.

The location of the site of ORN is also important. If the mandible is the only site reported, the incidence will be higher than if both maxilla and mandible are considered. This will become especially apparent if the total number of sockets is reported (31, 32).

Table 3
Values of ORN Found in the Literature

Author	Date	No. of Patients	Incidence of ORN	Rads Average or (Range)
Marciani	1986	109	3 (3%)	6500
Marx	1985	37 (HBO tx)	2 (5.4%)	6800
Coffin	1983	2853	25 (< %) to resection	—
Beumer	1983	120 (mandible)	17 (14.1%)	7000
		44 (maxilla)	1 (2%)	5200-12,000
Beumer	1983	45 (post-rad ext mandible)	13 (29%)	6500
		27	3 (11%)	3925- 7000
Morrish	1981	100	8 (8%)	8000
Horiot	1981	528	10 (2%)	
Murray	1980	404	77 (19.1%)	2000- 8000
		249	61 (24.5%)	2000- 8000
Starche	1977	62	1 (1.6%)	6000
Bedwinek	1976	381	54 (14%)	5000- 7999
Regez	1976	130	22 (17%)	6500
Cheng	1974	76		6000
Marcian	1974	220	23 (10.4%)	6000
Carl	1973	47 (86 maxilla teeth)	2 (4.2%)	3600-12,900
		101 (mandible)		3600-12,900
Daly	1972	304	66 (21.7%)	—
Rarkow	1971	176	12 (6.3%)	5000 (75%)
Solomon	1968	31	0 (0%)	—
Rahn	1967	120	53 (44.2%)	—
Grant	1966	176	66 (37.5%)	6000-13,000
MacComb	1962	93	19 (20%)	
Cook	1956	11	4 (36.4%)	
Wildermuth	1953	104	5 (6.0%)	
U. Brit Columbia	1940-49	180	9 (5%)	
Westfld St. San.	1937-57	491	26 (4.8%)	
Jacobsson	1948	277	15 (5.6%)	
Martin	1940	566	57 (10%)	
Watson	1938	1819	235 (13%)	

Variation between individual patients can also affect the reported incidence of ORN (e.g., tumor site, irradiation dosage, area irradiated, type of radiation, dental status, patient compliance). It becomes obvious that direct

comparison of individual studies would be inappropriate due to the numerous variables.

The importance of Marx's report (27) becomes evident in that a defined population having greater than 6800 rads with a common definition of ORN, a fixed treatment protocol, and a common endpoint, shows significant difference between HBO treatment and penicillin therapy. The presented data only support this work. No definitive conclusions could be drawn due to the retrospective nature of the data and the variability within the patient population and the treatments received.

However, questions can be raised by the presented data and review of the literature.

1. What is the incidence of ORN with the definition presented by Marx? It would be advantageous to know the incidence of ORN for various dosages of radiation so that unnecessary HBO therapy could be avoided.
2. What is the most effective time and dosage sequence for the administration of HBO?

These questions should be answered within defined populations so that definitive answers can be drawn.

SUMMARY

Twenty-five patients were treated with prophylactic HBO undergoing an average of 11.68 pre- and 13.26 postextraction dives with an average incidence of 8% (2). These data seem to support those of Marx. No definitive statements could be made due to the extensive variables within the literature and the presented data.

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ADJUNCTIVE USE OF HBO FOR CLOSTRIDIAL MYONECROSIS IN THE NEWBORN

A. T. Powers, I. Jacoby, F. P. Lynch, R. W. Coen, and T. S. Neuman

Clostridial myonecrosis is an anaerobic infection characterized by progressive destruction of the fascia, subcutaneous, and muscular tissues. Superficial signs of the infection often belie the extensive necrosis found under the dermis. The organism spreads quickly and the infection can become life-threatening.

Clostridial myonecrosis occurs rarely in the adult population and even less often in newborns. Standard therapy for clostridial myonecrosis consists of surgical excision of all infected tissues, appropriate parenteral antibiotics and, if available, hyperbaric oxygen (HBO) therapy. This case of a 6-d-old infant seems to be the youngest patient ever treated successfully with HBO for clostridial myonecrosis.

CASE REPORT

A 4500-g term infant boy was born to a 20-yr-old, gravida 1 female. The prenatal history was unremarkable. The infant was delivered with forceps after a 19-h labor. Apgar scores were 9 and 9 at 1 and 5 min, respectively. Because the peripheral hematocrit was 68%, an umbilical vein catheter was placed to accomplish a partial exchange transfusion. A central hematocrit of 58% negated the need for an exchange and the umbilical vein catheter was removed. The infant breast fed without difficulty and was discharged after 2 d.

On Day 5, the mother noticed an erythematous rim around the umbilicus and a bloody discharge from the umbilical area. She had been cleaning the umbilicus with alcohol regularly. On Day 6, the erythema spread and the infant was admitted to a local hospital that morning. The erythema progressed and the infant was transferred to Uuiversity of California San Diego Medical

Center for surgery and possible hyperbaric treatment.

Upon arrival, the infant's temperature was 99.4°C, pulse 118 beats/min, respiratory rate 64 breaths/min, and blood pressure 78 mmHg by Doppler. The infant was very irritable and seemed to be in pain. Approximately 15 × 20 cm of skin around the umbilicus was erythematous and indurated. A 3-cm area was blackened and definitely necrotic. The abdomen was tender, with few intermittent bowel sounds. White blood count was 38,900 and differential showed a slight shift to the left. A room air arterial blood gas showed a pH of 7.41, P_{CO_2} of 35, P_{O_2} of 85, and a -1.5 base deficit. Electrolytes were within normal limits. A chest roentgenogram was normal but an abdominal roentgenogram showed a single loop of distended bowel. Blood and cerebrospinal fluid cultures were drawn, and were later reported as negative.

A preoperative diagnosis of omphalitis with necrotizing fasciitis was made and a course of antibiotics consisting of penicillin 250,000 units every 4 h i.v., gentamicin 11.5 mg every 12 h i.v., and metronidazole 35 mg every 12 h i.v. were started. The patient was taken to the operating room that evening for a full thickness debridement of the cutaneous, subcutaneous, and anterior rectus fascia involving the anterior abdominal wall from the suprapubic margin to the subcostal margin. The umbilical cord and a 3 × 3-cm periumbilical area of rectus sheath and muscle layer also were removed. Cultures obtained during surgery subsequently grew *Clostridium sordellii*. Histopathologic exam of the resected abdominal wall showed many polymorphonuclear leukocytes and gram-positive rods with subterminal spores on Brown and Brenn staining. Diagnosis of clostridial myonecrosis was made based on these findings.

Hyperbaric oxygen, using a multiplace chamber, was started in the immediate postoperative period. Hyperalimentation was initiated also at this time. During the next 2 d the infant received a total of 4 hyperbaric treatments; 1 at 3 ATA (44 psig) for 159 min and 3 at 2.4 ATA (35 psig) averaging 115 min. The child was mechanically ventilated on a Baby Bird(R) ventilator from the time of surgery until postoperative Day 9. Initially, rates of 28 to 35 with an inspiration to expiration ratio of 1:3 were needed for adequate ventilation. Peak inspiratory pressures ranged from 20 to 30 mmHg. During HBO therapy, ventilator adjustments of rate and volume were determined by observation of chest excursion, auscultation, and intermittent arterial blood gas analysis. Arterial blood gases confirmed the readings of a Kontron(R) transcutaneous oximeter placed on the infant's chest, which continuously monitored oxygenation. During the first HBO treatment at 3 ATA, a maximum transcutaneous P_{O_2} of 895 mmHg was recorded. The remaining HBO treatments at 2.4 ATA were associated with transcutaneous P_{O_2} measurements of 700 to 1015 mmHg.

The 2nd hyperbaric treatment was complicated by lower-than-expected oxygen levels. A chest roentgenogram taken after this treatment showed that the endotracheal (ET) tube had slipped into the right bronchus, causing collapse of the right upper lobe of the lung and interferring with oxygenation during and after HBO. Adjustment of the ET tube reexpanded the lobe and

improved oxygenation. By the 4th hyperbaric treatment, higher ventilator pressures were needed to oxygenate the infant. Posttreatment arterial Po_2 was lower than before HBO therapy. A chest roentgenogram revealed left upper lobe atelectasis, possibly due to oxygen toxicity. HBO therapy was discontinued at that time because the wound was showing signs of improvement with no evidence of infection.

On postoperative Day 4, the infant returned to the operating room for further debridement of the wound edges and primary closure of the abdominal wall. He continued to receive hyperalimentation and betadine dressing changes during the remainder of his uncomplicated postoperative course. He was discharged from the hospital 17 d after admission. His large abdominal wound gradually healed through contracture and epithelialization during the ensuing months. Ophthalmologic examinations and neurodevelopmental testing at 6 mo. were normal.

DISCUSSION

Clostridium, a spore-forming, gram-positive anaerobic bacillus, is widespread in the environment, however only six species are known to cause myonecrosis (1, 2). *Clostridium sordellii*, an organism recognized in 1975 as a species separate from *C. bifermentans*, is one of these (2, 3). Only in the vegetative state is Clostridia able to produce the exotoxins that allow tissue invasion and necrosis. Trauma, ischemia, or the presence of a foreign body lower tissue oxygenation-reduction potential, which in turn stimulates the clostridial spores to convert to the vegetative state and proliferate.

Clinical signs of clostridial myonecrosis are severe localized pain and induration with a magenta-bronze color of the overlying skin. In an advanced infection, brackish, foul-smelling, fluid-filled bullae and crepitus occur (3, 4). Roentgenograms may show gas in the wound. Late systemic symptoms include fever and tachycardia. Ultimately, hypotension, disseminated intravascular coagulation, renal failure, metabolic acidosis, and shock occur (3, 4). Visual inspection of the infected muscle at surgery shows pale, devitalized tissue which does not contract when incised (3, 4).

Clostridia species have been cultured from umbilical cords 24 h after delivery (5) and *C. sordellii* has previously been reported as the pathogen in three fatal cases of omphalitis (6, 7). In all cases, the presenting picture was one of brawny discoloration around the umbilicus with rapid spread across the abdomen, despite the use of antibiotics.

Treatment for clostridial myonecrosis consists of surgery, antibiotics, and, if available, HBO. To limit the spread of tissue necrosis, complete surgical excision of all devitalized tissue is of primary importance. Repeated wound inspection and debridement may be necessary to ensure complete removal of the affected tissue. Penicillin G is the antibiotic of choice. Other antibiotics may be added to provide broad spectrum coverage of co-involved or opportunistic organisms. Although no prospective, randomized clinical studies have

been performed to evaluate adjunctive HBO therapy in treating this disease, there is strong evidence to suggest that it is beneficial (8-13). It is felt that HBO can decrease toxin production and, at high enough oxygen tensions, actually kill clostridial organisms.

Patients receiving HBO are at risk for barotrauma and oxygen toxicity. Barotrauma occurs when there are significant pressure gradients between closed air spaces and the chamber atmosphere. Most commonly affected air spaces are the middle ear and sinuses. Without pressure equalization, congestion, edema, and hemorrhage occur in these spaces. The tympanic membrane also may perforate. Pulmonary barotrauma is a more serious problem which, fortunately, occurs rarely. Expanding gases trapped in the lung on decompression can cause an air embolism or tension pneumothorax from obstructive air flow and pulmonary overinflation. Intubated patients need to be observed closely to prevent pulmonary barotrauma. Endotracheal tube placement and patency are critical. An endotracheal tube in the right or left mainstem bronchus may trap gas in the nonintubated side; similarly, lung secretions might occlude air flow through the tube. Both events could produce an air embolus or pneumothorax.

Our patient's ventilation was supported with a Baby Bird ventilator during treatment in the hyperbaric chamber. Because gas density changes in direct proportion to chamber pressure, i.e., compression increases gas density and vice versa (Boyle's Law), air flow through the gas-powered Baby Bird ventilator changes inversely with gas density, affecting rate and tidal volume. Higher ventilator pressures and volume settings are needed to compensate for increased gas density. The reverse is true also. Hypoventilation or pulmonary overinflation might occur if ventilator settings are not recalibrated with each change in chamber pressure.

Hyperoxia can have a deleterious effect on the lungs, central nervous system, and, in premature infants, the retina. The actual mechanism of oxygen toxicity is not clearly understood, but it is believed that antioxidant enzymes become inadequate to handle the excess accumulation of oxygen-free radicals and oxidation of essential cellular components occurs (14). Alveolar damage, atelectasis, respiratory distress syndrome, and retrolental fibroplasia in premature infants (15) are associated with prolonged exposure to high ambient oxygen concentrations. Extremely elevated oxygen tensions, achieved only in a hyperbaric environment, may induce central nervous system toxicity symptoms manifested by grand mal seizures.

Reports of HBO being administered to newborns are sparse (16-20). Potential problems need to be considered before initiating HBO therapy in this group of patients. A hyperbaric nurse, neonatal nurse, and respiratory therapist are needed inside the chamber with each treatment to provide continual assessment and support of the infant.

In newborn infants, several factors increase the likelihood of barotrauma. Sedatives and paralyzing agents which are employed during mechanical ventilation prohibit the infant from voluntarily equalizing middle ear pressure by crying, yawning, sucking, or swallowing. Our infant's tympanic membranes

had no gross anatomic changes after HBO treatments, even though myringotomies or pressure equalization tubes were not placed. Brainstem auditory evoked responses (BAER) were normal after therapy.

Because this infant was intubated and mechanically ventilated, frequent auscultation of breath sounds and meticulous pulmonary toilet were performed by the nurses and respiratory therapist to minimize the risk of lung barotrauma. As an additional measure, a Mapleston anesthesia bag was used for manual ventilation during increases or decreases in chamber pressure until mechanical ventilator settings could be recalibrated to accommodate the change in gas density. Moreover, it was felt that slight changes in lung compliance, endotracheal tube placement, or patency might be detected immediately by using the Mapleston anesthesia bag during the critical time of decompression. Intermittent arterial blood gases, continuous transcutaneous oximetry, and posttreatment chest roentgenograms were performed to monitor the infant's pulmonary status.

Even with the above precautions, a right mainstem intubation during the second hyperbaric treatment was not recognized until the posttreatment chest roentgenogram. The resulting right upper lobe atelectasis reexpanded with endotracheal tube repositioning. Fortunately, no serious pulmonary barotrauma occurred.

The infant had no indications of ocular or central nervous system oxygen toxicity. An examination of the infant's retina showed no abnormalities, and an electroencephalogram, performed the day after terminating HBO therapy, was normal. After the 4th treatment, left upper lobe atelectasis and low posttreatment arterial Po_2, 70 to 80 mmHg on 100% oxygen at 1 ATA, were thought to be due to pulmonary oxygen toxicity. Gradual improvement in oxygenation occurred after cessation of HBO therapy. There were no other pulmonary problems.

The long-term effects of HBO for this age group are not documented. However, in this case, neurodevelopmental testing was normal at 11 wk and 6 mo. of age.

Although rare, the use of HBO in the newborn may be necessary. Close monitoring and attention to the unique problems confronting physicians and nurses by patients in this age group can minimize risks associated with HBO. When used with surgery and antibiotics, HBO can be used as an adjunct to successfully treat clostridial myonecrosis in the newborn.

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PHYCOMYCOTIC GANGRENOUS CELLULITIS (MUCORMYCOSIS): CASE REPORT AND REVIEW

G. H. Cohn

The name "phycomycosis" was suggested by Emmons and Binford (1) to designate an occasionally occurring human disease caused by fungi. These fungi belong to the class known as phycomycetes. The principal characteristics of these fungi are the almost complete lack of septae in the hyphae and the production of spores within enlarged terminal sacs called sporangium. Sluyter (2) described pulmonary phycomycosis in 1847, and in 1885 Paltauf (3) reported the disease disseminating to the brain. By 1903, Vuillemin (4) showed a disease caused by the species phycomycetes of the genus *Absidia*. The term mucormycosis has long been used synonymously with a newer name—phycomycosis; however, the genus *Mucor* are not often involved.

Phycomycosis, the name proposed by Emmons and Binford (1), provides a convenient inclusive name for any of the mycoses caused by a specific phycomycetes, including all those in which diagnosis was made from the morphology of the fungus in vivo without verification by culture. The name has been widely used with many modifications.

The term mucormycosis refers to clinically similar fungal infections caused by members of the order Mucorales and its families. The commonly encountered fungi are Mucoraceae (genera *Absidia*, *Mucor*, *Rhizomucor*, and *Rhizopus*). These 4 genera of Mucoraceae can not be distinguished in a histopathologic examination. The clinical diagnosis of mucormycosis must be substantiated by histopathologic biopsy and culture. Yet, the term mucormycosis has been a useful name for a mycosis in which no cultures have been obtained, but where the morphology of a fungus in sections is that of a "phycomycete," by morphologic appearance (as described in paragraph one).

Clark (5) proposed to revert to the name mucormycosis, a designation never really abandoned, for those mycoses caused by fungi belonging to the

order of Mucorales. Although the name phycomycosis has generally been rejected by taxonomists, medical mycologists continued to use it to include a wider range of pathogenic fungi.

The fungi of phycomycosis are characteristically 6 to 50 μm and with ribbonlike non-septate. On biopsy, phycomycetes show broad, non-septate hyphae. It is easily seen with hematoxylin and eosin (H&E) stain. The stains which are so often useful in other fungal diseases such as periodic acid Schiff do not do as well as the H&E stain. The organisms are ubiquitous. They are usually found as saprophytes in the human respiratory and gastrointestinal tract and are commonly found in molds that are airborne, in the soil and manure, in insects, and in bread and fruit molds. Spores may become airborne and drawn into the sinuses, or they may be ingested with foods and initiate infections in the intestinal tract or be inoculated s.c. *Given the right conditions, and a compromised host*, these seemingly innocuous organisms can be devastating as they invade the host.

The mucormycosis is characterized by striking hyphal invasion of and through the blood vessels with hemorrhagic thrombosis and infarction, nerve invasion, invasion of tissue planes, necrosis, and sometimes intense polymorphonuclear leukocyte infiltration. A cellular reaction in the tissue from the phycomycetes may cause abscess accompanied by much necrosis. This invasion of the blood vessels by the organism permits hematogenous dissemination; thus it is understandable how rapidly disseminating disease can be so destructive and fatal even in a few days.

Epidemiologically the disease mucormycosis is rarely found in a patient with no predisposing conditions. The phycomycetes are distributed worldwide; there are no geographic limitations. The disease concentration is in places where personnel are making the clinical diagnosis, where autopsies are customary, where severe dietary deficiencies exist, and where certain types of therapy for systemic disease that renders persons more susceptible to phycomycosis are carried out. No preponderance for sex or age occurs and all age groups are equally vulnerable. The fungi that cause phycomycosis are commonly saprophytes in nature or frequently encountered as contaminants in the mycology laboratory. Fungi are contracted frequently by all human beings; they are not true pathogens, but rather opportunists capable of causing disease.

Mucormycosis is the most acute and fulminating fungal infection known. In the acidotic patient, death often occurs within 7 d. The pathogenesis of mucormycosis is strongly associated with diabetes mellitus (especially diabetic acidosis), renal insufficiency, uremia, patients receiving immunosuppressant therapy, severe cachexia, carcinoma, profound dehydration, septicemia, and with drug addicts and patients who are on massive steroid therapy. Patients with acidosis show a predilection to be infected with these fungi no matter what causes the acidosis (6).

Clinically uncontrolled diabetics with acidosis account for most of the cases and most of the cases are the rhinocerebral type (7-12). The course of the infection may fulminate within 10 d or less. Less common types of clinical

mucormycosis are the gastrointestinal, pulmonary, and burns. Histologic examination of surgical specimens is required to make the diagnosis; multiple organ failure is common. Unfortunately, diagnosis of the disease is usually recognized only at autopsy.

When the disease entity is first considered, a histologic examination by surgical biopsy should be done as soon as possible to make the diagnosis. When a seemingly normal patient is overwhelmed by injury such as a burn, multiple trauma, with impregnation with organic debris or soil, one must consider the diagnosis of phycomycotic gangrenous cellulitis in the differential (13-16). Examination of scrapings or biopsies are required to make the diagnosis of mucormycosis. Specimens should also be inoculated into Sabarou's media and glucose agar and incubated at 37°C and at room temperature.

The most common types of mucormycosis are the rhinocerebral types, which usually present clinically with unilateral headache, eye irritation, swelling, lacrimation, periodic numbness, nasal stuffiness, and epistaxis. The patient becomes lethargic, semicomatose, or comatose. Orbital facial cellulitis with proptosis is seen in approximately two-thirds of the cases. The rhinocerebral mucormycosis usually seen in the diabetic, although rare, is common enough to be stressed in training of otolaryngologists and endocrinologists. Gastrointestinal and respiratory mucormycosis are usually associated with diseases other than diabetes (13-19). One must keep a high degree of suspicion to make the diagnosis. Examinations of large amounts of sputum and cultures of respiratory secretions are frequently negative. If positive, it still requires histologic confirmation, so that fiberoptic bronchoscopy with brushing of the tracheal biopsies is often necessary to make a diagnosis of pulmonary mucocmycosis. Mucormycosis frequently disseminates from the lungs and spreads to the central nervous system, and in a similar manner the gastrointestinal mucormycosis or cases from infected burns or immunocompromised hosts will frequently metastasize via the blood stream. No matter what type of mucormycosis is seen, one must first think of the diagnosis and confirm it by biopsy.

Our case represents a very rare type of mucormycosis which is more frequently associated with infected burns or infected wounds, particularly after local or diffuse trauma. Vascular invasion and thrombosis are an important aspect of these infections. The common denominator in these cases seems to be massive acute compromise of the host, associated shock with hypoperfusion, hypoxia and secondary acidosis, and impregnation of the opportunistic fungi in the compromised host.

Case Report

Our patient was an 11-yr-old boy who sustained multiple injuries and traumatic shock when he fell into a silage forage wagon mechanism and was caught between the augers. At initial staging the patient was intubated and started with fluid resuscitation. When he was removed from the machinery, he was transferred by helicopter to our emergency room, stabilized, given

appropriate diagnostic studies, and taken to the operating room. He was noted to have an anterior and posterior rupture of the left diaphragm with omental evisceration through the left chest wall, a right rectus abdominis injury down to the peritoneum, a retroperitoneal hematoma, a posterior splenic laceration, two left axillary muscle wounds, left side abdominal muscle wound, a left hip wound with communication to the left greater trochanter with exposed periosteum, left lateral thigh laceration, a right posterior thigh laceration, division of the right semimembranous and semitendinous biceps, and a scalp laceration with periosteal exposure. The patient underwent exploratory laparotomy with repair of the left hemidiaphragm, a splenorrhaphy, a suprapubic cystotomy, a transverse colostomy with mucous fistula and debridement irrigation, packing of wounds, suture of the scalp laceration, and placement of a left anterior chest tube. He received vigorous fluid resuscitation including packed red cells, crystalloids, and plasma.

Four days after admission he was taken back to the operating room for further debridement. At this time he had initial evaluation by hyperbaric medicine and started on treatment immediately postoperatively with high pressure oxygen therapy.

At this time, the 4th d after the injury, on the basis of the surgeon's clinical suspicion, a biopsy was taken and a diagnosis of mucormycosis was made. Immediately upon opening the abdominal wall it was apparent that the fungal infection had traversed the entire thickness of the abdominal wall and indeed had spread intraperitoneally. The abdomen was generally explored and most of the visible large fungal growths were excised. It was not possible to remove all of the infected growths because the infection was very widespread.

The infected portions of the anterior abdominal wall were resected. Upon debridement of the wound near the pelvis, it became apparent that the fungal infection was tracking along the suprapubic tube and there was probable intracystic involvement. After completing the abdominal wall resection it was apparent that the wound would not be able to be closed. A burn pad soaked with warm saline was placed over the open abdomen and sewn into place to the remaining fascia. The bowel and liver were essentially completely uncovered and seemed to maintain viability without evidence of fungal infestation.

The patient remained in septic shock, and clinically it was felt he would not survive. Further wound debridement and dressing changes were done almost daily in the operating room. He was treated vigorously with radical surgical debridement and given massive doses of intravenous antibiotics, including actinomycin.

He was treated vigorously with hyperbaric oxygen at 2.8 ATA for 2 h at a time, on a ventilator, twice a day for 5 d. When the patient was no longer in septic shock, he was started on a "wound-healing regimen" of 2.0 ATA for 2 h daily for a total of 38 treatments.

The patient required multiple surgical procedures under general anesthesia for dressing changes and repeated extensive debridement. The latter was done

to limit the spread of infection. The patient was treated with high pressure oxygen twice a day, working around the operating room schedules during these first 5 d when the patient was so critically ill.

Skin grafts were started after 7 d of hyperbaric treatment (Day 13 from the time of injury). Repeated debridement and dressing changes were continued on a daily basis, including the left thigh, left flank, and left axilla. There was a large amount of fungal material posterior to the abdominal wall adherent to the bowel.

Reinforced Silastic sheets were placed over the abdominal contents to retain fluid. After 19 d of hyperbaric therapy, granulation tissue had grown over the anterior abdominal wall and in the multiple injuries of arms, leg, and trunk enough to prepare for skin grafting. It was noted that a fibrinous material containing granulation tissue was present over the bowel and formed a "connective-tissue layer" over the entire abdominal viscera. On Day 28, a split thickness mesh graft from the lower extremities was placed directly over the entire abdominal wound, and postoperatively the patient continued to have hyperbaric oxygen therapy. On Day 38 the dressings were changed under general anesthesia and it was noted that the graft had taken very well and there was a layer of epithelium over the entire abdominal surface.

The patient went on to heal his multiple grafts, and hyperbaric oxygen therapy was discontinued after his 38th treatment; he was discharged after 54 hospital days and has had an amazing clinical recovery. He is back to school and participating in many activities, including Boy Scouts.

DISCUSSION

We have presented a case of a patient who had overwhelming injuries, associated septic shock, impregnation with plant matter, and impairment of host defense mechanisms which allowed a saprophytic infection of mucormycosis to invade the body and almost cause death. The patient's immune mechanism and response to sepsis, shock, and injury came about acutely rather than the classic, chronically impaired host seen in the diabetic, the cancer patient, or the immunosuppressed host. This acute, overwhelming compromise of the host defense mechanisms, plus the opportunistic impregnation of plant matter from the silage machine, set the stage for this very aggressive saprophytic infection to occur.

It cannot be stressed enough that a multitude of factors contributed to the patient's survival. First, were the skills and clinical abilities of our trauma surgeon. Second, excellent ventilatory resuscitation and fluid resuscitation stabilization at the scene of the accident. Third, fluid resuscitation and stabilization of the septic shock in our intensive care unit, and, finally, the thorough evaluation and aggressive treatment by our infectious disease team.

The roles of hyperbaric oxygen therapy are adjunctive to all of the above modalities of treatment. These adjunctive roles of high pressure oxygen therapy are that it a) increases oxygen diffusion to hypoperfused hypoxic tissue, b)

reduces tissue acidosis of hypoxia, c) decreases rate of growth of fungi [if concentrations of oxygen are high enough—fungistatic if not fungicidal (20-29)], d) enhances synergistic activities of antimicrobial agents (29). In the second phase, hyperbaric oxygen plays a very significant role of enhancing angiogenesis in preparing these large wounds for graft and assuring the graft take.

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PATHOHISTOLOGIC STUDIES ON THE EFFECT OF HBO ON MULTIPLE ORGAN FAILURE

Y. Fujiwara and Y. Nanbo

Hyperbaric oxygenation (HBO) raises the O_2 tension of blood and then that of tissues. This higher level of O_2 tension protects the structure and physiology of the tissue cells from hypoxic derangement upon their exposure to prolonged anoxia or critical circulatory failure, such as shock, intoxication and/or multiple organ failure (MOF).

We studied experimentally the effect of HBO treatment on these pathologic derangements in the tissue cells by the pathohistologic observation of the main vital organs of animals that were artificially induced into a state of acute MOF.

METHOD

The state of acute MOF was induced in male rabbits by i.v. injection of D-galactosamine, 1 g/kg body weight. The animals for the HBO group were given HBO 8, 16, and 24 h after the onset of MOF by placing them in a hyperbaric chamber with 2 ATA of air with 100% O_2 inhalation for 1 h. Those in the control group were not given HBO but all other treatments were the same.

About 30 h after induction, the animals of both groups were killed and samples were obtained from the brain, liver, and kidney and prepared for pathohistologic observations. Blood samples were also taken for laboratory data. The results from the 2 groups were compared.

Intracranial pressure (ICP) was monitored continuously during and after the HBO, using a small intracranial balloon containing saline inserted into the extradural space through a Burr-hole and connected to a P50 transducer.

RESULTS

In the liver, degeneration and necrosis of the centro-acinar hepatic cells, with karyorrhexis and poor leukocytic infiltration, were observed in the control group (Fig. 1, *left*), whereas in the HBO group the cellular structure was fairly well maintained with basophilic fine globular substances in the cytoplasm (Fig. 1, *right*).

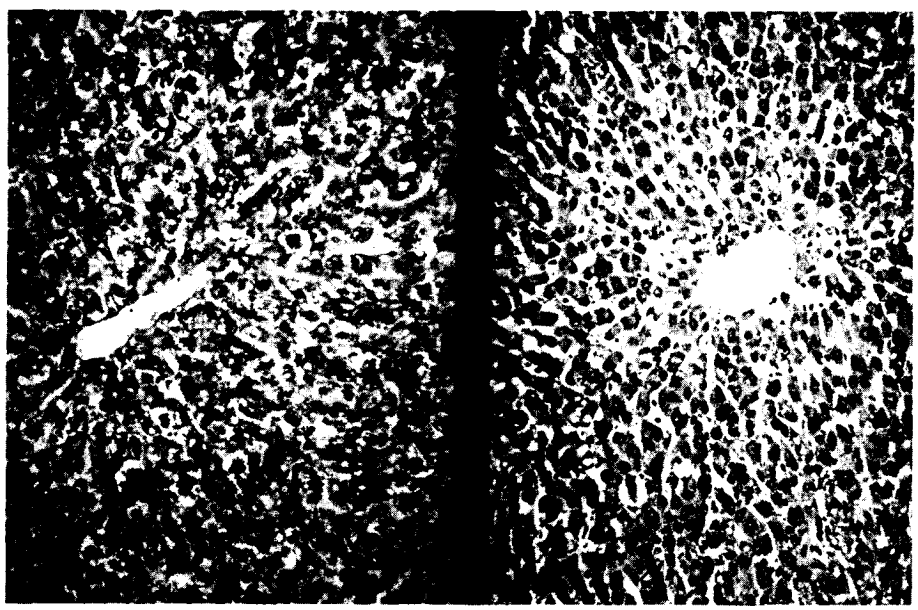


Fig. 1. Hepatic cells in MOF: control (left) vs. HBO (right).

Electron microscopy revealed a fine globular substance, so-called ring mitochondria, which has been reported in livers damaged by such substances as carbontetrachloride or alcohol (Fig. 2). The ring mitochondria was noted only in the HBO group.

In the kidney, vacuolar degeneration of the epithelial cells of the proximal tubules was observed more often in the control group (Fig. 3, *left*) than in the HBO group (Fig. 3 *right*).

Intracranial pressure during HBO treatment declined as the chamber pressure was raised, and then leveled off to a stable line. After decompression of the chamber, the ICP gradually returned to normal. If the chamber was decompressed very slowly, "rebound rise" of ICP was not observed.

From the laboratory data it was determined that: rising rates of glutamic-oxaleacetic transurinase, grutamicpyruvic transminase, prothrombin time, and activated partial prothrombin time and the depletion of platelets were less in the HBO group.

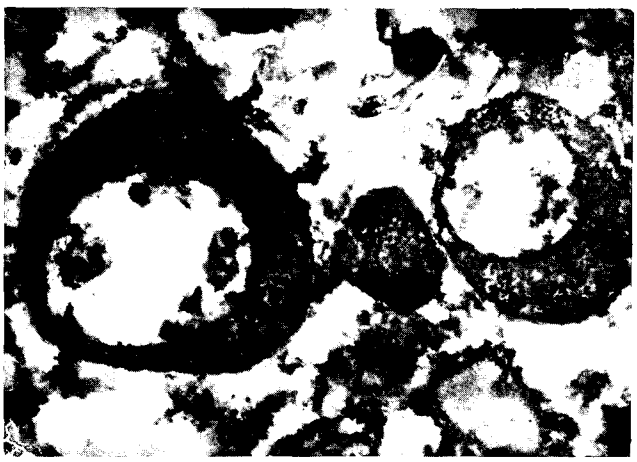


Fig. 2. Ring mitochondria, observed in the HBO group.

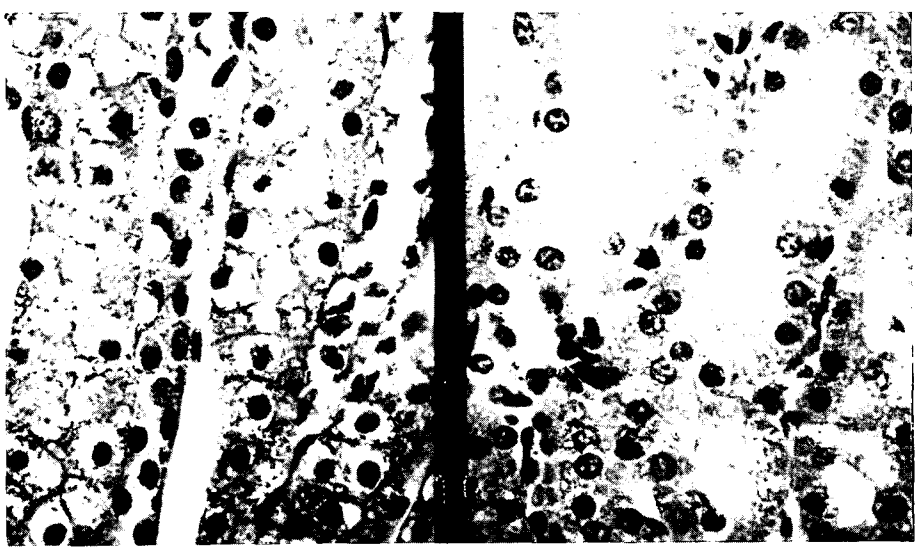
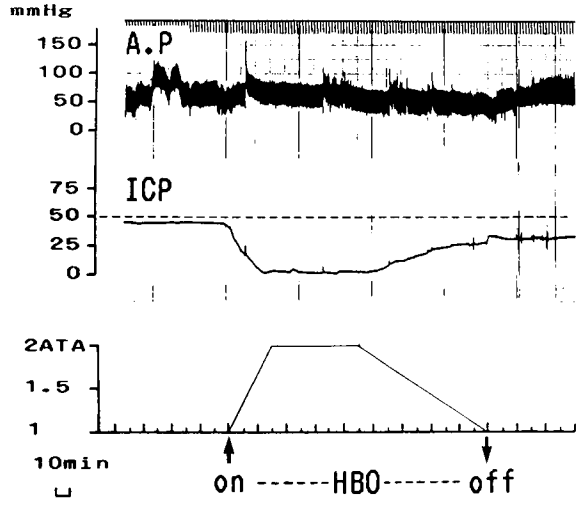


Fig. 3. Epithelial cells of the proximal tubules of the kidneys, control (left), HBO (right).

DISCUSSION

Hepatorenal failure occurring in patients with MOF is one of the fatal complications. The most commonly observed pathologic findings are centroacinar necrosis occurring widely in the hepatic cells, vacuolar degeneration of the epithelial cells of the renal tubules, and cerebral edema with intracranial



4. The effect of HBO on ICP.

hypertension and coma. These are the results of circulatory failure and hypoxia in those vital organs. Disturbance of the microcirculation is also manifested by the increased fibrin and fibrinogen degradation product and depleted platelets.

For clinical treatment, maintenance of the circulation to the organs and cellular oxygenation are of extreme importance for the prevention and cessation of the progress of those pathologic changes in the cells. In other words, HBO is a treatment of choice because it maintains the high level of Pa_{O_2} and Pt_{O_2}, supporting the cellular oxygenation.

Soviet researchers (1-7) evaluated the effects of HBO on a variety of liver diseases, and most of them reported favorable effects, including less morphologic and biochemical changes of the hepatic cells (2, 3), decrease in blood acidosis (3, 4), increase in blood O_2 saturation and tension leading to clinical improvement (4, 5), and a 50% decrease in the lethal outcome of acute hepatic failure (5). Mader (8) reported the phagocytic killing and bacteriocidal effects of HBO, preventing secondary infection.

In our experiments using rabbits in MOF, HBO treatment was also considered effective in preventing degeneration of hepatic cells, preventing ring mitochondria from vanishing, and protecting the renal tubular cells from vacuolar degeneration. HBO is also reported by many (9-13) to be effective in reducing cerebral edema and ICP. Our monitoring of ICP during HBO via an intracranial balloon showed an initial decline of ICP, followed by a plateau, and then by a gradual return. Rebound rise of ICP was avoided by slower decompression of the HBO chamber. The decline of ICP during HBO is probably due to the decrease in cerebral blood flow caused by high Pa_{O_2} and the secondary cerebral vasoconstriction (12, 13).

CONCLUSION

Hyperbaric oxygen is a valuable therapy for MOF and hepatorenal failure because it protects the cell structure and physiology from anoxic degenerative changes and reduces ICP and pathologic changes in the central nervous system.

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EFFECTS OF HYPERBARIC OXYGEN THERAPY IN ISCHEMIC CEREBROVASCULAR DISEASE IN CHINA

M. Z. Hao, Z. Q. Liu, and C. K. Yu

Hyperbaric oxygen therapy (HBO) has been used to treat ischemic cerebrovascular disease in China since 1972. We have accumulated a large quantity of data from the Chinese National Hyperbaric Oxygen Therapy Association and developed a protocol for treatment. This report presents results of 978 cases which were obtained from 1972 to 1982.

METHODS OF TREATMENT

A total of 978 patients, ages ranging from 2 to 75 yr, were treated with pure or mixed oxygen in a pressurized chamber. Two different types of hyperbaric chambers were employed: a) A large multiplace chamber where patients used an oxygen mask to breathe pure oxygen, or mixed oxygen-carbon dioxide, for 40 to 60 min at 2 to 2.5 ATA, with 10-min breaks for air breathing at 20-min intervals. The treatment cycle was 7 to 10 d with one or two sessions per day. b) A monoplace chamber, model SG-73, 74, 75, pressurized with pure oxygen to 2 to 2.5 ATA was employed. Patients received 60 to 90 min treatments daily for 7 to 10 d as 1 treatment cycle. Two to three cycles were administered depending on degree of improvement. Patients received an average of 30 d of treatment.

DISCUSSIONS

Ischemic cerebrovascular disease is caused by necrosis of cerebral tissue due to cerebral edema or insufficient blood supply in the brain. The fatality rate is 30 to 60% (1). The surviving patients usually are crippled by massive neurological defects after acute ischemic cerebrovascular seizures (2). About one-third to one-quarter of the 30 to 60% fatalities were caused by necrosis of

Table 1
Effects of HBO Therapy on 978 Cases of Ischemic Cerebrovascular Disease

	Cerebral Thrombosis		Cerebral Embolism		Cerebral Arteriosclerosis		TIA		Total	
	Case	%	Case	%	Case	%	Case	%	Case	%
Cured	129	15.6	0	0	6	4.0	0	0	135	13.80
Markedly improved	190	24.3	8	50	61	40.9	19	61.29	278	28.42
Improved	313	40.0	4	25	66	44.3	9	29.03	392	40.10
No effect	150	19.2	4	25	16	10.8	3	9.68	173	17.68
Total	781	100.0	16	100	149	100.0	31	100.0	978	100.0

cerebral tissue. Although motor functions of 50 to 70% of the surviving patients were not impaired, 20 to 30% of the patients suffered from serious and chronic functional disability (3).

The use of HBO treatment increases oxygen content in the blood, raises partial pressure of oxygen in the brain tissue, reduces accumulation of lactic acid, promotes aerobic metabolism, reduces cerebral edema, and thus provides favorable conditions for regeneration nerve tissue and of circulation in the brain. As shown in Table 1, of the 978 cases, 13.8% were cured, 28.4% showed marked improvement, 40.1% improved, resulting in a total treatment effect of 82.3% improvement against 17.6% no effect. It is important to point out that one-third of the cured patients in the acute category after receiving HBO treatment were free from infection of lungs and the urinary system and of bed sores.

In selecting patients and determining duration of treatment, HBO treatment centers in China have agreed that to achieve better results a) HBO should be administered as soon as possible, b) the younger the patients the better the results, and c) treatment cycle should be sufficient but not prolonged; generally a 2- to 4-treatment cycle is preferable. In treating patients, mixed oxygen (3% carbon dioxide:97% pure oxygen) at 2 ATA was found to have the advantage of a lower chamber pressure for treatment, no need to prescribe drugs for blood vessel dilation, a reduced treatment cycle, and a saving of oxygen. The method of mixed oxygen has been adopted by all HBO treatment centers throughout China.

There are several successful blood vessel by-pass operations in the cured group following recovery of functions in the brain, which supports the notion that cerebral necrosis is not irreversible and thus suitable for a cerebral blood vessel by-pass operation. However, other physicians in China believe that for this type of disease, the by-pass operation should be performed before HBO treatment. At present, there is insufficient data to support either side, and more evidence is needed before drawing any conclusions.

Of the 978 cases, some patients had been administered Chinese herbal medicine to prevent "reflective" blood vessel contraction. Overall, there was no report of deterioration nor recurrence of the disease due to HBO treatment. There were, however, cases of failing to treat cranial infection for the patients in the younger age bracket, resulting in a delay or failure to achieve HBO treatment effects.

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COMBINATION THERAPY FOR THE EXPERIMENTAL TUMOR WITH HYPERBARIC OXYGENATION AND ANTITUMOR ANTIBIOTICS

H. Sugiyama and K. Kamiyama

Hyperbaric oxygen is said to produce active oxygen in the tissues (1), which has three reactions (2). First, it reacts with the lipids of cell membranes, producing lipid peroxide which leads to cell membrane breakdown (3, 4). Second, it reacts with nucleic acids, such as DNA or RNA, and makes them breakdown or change. Third, it causes damage to various enzymes; for example, superoxide dismutase (SOD) and catalase are inactivated by active oxygen. These reactions of active oxygen are powerful weapons for cancer.

Antibiotics exist that generate active oxygen and act as antitumor agents releasing active oxygen to the tumor (5). Such drugs are called antitumor antibiotics and have a quinone nucleus (6). The effectiveness of this antitumor agent with HBO is enhanced when the active oxygen is increased (7). Our combination therapy of Mitomycin-*C* (MMC) with HBO to the S-180 of mice enhances the active oxygen to cure the cancer.

MATERIALS AND METHODS

The experimental hyperbaric chamber (Hanyuda Iron Company) is cylindrical, 40 cm in diameter, and 100 cm in length, and has a window to observe the mice. After a 5-min flow of pure oxygen; the concentration of oxygen in the chamber rose to 100%. The oxygen concentration in the chamber was measured regularly. DdY-mice were used and active oxygen in the blood during HBO treatment was measured. The mice had generalized convulsions, called oxygen intoxication, which paralleled the atmospheric pressure and the duration of exposure. Clinical signs of oxygen intoxication means there is an increase of active oxygen. When the pressure goes up, generalized convulsions occur within a shorter exposure time (Fig. 1). This means the active oxygen

parallels the atmospheric pressure and exposure time. For these experiments, HBO for 1 h under 4 ATA was chosen because with this exposure schedule the side effects from hyperbaric oxygenation do not occur in the experimental mouse and much active oxygen is produced.

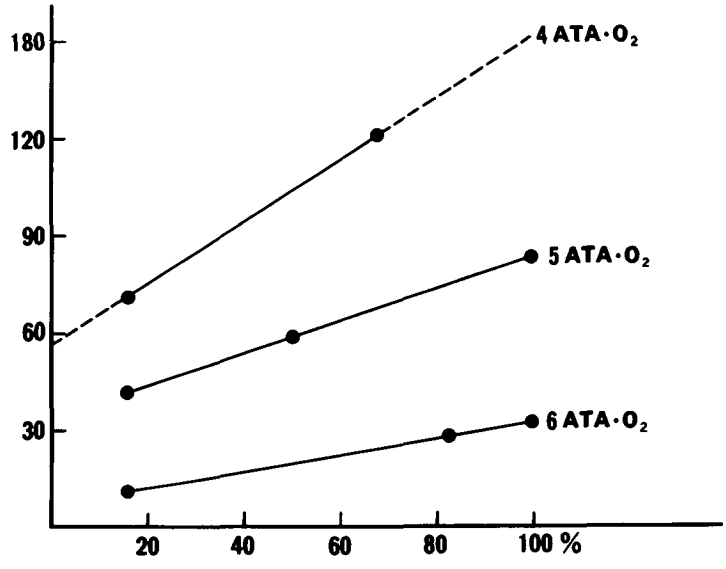

Fig. 1. Generalized convulsion under different atmospheric pressures.

The average body weight of the mouse was 20 g. S-180 was chosen as the experimental tumor because it has sensitivity to MMC (7), responds to the drug, can be measured easily, and can be transplanted to the abdominal wall of the ddY mouse. The length and width of the tumor were measured, and the tumor volume calculated, long diameter times short diameter squared times one half. The side effects of the antitumor drug and HBO were clearly shown from the number of white cells in the blood in relation to body weight.

Mitomycin-C is used as the antitumor drug, because it (is one of the antitumor antibiotics that exhibits antitumor effects by the so-called free radical reactions (7). MMC also has fewer side effects than other antitumor antibiotics. The method of its application to the mouse is very simple. The LD_{50} of MMC to the mouse is 8.4 mg/kg. After several preliminary studies, 6 mg/kg showed maximum effects of antitumor for the S-180, and was given via the peritoneal cavity. No deaths occurred after 1 wk of HBO. The mice were divided into 4 groups of 8 mice each. There was a control group (C), a HBO group (O), a MMC group (M), and a MMC + HBO group (M+C).

RESULTS

The decrease in body weight after 3 exposures is shown in Fig. 2. When compared to the control group, there were statistically significant decreases at the level of $P < 0.05$, for group M and $P < 0.01$ for group O+M. Leukopenia was found in group M, but the statistical significance between group C and group O, M, or O+M was not significant (Fig. 3). No mice died during these experiments, and their health was good.

Sizes of the tumors were 68 ± 7.8% in the HBO group, 45 ± 5.6% in the MMC group, and 31 ± 2.1% in the HBO + MMC group when compared with the control group (Fig. 4). A statistical analysis between the MMC and HBO + MMC groups indicated a significance of $P < 0.05$ (Fig. 5).

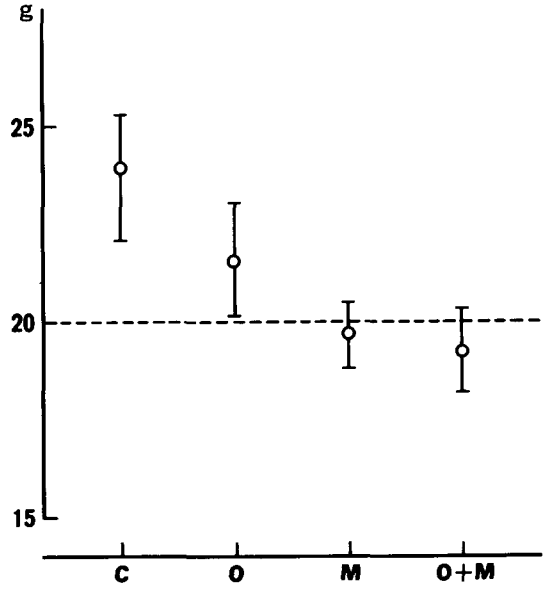

Fig. 2. Changes of body weight of mice among different groups. C = control group, O = HBO group, M = MMC group, and O+M = HBO + MMC group.

DISCUSSION

Hyperbaric oxygen is less powerful in curing malignant tumors than when used in combination with other therapies. HBO with radiation therapy was done first by Gray in 1953 (8), and was followed by many reports employing combination therapy (9). In 1977, Dische (10) reported clinical trials coordinated by several cancer research centers in which head and neck, cervical, bladder, and bronchial cancers were treated with HBO and radiation. More than 1000 patients participated in this study, and clinical results were disappointing.

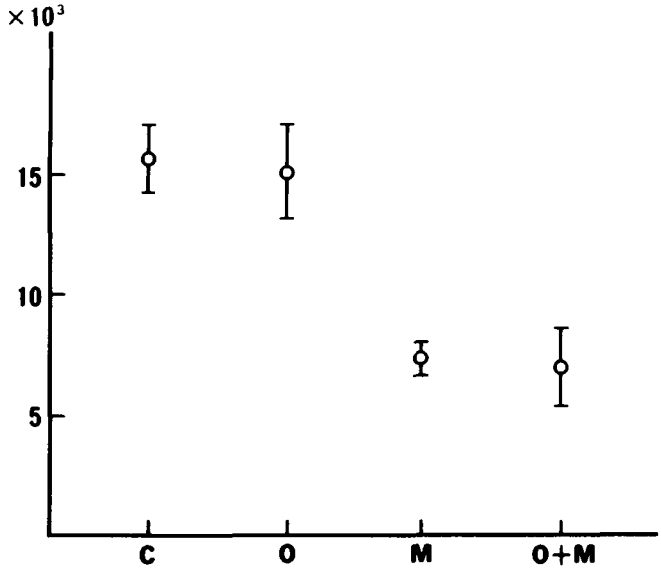

Fig. 3. White cells in the blood of mice in the different groups.

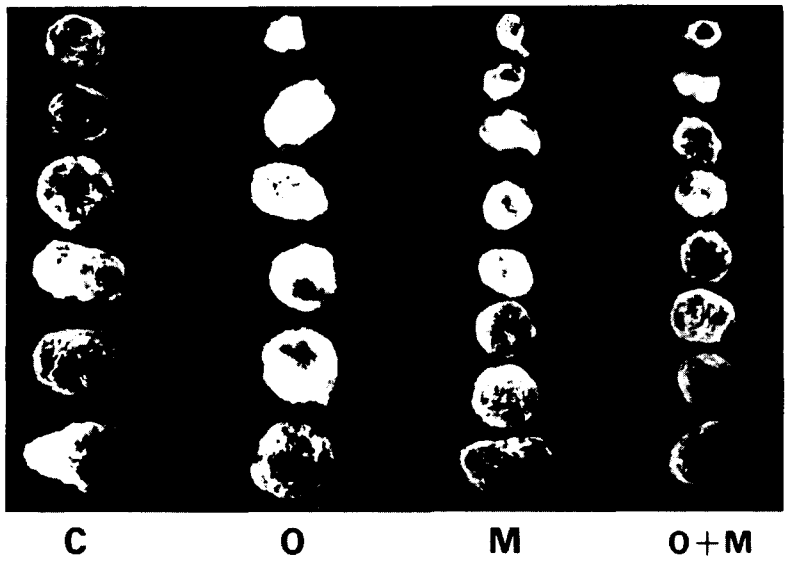

Fig. 4. Sarcoma-180 on the abdominal wall after 7 d implantation.

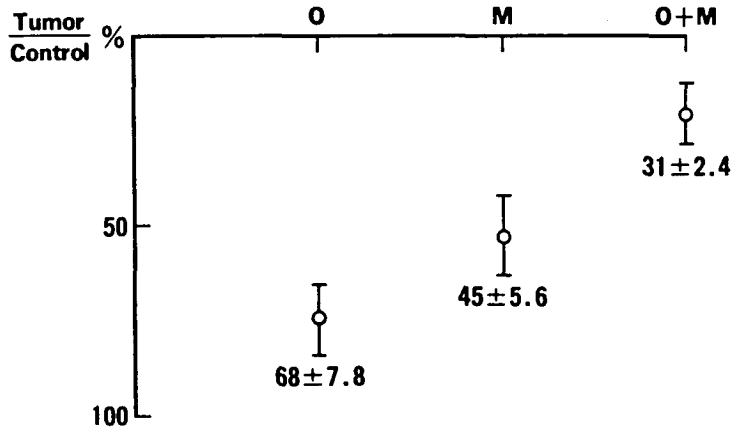

Fig. 5. Tumor sizes compared with control group.

Combination therapy using HBO and antitumor drugs such as nitrogen mustard were mostly experimental (5, 11). In 1980, Takenaka et al. (12) used Bleomycin with HBO to treat Ehrlich ascites carcinoma in mice abdomens, and succeeded in augmenting the antitumor effect with oxygen without a statistical increase of side effects to lung and metastasis. The effectiveness of oxygen to the cancer can be easily understood from the biological activity of active oxygen. To generate active oxygen into the body in sufficient amounts to damage the tissue also enhances the antitumor effect. Generalized convulsion in the chamber signifies toxic, active oxygen and its effect on the CNS. Just before convulsions there is an increased amount of active oxygen in the body. We used 4 ATA of oxygen for 1 h in our mice, and we found that exposure to be sufficient to generate active oxygen in the body.

We chose S-180 as the experimental tumor because it is sensitive to MMC and we could therefore judge tumor growth within a week. At the same time, HBO responded to S-180. This meant it had a sensitivity to active oxygen and MMC. On the other hand, the Ehrlich ascites carcinoma had no sensitivity to HBO. S-180 was the best experimental tumor for responsiveness to HBO.

For maximum antitumor effect, we chose 6 mg/kg MMC i.p. in one infusion. The decrease of both white cells in the blood and body weight were seen as side effects of MMC, but HBO did not worsen these effects. These causes were not due to the active oxygen. On the other hand, lung fibrosis occurs after exposure to active oxygen. Bleomycin also induces lung fibrosis (8). In our experiments, pathologic lung fibrosis was not in evidence.

The 31% tumor-to-cancer (T-C) values found after data analysis did not satisfy us, because we had expected a smaller T/C value. However, we felt that the conditions of HBO, the dose and method of applying MMC, the experimental tumor, and the test animals were ideal. If we want to find a way to cure tumors with HBO, we will have to find a method to protect the normal tissues from active oxygen.

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HYPERBARIC PULMONARY LAVAGE IN PULMONARY ALVEOLAR PROTEINOSIS

D. J. Bakker, H. M. Jansen, W. W. A. Zuurmond, J. J. Schreuder, and C. M. Roos

Pulmonary alveolar proteinosis (PAP) is an uncommon disease of unknown origin, characterized by accumulation of lipid- and protein-rich insoluble material in the alveoli of the lung. This disease was first reported by Rosen et al. (1). Since then, several papers on diagnosis, pathogenesis, and ways of treatment of this disease have been published (2-6).

The clinical course and prognosis of the disease are extremely variable. Bronchoalveolar lavage is an effective treatment for symptomatic PAP patients in whom spontaneous remission does not occur, and dyspnea and progressive deterioration of arterial oxygenation develops. This lavage by which the proteinaceous material is removed from the alveoli was introduced by Ramirez et al. in 1966 (7). Following therapeutic lung lavages, significant improvement in symptoms as well as pulmonary function tests and blood gases were observed. However, lavage procedures with ventilation of one lung while repeatedly lavaging the other lung can be impossible to perform on patients with severe respiratory failure because adequate oxygenation cannot be maintained.

Alternative approaches, such as lavage procedures during extracorporeal membrane oxygenation (ECMO) or partial cardiopulmonary bypass, are very invasive (8-10), and sequential lobar lavage through a fiberoptic bronchoscope (11) under local anesthesia is rather ineffective.

To avoid these problems, lung lavages under hyperbaric oxygen ventilation in a large, multiple, walk-in hyperbaric chamber were carried out. Severely afflicted patients could safely be treated in this way.

MATERIALS AND METHODS

Massive unilateral bronchopulmonary lavage under hyperbaric conditions was performed 4 times in 2 patients with severe respiratory insufficiency due to PAP.

Patient A

Male, age 29 yr, who during the preceding 14 mo. had developed progressive respiratory insufficiency, at first on exertion only, later also at rest. Diagnosis of PAP was made by open lung biopsy, showing the alveoli filled with granular, periodic acid Schiff-positive, alcian blue negative material. High-dose corticosteroid therapy for 2 mo. was unsuccessful, and the patient was referred to our institution for further evaluation and treatment.

The patient was a professional road-maker from a rural area, smoking up to 40 cigarettes/d for more than 10 yr. Physical examination showed peripheral cyanosis, clubbing of the digits, and the use of accessory respiratory muscles. Blood pressure was 120/80 mmHg, pulse rate 100/min, temperature 37.0°C, respiratory rate 20/min. Auscultation of the lungs only revealed some late inspiratory bibasic crackles.

Chest x-ray: diffuse bilateral alveolar nodular pattern, coalescent in the center of the butterfly shadowed picture. ECG: regular sinus tachycardia. Laboratory: Hb 9.7 mmol/liter. erythrocyte sedimentation rate 3 mm, pH 7.46, P_{O_2} 44 mmHg (in room air at rest), P_{CO_2} 29 mmHg.

Pa_{O_2} was 109 mmHg at 100% O_2, giving a calculated alveolar-arterial oxygen difference of 544 mmHg, equivalent to a right-to-left shunt of 26% of the cardiac output. There was a restrictive ventilatory disorder with severely depressed diffusing capacity for carbon monoxide (DCCO) of 23% of the predicted value.

Patient B

Male, age 46 yr, with a progressive shortness of breath without coughing for 16 mo. Diagnosed PAP was by open-lung biopsy. The patient was a florist without previous airways disease or respiratory infections. He smoked up to 25 cigarettes/d for more than 20 yr and had stopped smoking 1 yr earlier. Physical examination showed no cyanosis or clubbing of the digits. Blood pressure was 135/80 mmHg, pulse rate 92/min, temperature 37.5°C, respiratory rate 14/min. Auscultation of the lungs gave some fine bibasic crackles. His chest x-ray showed a diffuse, bilateral alveolar nodular pattern.

Laboratory: Hb 10.8 mmol/liter, erythrocyte sedimentation rate 17 mm. Arterial pH was 7.43, P_{CO_2} 28 mmHg, P_{O_2} 57 mmHg, breathing room air at rest.

A right-to-left shunt was 22% of the cardiac output and DCCO was 39% of the predicted value. There was a restrictive ventilatory disorder. ECG was normal, but a dilated right ventricle without an optimal contractility pattern was found. Further studies revealed a decreased right ventricle ejection

fraction of 33%.

METHODS

Both patients underwent hyperbaric pulmonary lavage twice under general anesthesia in a large hyperbaric chamber. Patients were pretreated with hydrocortisone 250 mg i.v. Arterial blood pressure, ECG, heart rate, central venous pressure, pulmonary artery pressure, mixed venous oxygen saturation, expired carbon dioxide, and body temperature were monitored continuously. Arterial and mixed venous blood gases and pulmonary artery wedge pressure were measured intermittently.

After preoxygenation, patients were given etomidate 20 mg, fentanyl 350 mg, and pancuronium bromide i.v. Anesthesia was maintained with a continuous etomidate infusion. Patients were intubated with a double lumen intubation tube and placed in a lateral decubitus position. After checking the tube position, 100% O_2 ventilation at 2 bar was started, and unilateral lavage procedure begun, using 20 to 25 liter saline solution (500-750 ml of saline/lavage cycle) through a sump drain into the collapsed lung. The entire procedure at pressure for both patients lasted no longer than 240 min.

After lavage procedures and repetitive suctioning of the lavaged sides, the two lungs were ventilated with a Fi_{O_2} of 1.0 rapidly tapering off to 0.5 with 10 cm positive end respiratory pressure tapering off to zero.

Extubation could be accomplished about 60 min after lavage and patients were then treated with oxygen by nasal prongs (5 liter/min).

RESULTS

Data during and results after this treatment can be seen in Figures 1-6.

DISCUSSION

The first lavage in these patients was performed on the most affected left lung, leaving the larger right lung to support as much as possible gas exchange. Lavage procedures were well tolerated and did not add to morbidity. Bacterial and fungal cultures of the recovered samples were all negative.

An Fi_{O_2} of 1.0 at 2 ATA gave rise to an increase of the arterial oxygen tension (Pa_{O_2}) from 60 mmHg with one lung blocked 550 to 650 mmHg.

Mixed venous oxygen tension ($P\bar{v}_{O_2}$) rose from 42 to 45 mmHg to 60 to 65 mmHg during left lung and right lung lavage, respectively.

The Fi_{O_2} could soon be reduced to 0.5 based on these data. A slight increase in Pa_{CO_2} was seen as well, due to the presence of less-reduced hemoglobin which transports CO_2. However, this was, together with an acid shift in pH, of no clinical significance (12).

For technical reasons it was impossible to measure absolute values of the $PA\text{-}a_{O_2}$ difference at 2 ATA. Also, O_2-content measurements were not done.

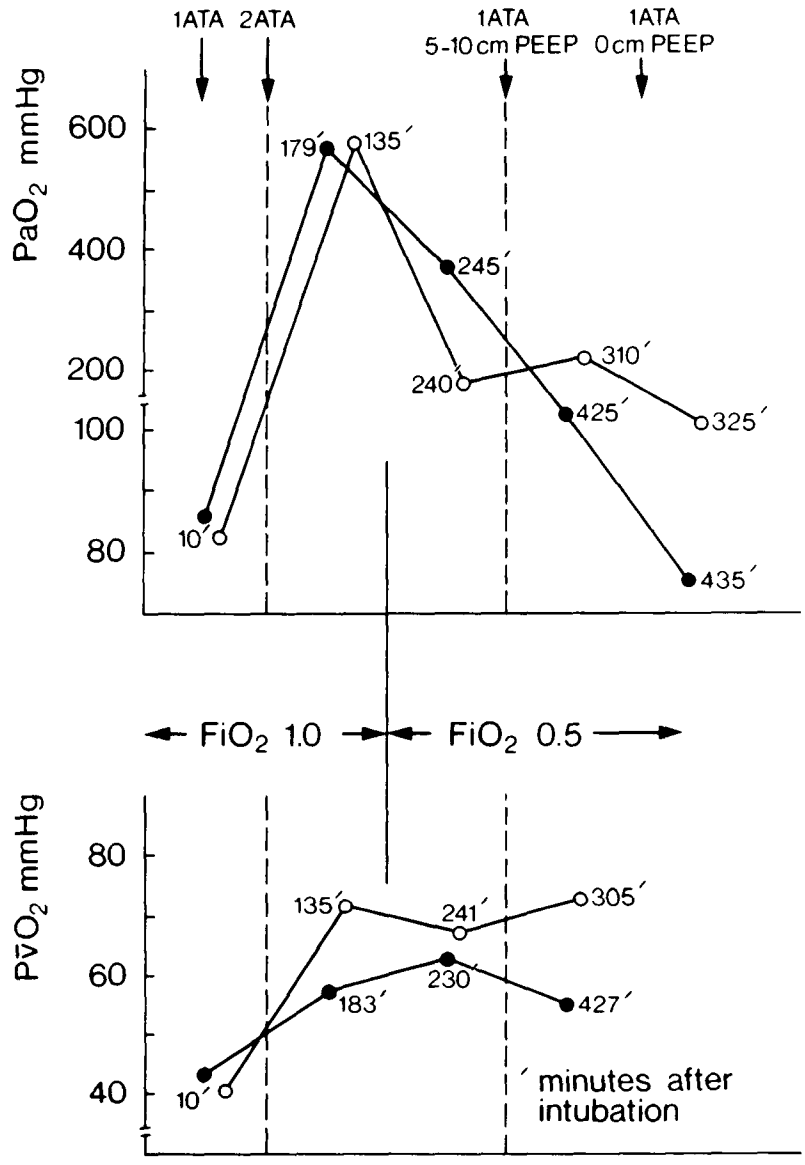

Fig. 1. Pa_{O_2} and $P\bar{v}_{O_2}$ during left lung lavage (•—•) and right lung lavage (o—o), before, during, and after lung lavage.

These will be included in future lavages because only in this way will it be possible to calculate the absolute amount of right-to-left shunting. This shunting appeared to be maximal during periods of lavage fluid drainage (Fig. 3).

In the literature, there seemed to be a direct correlation between low initial Pa_{O_2} and consecutive high initial $P(A\text{-}a)_{O_2}$ pressure difference and the ultimate outcome of the disease (13).

The concomitant further decrease of oxygen exchange during conventionally performed lavage procedures might be one of the causes of this. Therefore, we believe that the combination of massive pulmonary lavage under hyperbaric conditions, during which a further, longstanding decrease of Pa_{O_2} and $P\bar{v}_{O_2}$ can be prohibited, represents a significant therapeutic advantage. It also makes unilateral lavage more effective because increasing amounts of lavage fluid can be used.

Follow-up studies on pulmonary function (Fig. 4) showed improvement of pulmonary volumes ($VC + FEV_1$) with an increase of residual volume (RV) and total lung capacity (TLC). A striking improvement of carbon monoxide transfer factor (DLCO, corrected for Hb content) developed. In addition, blood gases and chest x-rays showed significant improvement on follow-up.

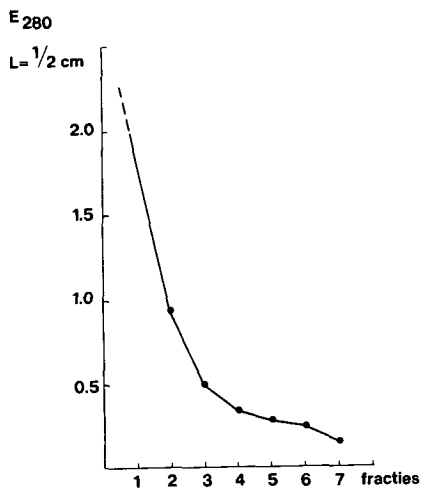

Fig. 2. Course of A-280 absorption of subsequent recovered samples of lavage fluid demonstrating decreasing protein content during the procedure. Ordinated units are absorption units.

CONCLUSIONS

Hyperbaric bronchoalveolar lavage in PAP is superior to standard procedures because:

- It seemed to be more effective in that Pv_{O_2} and $Vsat_{O_2}$ during the procedure increased far above safe lower limits.
- It is a safer procedure in severely afflicted patients with severe respiratory insufficiency.
- It is easy to perform is a multiplace hyperbaric chamber as far as anesthesia, lavage performance, and monitoring are concerned.

This treatment needs further development for other indications such as: lipid pneumonitis, severe exposure to inorganic dust, and alveolar microlithiasis.

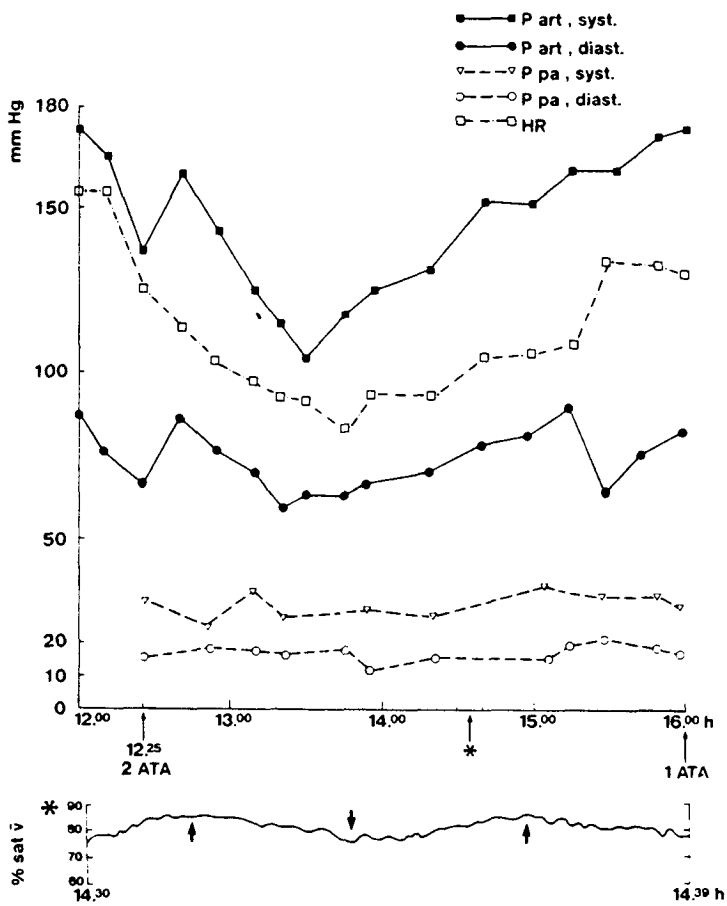

Fig. 3. Data during right lung lavage (systolic and diastolic blood pressure, pulmonary artery pressure, and heart rate). Note: The striking difference in the $\bar{v}$ sat between the empty and the fluid-filled lung (lower curve).

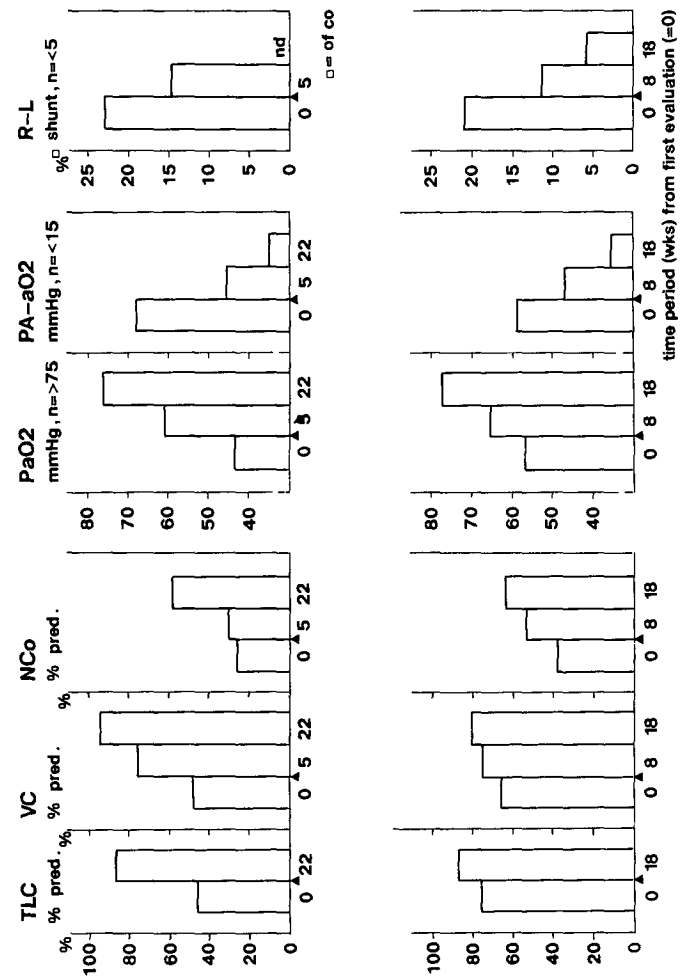

Fig. 4. Data on pulmonary function and blood gas levels, before and after 5 and 22 wk (patient A, top), respectively, 8 and 18 wk (patient B, bottom), after bronchoalveolar lavage. There is a significant improvement in pulmonary function in this follow-up period.

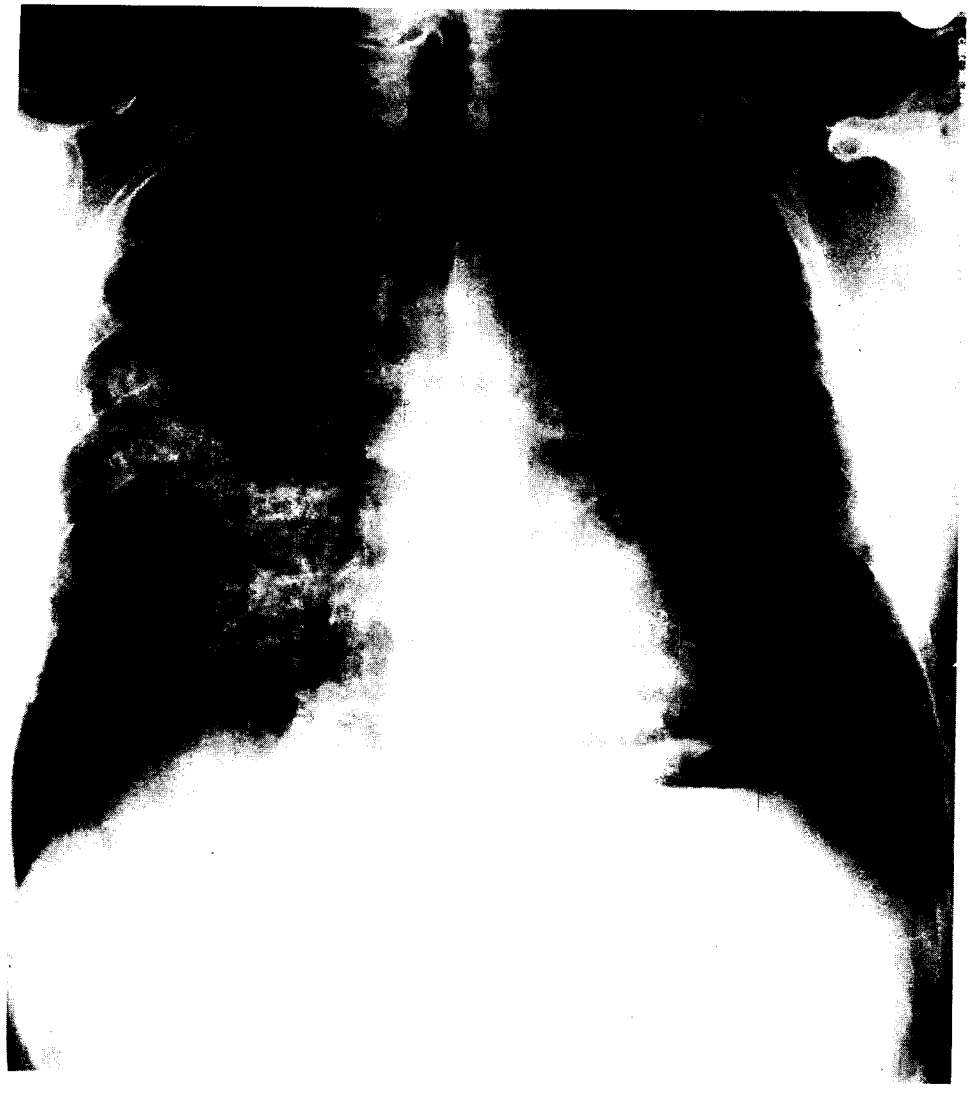

Fig. 5. Chest x-ray of patient A before lung lavage.

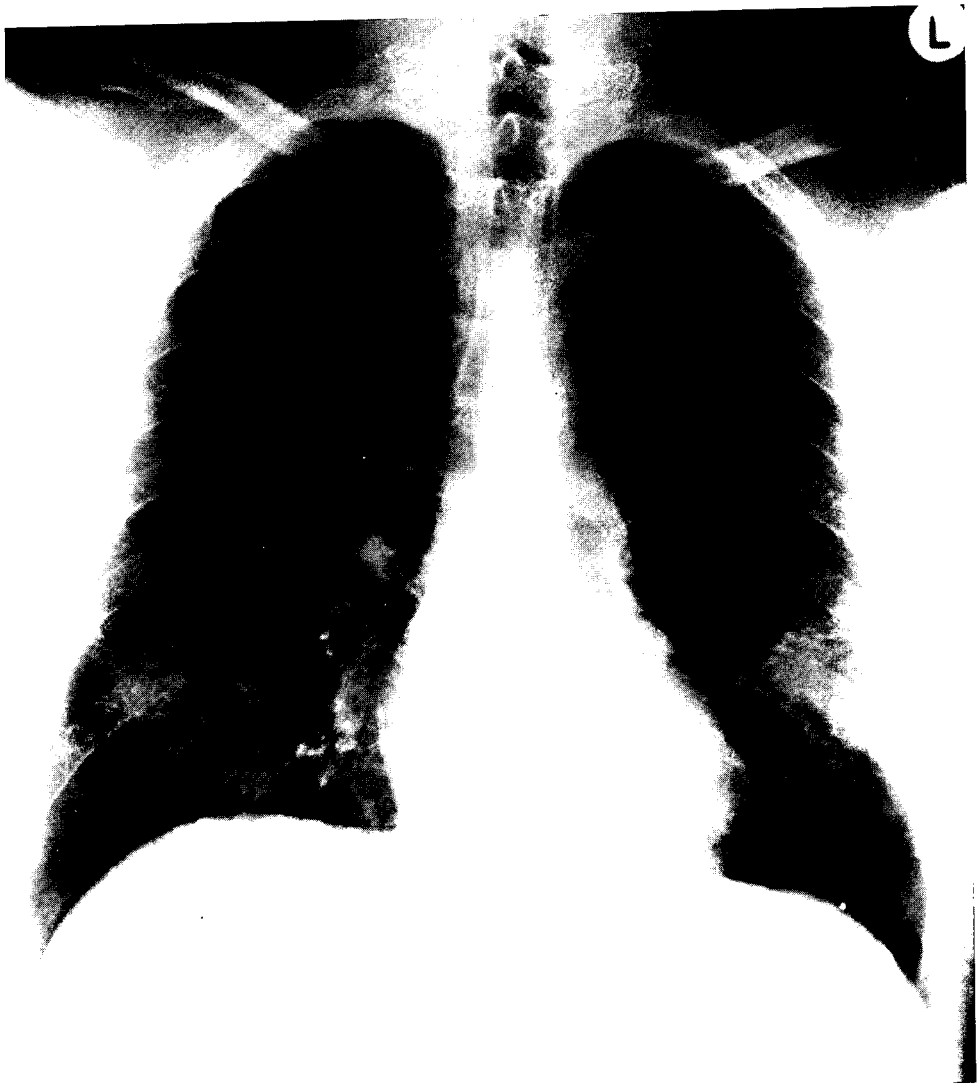

Fig. 6. Chest x-ray of patient A after lung lavage.

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PREDICTION OF ARTERIAL Po_2 DURING HYPERBARIC TREATMENT

R. E. Moon, E. M. Camporesi, and D. L. Shelton

Inasmuch as the main object of hyperbaric oxygen therapy (HBO) is to increase tissue Po_2, the likelihood of success of treatment is related to the arterial Po_2 (Pa_{O_2}) which may be obtained. Ideally, one would tailor the ambient pressure[2] during HBO to attain a given measured arterial or tissue Po_2. In lieu of measurement it would be advantageous to have a method by which Pa_{O_2} at pressure could be predicted from values measured at 1 ATA. It has been previously shown at 1 ATA that arterial Po_2 values measured while breathing a given inspired O_2 concentration (FI_{O_2}) can be used to predict Pa_{O_2} while breathing any other FI_{O_2} using the[2] arterial:alveolar oxygen tension ratio (Pa_{O_2}/PA_{O_2}), which remains relatively constant in any given patient (1). This[2] technique has not been applied to the hyperbaric environment. We present here a series of measurements performed on normal volunteers and patients with varying degrees of lung disease to determine the applicability of this principle to atmospheric pressures higher than 1 ATA.

METHODS

Arterial blood gases (ABG) were measured at rest from samples collected anaerobically from a radial artery catheter in 5 healthy, informed volunteers. The protocol for this study was approved by the Institutional Review Board of Duke University. The subjects' blood was sampled at 1 ATA, breathing air and 100% O_2, and at 2.82 ATA (18.3 msw), breathing compressed air and 100% O_2. ABG were measured also for clinical purposes on 37 patients receiving hyperbaric O_2 treatment at various ambient pressures. Oxygen was usually administered through a Duke head tent, with a rubber seal around the neck. The tent was connected to a semiclosed breathing circuit. O_2 and CO_2 levels in the tent were monitored, and inflow of O_2 was adjusted to maintain $Fi_{O_2} > 0.98$.

Two patients had oxygen administered via a cuffed tracheostomy tube. All patients were breathing spontaneously. After appropriate equilibration times (10-30 min), Pa_{O_2}, Pa_{CO_2}, pHa were measured in each case at the depth of collection using electrodes inside the pressure chamber. Calibrations for O_2 and CO_2 were performed at the depth of measurement with appropriate gas mixtures bracketing the physiologic values expected. Each ABG measurement was preceded and followed by a calibration determination. For each subject, at each exposure pressure and FI_{O_2} studied, PA_{O_2} was calculated using the following formula:

$$PA_{O_2} = PI_{O_2} - PA_{CO_2}\left[FI_{O_2} + \frac{1-FI_{O_2}}{R}\right] \quad (1)$$

where: PA_{O_2}, PA_{CO_2} = alveolar gas tensions

PI_{O_2} = inspired O_2 tension

Pa_{CO_2} = arterial Pco_2

FI_{O_2} = fractional inspired O_2 concentration

R = respiratory quotient = $\dot{V}CO_2/\dot{V}O_2$

and assuming $PA_{CO_2} = Pa_{CO_2}$ and $R = 0.8$. This allowed calculation of Pa_{O_2}/PA_{O_2} (a:A ratio) for each ABG value measured. Additionally, the a:A ratio obtained while breathing room air at 1 ATA was used to multiply PA_{O_2} values measured for each subject in the various conditions, to obtain a predicted Pa_{O_2} value. Subjects and patients were arbitrarily classified as "normal" (group A, a:A ratio $\geq$ 0.75: breathing air at 1 ATA) or "abnormal" (group B, a:A ratio $<$ 0.75).

RESULTS

The range of exposure pressures is shown in Table 1. All subjects tolerated the procedure well. Pa_{CO_2} values ranged from 23 to 43 at 1 ATA and 25 to 40 breathing 100% O_2 at pressure. There was no significant change in Pa_{CO_2} between 1 ATA (air) to pressure (100% O_2); mean change was -1.8 Torr. Table 2 describes the distribution of the variables.

A plot of all actual *measured* Pa_{O_2} (other than air, 1 ATA) vs. the corresponding Pa_{O_2} *predicted* from the 1 ATA measurements of PA_{O_2} and Pa_{CO_2} using the described equation is presented in Fig. 1.

Table 1
Measurement Pressures (ATA) and Number of Individuals Exposed

Pressure	No. of Measurements
2.0	29
2.4	8
2.8	6
3.1	1

Table 2
Pa_{O_2} and a:A Ratio Obtained from ABG Determinations at 1 ATA, Breathing Room Air

	Group A	Group B
a:A ratio	≥ 0.75	< 0.75
No. subjects	6	36
No. sets of measurements	7	37
No. ABG determinations	22	99
Pa_{O_2}, mean ± SD	81.4 ± 4.3	67.3 ± 8.8
Pa_{O_2}, range	75-85	48-83
a:A ratio, mean ± SD	0.79 ± 0.02	0.62 ± 0.08
a:A ratio, range	0.77-0.81	0.46-0.74

DISCUSSION

The use of a:A O_2 ratio as an index of gas exchange has been described with changing values of inspired oxygen concentration (1). As such, the ratio has been used at 1 ATA to predict the anticipated oxygen tension at a given FI_{O_2} from arterial blood gas data obtained at another FI_{O_2}. We have shown that the principle can be extended to ambient pressures above 3 ATA and can successfully predict Pa_{O_2} even in the presence of baseline gas exchange abnormalities. While PA_{O_2}-Pa_{O_2} (A-a gradient) is sensitive to Fi_{O_2}, a:A ratio remains nearly constant due to the relative linearity of the Hb-O_2 dissociation curve above 95% saturation.

If it is assumed that $R = 0.8$ then a:A ratio can be calculated from the room air blood gas values obtained at 1 ATA and eq. *2*.

$$\text{a:A} \approx \frac{Pa_{O_2}}{0.21(P_{Bar}{}^{-47}) - 1.2\ Pa_{CO_2}} \qquad (2)$$

where: P_{Bar} = barometric pressure (mmHg).

Equation *2* is an approximation (assuming $R = 0.8$) of the full *3* which can be used if the patient is breathing any O_2 concentration.

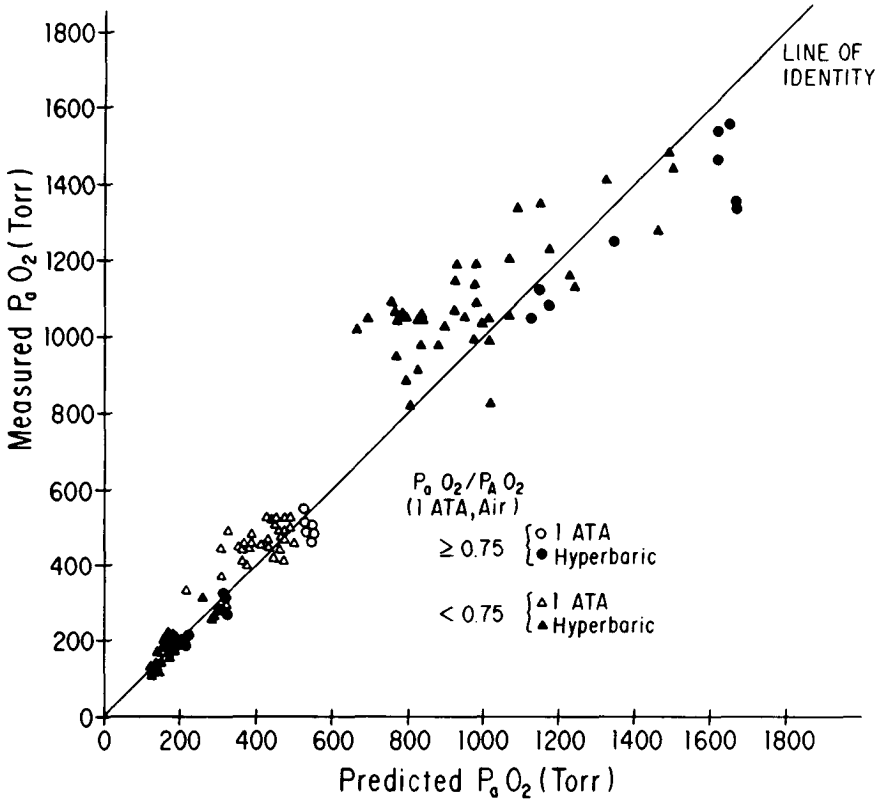

Fig. 1. Predicted vs. measured Pa_{O_2} from 1 to 3.08 ATA. The regression equation is:

$$PO_2 \text{ (meas)} = 0.974\ PO_2 \text{ (pred)} + 59.6 \ (r = 0.96, P = 0.0001)$$

where: PO_2 (meas) and PO_2 (pred) are the measured and predicted Pa_{O_2} values, respectively. All points scatter around the identity line, both in normal subjects and in patients. Regressions of actual vs. predicted Pa_{O_2} values did not differ significantly from the identity line for both sets of measurement, despite the variability in room air Pa_{O_2} reported in Table 1.

$$a{:}A = \frac{Pa_{O_2}}{FI_{O_2}\,(P_{Bar}{}^{-47}) - Pa_{CO_2}\,(1.25\text{-}0.25\ FI_{O_2})} \qquad (3)$$

Predicted Pa_{O_2} at pressure, breathing 100% O_2, can then be calculated from eq. *4*.

$$Pa_{O_2}(\text{pred}) = (a{:}A)\,[(760\ P_{ATA}-47) - Pa_{CO_2}] \qquad (4)$$

where: P_{ATA} = ambient pressure in ATA; Pa_{O_2} is measured in mmHg.

We propose the use of the a:A ratio for the prediction of Pa_{O_2} during hyperbaric treatment when actual measurement is not feasible. This principle seems to be valid for individuals with either normal or abnormal gas exchange.

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ROLE OF HYPERBARIC OXYGEN THERAPY ON ATHLETES' PERFORMANCE: FIRST EVALUATIONS

P. Longobardi, R. Mastroianni, G. Medolla, and F. M. Pallotta

Literature reports that by supplying normobaric O_2 to tissue perfusates (1) or to patients (with peripheral vascular occlusive disease, i.e., suffering from muscular hypoxia in a similar way to normal subjects under strain) who underwent muscular biopsy (2) an increase in glycolysis and Krebs cycle enzymes and particularly in creatine phosphokinase (CPK), in pyruvate-lactate rate (with less production of lactate), in phosphocreatine, and in ATP-ADP rate can be noticed. The results show the importance of Po_2 in the regulation of the energy and redox state of the muscular tissue (Figs. 1-5).

Hence, a hypothesis was formulated: By supplying hyperbaric O_2 to athletes whose training consists of medium- or long-time exercise (3, 4) there is a reliance on aerobic metabolism because training as well as other factors determine the differentiation of muscular fibers toward a type with mainly oxidative metabolism [slow fibers or type I fibers (5)], resulting in an increased production of ATP and phosphocreatine, i.e., of energy state. Consequently, after HBO the athletes could carry out their own performance in aerobiosis, accumulating less lactate and less oxygen debt.

MATERIALS AND METHODS

Two groups of experienced judo wrestlers were selected from among the athletes of Kodokan Sports Centre (FILPJ). The 14 athletes in each group were randomly selected. One group was treated with HBO and the other group served as a control. During the experiment the 2 groups trained together so that they differed only in hyperbaric treatment.

The experiment was carried out by using a Galeazzi multiseater compression chamber equipped with an airlock and all the necessary instruments for

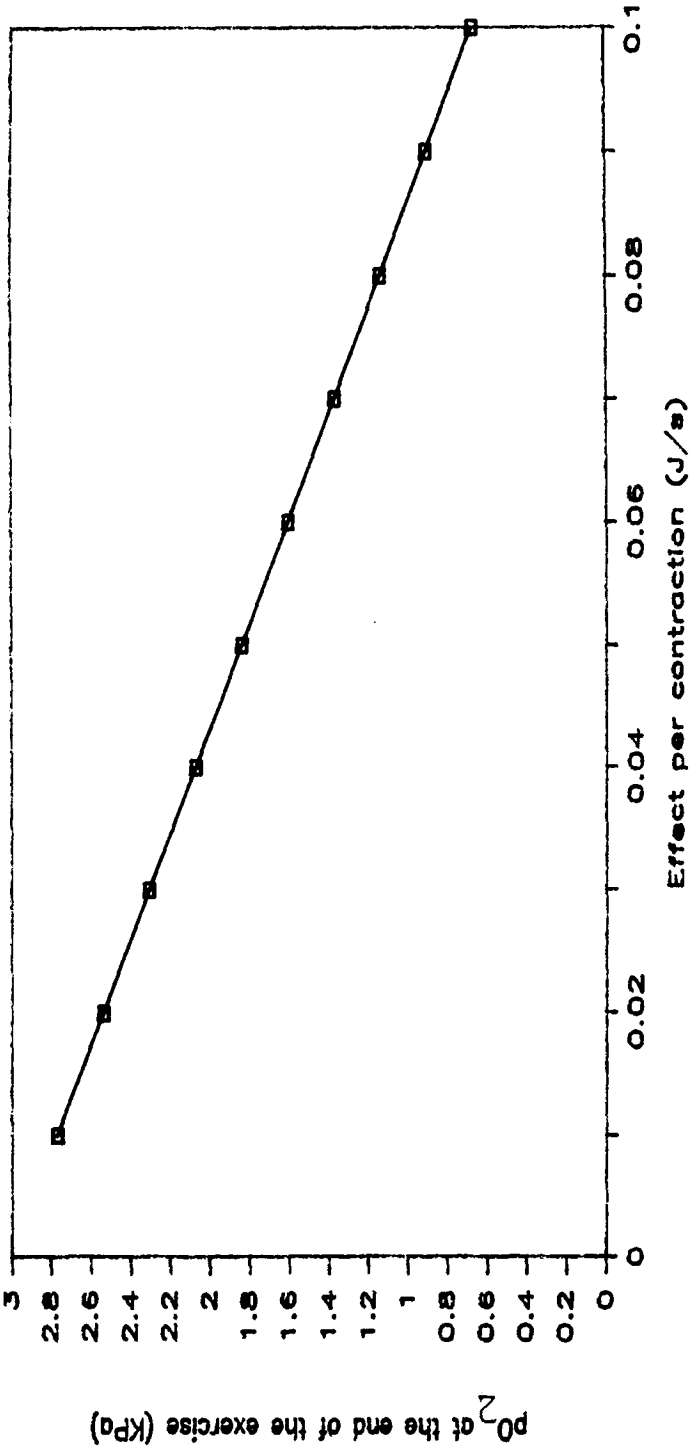

Fig. 1. Relationship between the intensity of exercise expressed as effort (W) per contraction and the intramuscular PO_2 at the end of exercise. The line of best fit to the data is $y = 3 - x\,(2.8/1.12)$; $P < 0.005$.

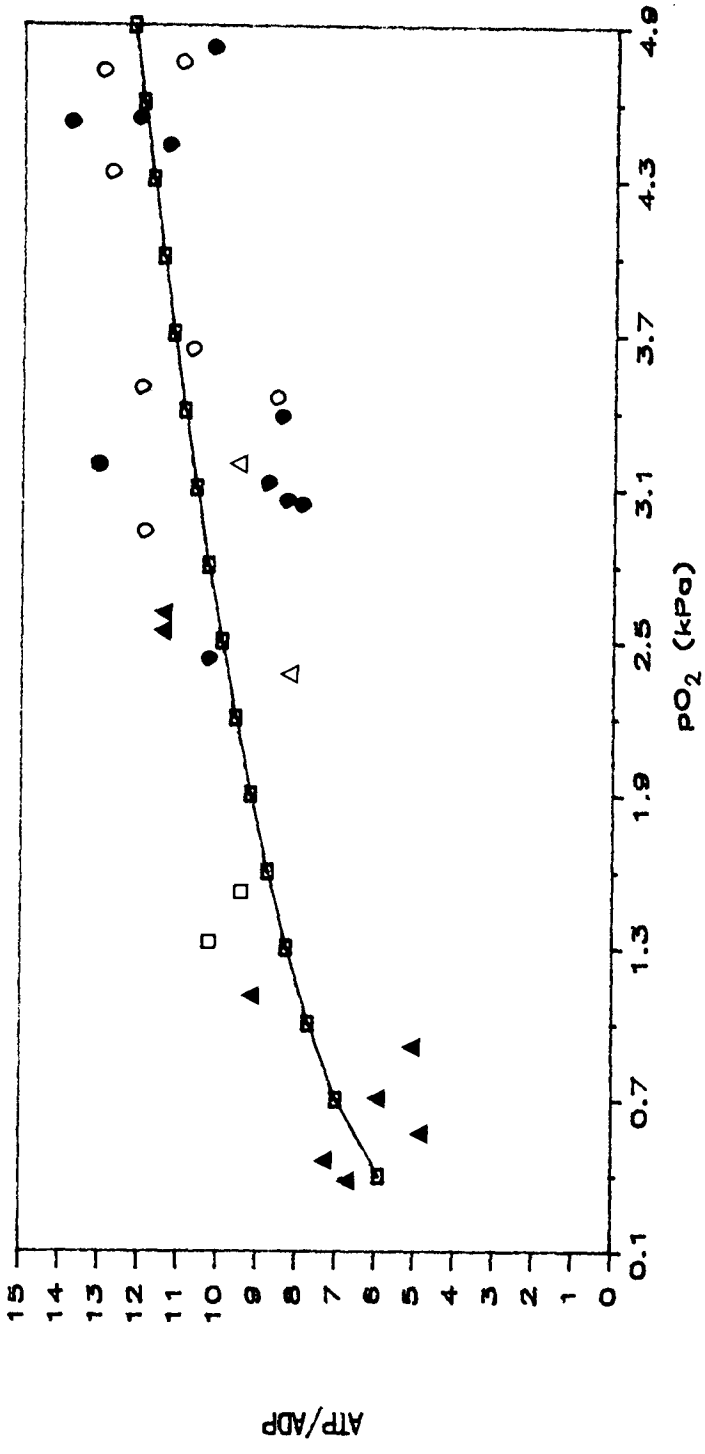

Fig. 2. Relationship between intramuscular PO_2 and the ATP: ADP ratio in muscle tissue at rest and during exercise. Symbols: Open circle, triangle, and square = controls; solid circle and triangle = athletes treated with HBO; open and solid circles = rest; open and solid triangles and open square = exercise. The line of best fit to the data is $y = 3\sqrt{x} + 4.8$; $P < 0.001$.

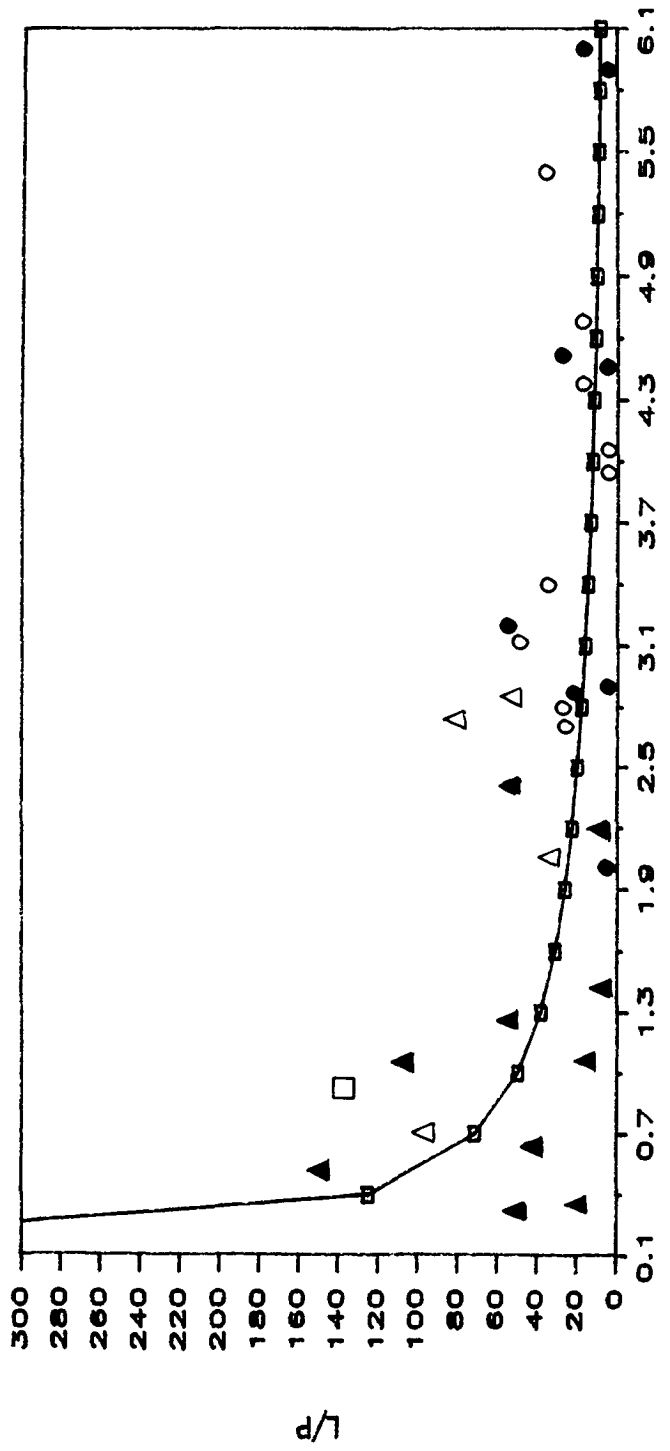

Fig. 3. Relationship between intramuscular PO_2 and the lactate:pyruvate (L:P) ratio in muscle tissue at rest and during exercise. Symbols are defined in Fig. 2. The line of best fit to the data is $y = 50/x$; $P < 0.001$.

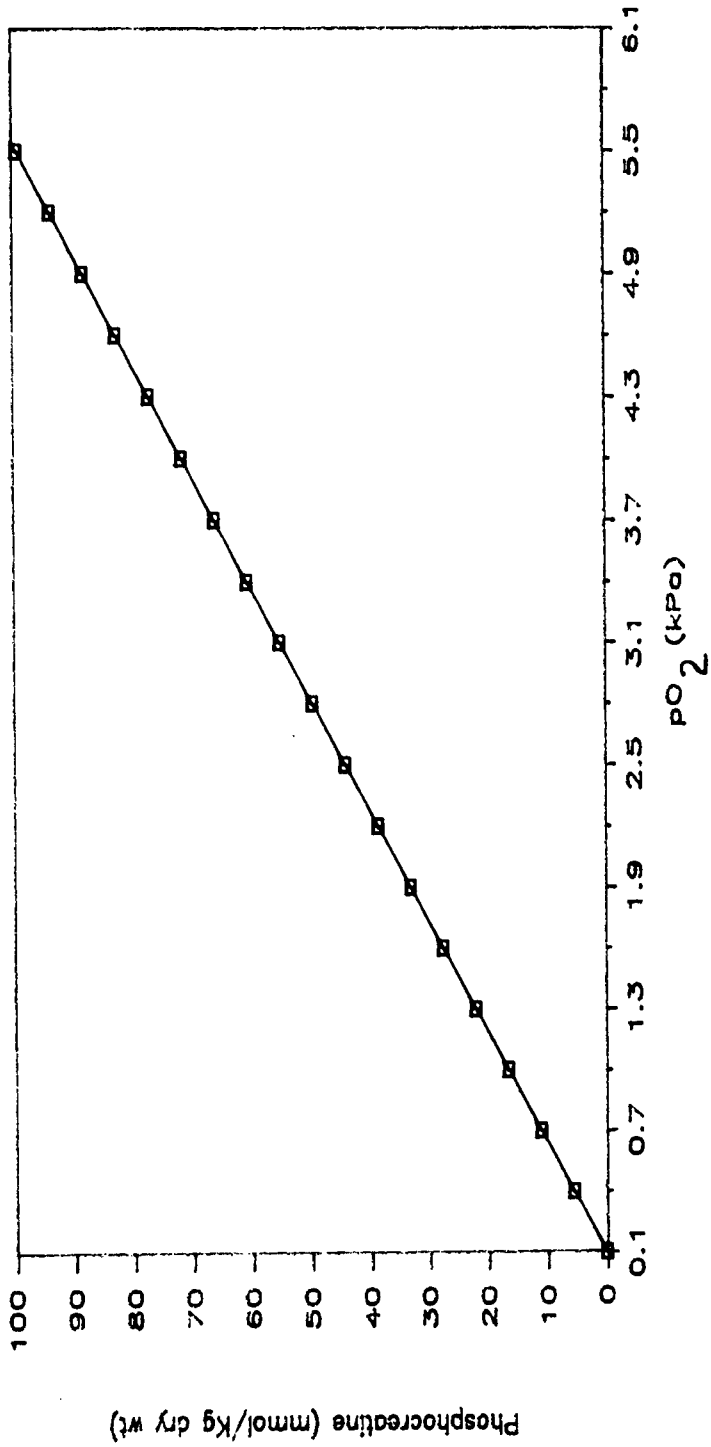

Fig. 4. Relationship between intramuscular PO_2 and the phosphocreatine concentration in muscle tissue at rest and during exercise. Symbols are defined in Fig. 2. The line of best fit to the data is $y = (x - 0, I)\ 90/(5 - 0, I)$; $P < 0.001$.

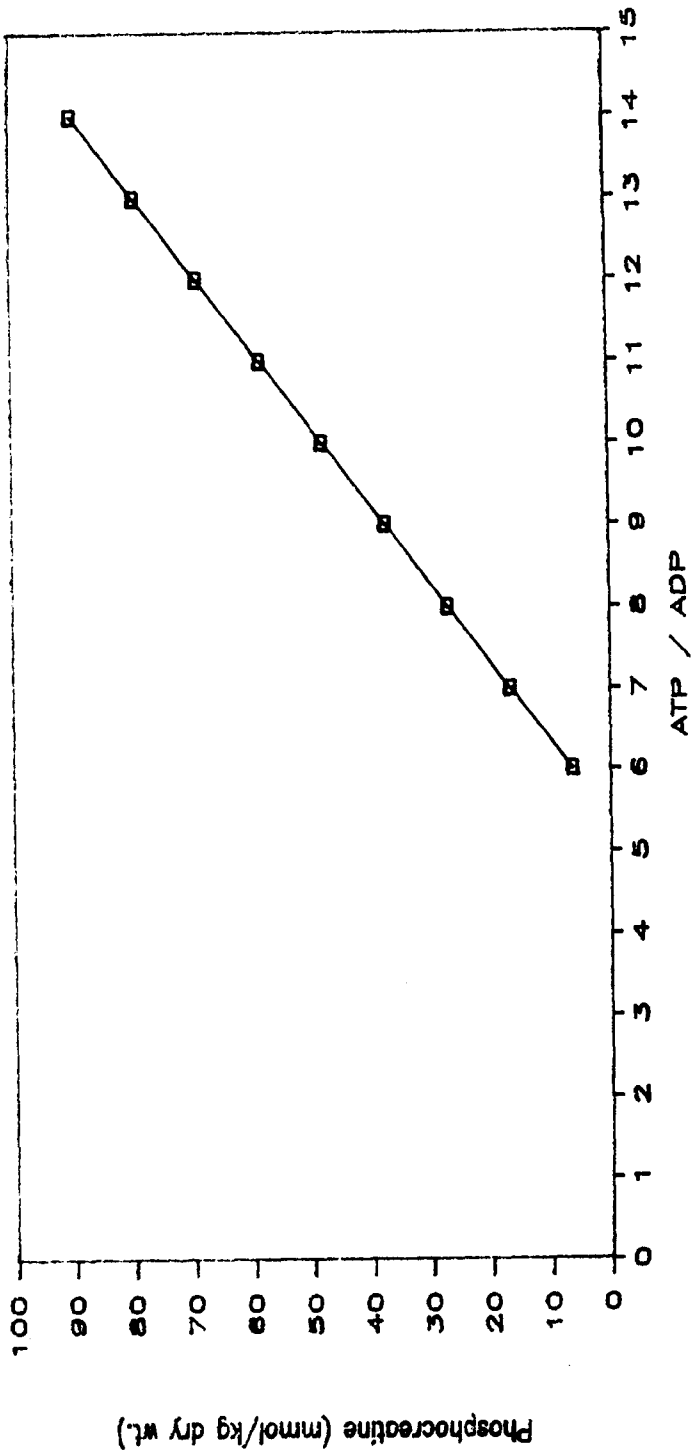

Fig. 5. Relationship between the ATP:ADP ratio and the phosphocreatine concentration in muscle tissue at rest and during exercise. Symbols are defined in Fig. 2. The line of best fit to the data is $y = 100/9.6\ (x - 5.4)$; $P < 0.005$.

the athletes' and the medical staff's assistance and safety, at the Istituto di Studi e Ricerche Subacquee ed Iperbariche dell'Amministrazione Provinciale di Napoli (Institute of Underwater and Hyperbaric Research of Naples) directed by Prof. R. Pallotta.

The group was treated with 8 hyperbaric oxygen sessions at 2.4 ATA, during each of which the athletes breathed 100% oxygen for 40 min, divided into 2 periods of 17 and 20 min, with 3 min of air breathing between them. The 8 sessions were carried out within 2 wk; 4 sessions a week on consecutive days. Normative values relative to unit pulmonary toxicity dose (UPTD) were recorded. Signed consent forms were required from each participant. The following physiologic data for each athlete, independent of his own group, were checked: radial pulse frequency at rest, after exercise, and 1 min later, and arterial pressure at rest, after exercise, and 1 min later. The same parameters were checked at the beginning of the last week of treatment and at the end of the hyperbaric sessions.

The effort considered for the evaluation of the above-mentioned parameters consisted of two matches, each lasting 5 min with 5 min rest between the matches. Data were recorded at the end of each match. Moreover, at the end of the last match of the series, which followed the last hyperbaric treatment, each athlete, independent of his own group, had 10 ml of blood drawn to evaluate the following biochemical parameters: lactate, pyruvate, CPK, lactate dehydrogenase (LDH), and electrolytes (Ca, Na, K).

The athletes who wrestled in the matches were chosen and paired in accordance with the level of their athletic training, so that the two wrestlers were equal in physical conditioning and devoid of any psychologic influences resulting from being in the HBO group.

RESULTS

Although the first results are useful to verify the experimental hypotheses, they are not suitable for a realistic statistical or graphic interpretation. The different features of each athlete and the need to integrate the experience with the athlete's normal training did not permit any standardization. For instance, there were no hematochemical data in the literature obtained from athletes with similar training or training backgrounds. Interesting data can be drawn from this first sampling (Tables 1 and 2).

The three evaluations of the parameters showed that in the athletes who did not receive HBO, pulse frequency reached high values after exercise and decreased 1 min later. Moreover, a marked decrease was noted in their ability to recover after the last match of each series. Conversely, pulse frequency of athletes who received HBO was nearly the same when checked after exercise and when checked at rest. One minute after exercise the heart rate reached high values (ca. 120 beats/min), then decreased to control values at the beginning of the last match of each series. The same occurred at the end of that match. This was seen in the HBO group from the second evaluation, i.e.,

at the beginning of the last week of treatment, and these values did not vary markedly at the end of the programmed hyperbaric session. At the end of each series of two matches arterial pressure reached high values in the athletes who did not receive HBO. On the contrary, in the subjects treated with HBO, arterial pressures were nearly the same when checked after the effort and when checked at rest. In the biochemical data, a decrease in lactate-pyruvate rate and increases in LDH and CPK were noted in the HBO group; on the other hand, no remarkable variation of the same parameter was observed in the control group.

Table 1

	At Rest		Match 1		Match 2		
	Pa	Fc	Fc Immediately After	Fc After 1 min	Fc Immediately After	Fc After 1 min	Pa
HBO Group							
S.S.	130/80	92	84	72	84	92	130/60
M.L.	120/80	72	72	120	84	120	110/60
M.S.	110/70	76	68	84	96	128	110/70
S.P.	110/70	74	74	100	80	112	110/70
C.N.	140/100	60	84	84	88	72	120/60
R.P.	140/90	72	80	88	88	60	145/90
F.M.	120/80	64	96	112	84	72	130/40
C.S.	140/100	72	90	108	94	86	140/100
P.M.	110/70	68	72	110	84	102	120/80
Control Group							
L.C.	140/100	100	100	80	96	136	140/90
R.N.	120/80	68	136	136	100	130	140/90
D.B.	110/70	94	120	102	136	120	130/80
P.L.	140/90	100	130	106	138	118	140/90
A.L.	130/80	92	124	104	130	106	140/90
C.P.	120/80	72	134	102	140	106	145/90
N.A.	130/90	82	118	96	124	100	130/80
N.I.	120/80	74	120	94	132	102	140/90
G.P.	110/70	68	118	88	122	104	120/90

Fc = radial pulse frequency; Pa = arterial pressure.

Table 2

Name	HBO	Lactate n.v. 5, 7 to 22 mg/100 ml	Pyruvate n.v. 0.36 to 0.50 mg/100 ml	Electrolytes Na+	K+	Ca++	LDH	CPK or CK-NAC
B.L.	No	14.7 n	0.36 n	147	4.8	9.45>	569 >	170 n
R.P.	No	7.0 n	0.40 n	148	4.6	10.1 >	555 >	215 >
P.M.	No	17.7 n	0.43 n	147	4.5	9.6 n	488 >	230 >
R.N.	No	21.5 n	0.465 n	148	5.2	9.45n	350 n	600 >
D'E.F.	No	15.0 n	0.38 n	146	4.6	9.8 n	515 >	204 >
L.C.	No	17.0 n	0.29 <	145	4.6	9.4 n	407 n	249 >
P.D.	No	17.7 n	0.48 n	144	5.3	9.7 n	660 >	317 >
C.C.	No	19.0 n	0.25 <	145	5.4	10.1 >	528 >	226 >
C.S.	Yes	9.2 n	0.35 <	144	4.7	9.1 n	434 n	170 n
M.S.	Yes	2.5 <	0.22 <	145	4.7	9.0 n	450 n	147 n
S.P.	Yes	7.0 n	0.34 <	144	4.7	10.7 >	515 >	170 n
M.L.	Yes	11.5 n	0.34 <	146	4.3	9.2 n	610 >	410 >
F.M.	Yes	13.0 n	0.26 <	143	4.1	9.0 n	435 n	91 n
C.N.	Yes	15.0 n	0.345 <	145	4.7	9.5 n	379 n	330 >
S.S.	Yes	14.5 n	0.36 n	145	5.2	9.7 n	435 n	205 >

Na+ n.v. 135 - 155; K+ n.v. 3.6 - 5.5; Ca++ n.v. 8 - 10; LDH n.v. 70 - 550; CPK or CK-NAC n.v. 24 - 195.

CONCLUSIONS

In the absence of any statistical analysis of the data, we will attempt to propose some general conclusions. The action of hyperbaric oxygen produces oxidization of the energy substrata of muscle cells, with the energy state tending to an ideal rate of 1 (ATP-ADP + Pi). ATP phosphoric bindings with a high energy level transferred to phosphocreatine, which is a steadier reserve of energy than unsteady ATP.

The biochemical data obtained during our experience agree with this hypothesis, as well as with other authors' suggestion that oxygen stimulates the production of oxidative and Krebs' cycle enzymes. During high exercise levels there seems to be a prevalence of aerobic-type cellular metabolism, along with the features required for the choice of the athletes, using up the energy reserves of phosphocreatine and changing into anaerobic metabolism only when they are exhausted, resulting in a lactate and oxygen debt.

Such considerations, which are quite new and to some extent revolu-

tionary for sports medicine, allow us to maintain that resistance to exercise stress can be increased through hyperbaric oxygen exposure, even with fewer hyperbaric sessions and with less severe bathymetric conditions. In fact, HBO creates conditions in the muscular tissue for better metabolism and for a delayed production of the residues of anaerobic metabolism (lactate in particular), which increases the need for blood and nourishment, with cardiocirculatory overload and consequent fatigue, the worst enemy of athletes. However, further research is considered necessary.

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IS BONE NECROSIS IN DIVING PAINFUL?

M. R. Cross

In the course of 2 yrs of clinical work with the commercial diving population it was our impression that aches and pains, both during and after diving, were more common among divers than was generally recognized. One reason is that those divers who experienced such symptoms tended to take little notice of them, and indeed regarded them as a natural occurrence to be expected. We were concerned that in some circumstances decompression sickness might go unrecognized, or at least unreported. In an earlier study a large number of commercial divers admitted to not always reporting "niggles" (1). The knowledge that bone infarction in sickle cell disease is painful made us wonder if similar pain occurred during the process of infarction in divers, and in 1979 we issued a questionnaire to a large group of divers (261) to determine if they reported any pains at sites where aseptic bone necrosis subsequently developed. We were particularly aware that bone infarction in sickle cell disease is painful and pain is well described. We first obtained the decompression sickness history of the divers and then asked them about the occurrence of any other aches and pains. Questions were asked as to the site, character, and quality of the pains and if they were "unlike those of a bend." We have determined that divers report the occurrence of pains unlike those of bends quite frequently. The pains occur during decompression and after surfacing and usually the divers do nothing about them.

DATA COLLECTION

To investigate the extent to which divers actually experienced various aches and pains during a dive and subsequent decompression, a questionnaire was devised and administered randomly to divers presenting for their annual diving medical examination. The questionnaire was given to the diver by one of

the physicians qualified to perform medical examinations of commercial divers.

Participation in this study was on a voluntary basis by both diver and physician. Physicians were asked not to select divers on the basis of any criteria, except to exclude beginners and those with no commercial diving experience. It was requested that the form be administered to the diver with a brief explanation of its purpose. Physicians were also requested not to administer the questions directly, but to leave the diver to fill in the answers himself.

Data obtained from each diver included age, length of time that he had been diving, and the particular types of diving he had performed. He was also asked for the commonest depths to which he usually dived and the maximum depth achieved in his career. Specific inquiry was made into any history of Type I decompression sickness, including the number of times he had experienced it and its location. Separate sections were to be filled in for both "bends" and "niggles." The final section of the questionnaire asked whether any other aches and pains were experienced, either during the dive or afterward. Separate questions dealt with the frequency, character, and duration of the pain for bends and niggles. Finally, the divers were asked about pain associated with types of diving, any action taken by the diver if such other aches and pains occurred, and to indicate on a diagram of the body the precise location of these symptoms.

RESULTS

Forms were obtained from 261 divers who had medical examinations between January and December 1979. Tables 1 and 2 present the data which characterizes the population with respect to types of diving and ages.

An initial analysis was made of the men who reported having experienced any type of pain associated with diving; those who reported definitely experiencing a bend or niggle; and those who reported having experienced any other ache or pain. This first analysis is presented in Table 3.

As shown in Table 3, a large number of men in the sample have experienced Type I decompression sickness in the form of either a bend or a niggle (43%). The magnitude of this population incidence forms the basis of a previous study (1) in which a full analysis was made. Of particular interest in the context of this study is the large group, 32%, who report having experienced "any other ache or pain." A considerable overlap between those who have experienced Type I decompression sickness in some form and those who reported "any other ache and pain" is evident if one presumes that these people can meaningfully respond to questions such as "Are the other aches and pains unlike those of a bend? These reports are analyzed in Table 4, divided into groups according to their history of previous decompression sickness.

After questions relating to the similarity or otherwise of these other aches and pains to previous decompression sickness, we asked about the actual point in the dive profile at which these symptoms were noticed. This infor-

Table 1
Diving Experience of the Population

	Numbers	Percentage of Whole Population
Air diving without decompression or with water stops	258	100
Air diving with surface decompression procedure	209	80.6
Air or oxy/nitrogen saturation	18	6.9
Oxy-helium bounce diving	141	54.4
Oxy-helium saturation	133	51.3
Other gases/mixtures*	54	20.8

* This group includes a number of divers who have specified trimix as an answer and also includes a small number with military experience of oxy-nitrogen mixtures.

Table 2
Mean Age of the Population Studied

	Age of Population, yrs		
	Mean	SD	Numbers
Whole population	30.07	5.67	258
Air divers	28.41	6.04	97
No decomp, or water stops only	26.15	3.69	40
Experience of surf decompression	30.00	6.84	57
Oxy-helium divers	31.66	5.21	161
Bounce diving experience only	31.81	9.07	27
Saturation diving experience	30.91	4.06	134

Statistical analysis shows that those who have only dived on air and either not performed decompression dives or used only in-water stops are younger than the remainder of the population and its subgroups, but the difference is barely significant, $0.1 > P > 0.05$.

mation is presented in Table 5, in which groups *A-D* refer to the same subgroups that were defined in Table 4.

Table 3
Preliminary Analysis of Men Who Have Experienced Any Ache or Pain

Total number of usable responses	259	
Men who have had ANY ache or pain during or after diving	130	(50%)
Men who have experienced Type I bend or "niggle" one or more times	110	(43%)
Men who also report "other aches and pains"	83	(32%)
Unusable responses	2	(1%)

Table 4
Other Aches and Pains Analyzed in Relation to
Previous Decompression Sickness History

Group		Number
	Men Who Have Experienced Type I Decompression Sickness Bend or Niggle, one or more times, $n = 63$	
A	Those who say other aches and pain is unlike their bend/niggle	37
B	Those who say other aches and pains like their bend/niggle or give no definite response (5 subjects)	26
	Men who have not ever experienced Type I decompression sickness, $n = 20$	
C	Those who say other aches and pains is unlike their idea of a bend	13
D	Those who say other aches and pains is like their idea of a bend or give no definite response (3 subjects)	7

Note: Subjects who do not give a definite response as to the similarity of the other aches and pains to a bend are included in the like groups in keeping with the tradition that any pain during diving is a bend till proven otherwise.

Note that in the subjects with a history of decompression sickness and in those without, other aches and pains have a predilection to occur either during or after decompression rather than at bottom depth, either during the working dive or while resting in the chamber. The enigmatic question remains, "If the pain occurs during or after decompression and was described by nearly two-thirds of the men who had experienced previous Type I decompression sickness as being like that of a bend, what did they think it was?"

Table 5
Occurrence of Other Aches and Pains in Relation to the Point in the Dive at Which They Occur

Group Letter and Number of Subjects	Occurrence of Other Aches and Pains			
	During Working Dive	Resting in Chamber	During Decompression	After Decompression
A 37	4	5	18	25
B 26	1	4	14	16
C 13	2	4	3	6
D 7	0	0	2	5
Totals	7	13	37	52

The answers to the question, "What do you do about these aches and pains?" suggest that we are dealing with a real phenomenon. Four alternative answers were offered: a) Undergo recompression. (The appropriate answer if the subjects attributed the pains to a dysbaric phenomenon.) b) Treat the symptoms themselves (i.e., with aspirin, etc). c) Seek medical advise. d) Do nothing. An analysis of the responses is presented in Table 6.

When asked how often the aches and pains occurred, most men said a few times only (Table 7).

We also asked them about the characteristics of the pain, which most respondents perceived as a dull ache in the bone (Table 8).

It is interesting and concerning that we have identified a group of divers (group A) who say that: a) they have pain "like those of a bend"; and b) this pain occurs during and/or after decompression, yet who say that in 30 out of 37 cases they do nothing about it.

The problem is further compounded by an analysis of the sites at which these other aches and pains occur and by comparing the distribution of the aches-and-pains sites with the distribution for both bends and niggles.

The frequency with which particular regions were described as being the location of a bend pain are shown in Fig. 1; Fig. 2 provides the same information for niggles; and Fig. 3 shows the frequency with which various body regions were indicated on the diagram presented to the divers in the questionnaire.

The remarkable similarity in the distribution of both the recognized manifestations of decompression sickness, i.e., bends and niggles, and of the aches and pains invites speculation as to the exact nature of the aches and

Table 6
Courses of Action Taken by Men Who Experienced
Other Aches and Pains—Subgroups as Per Table 6

Group Letter and Numbers	Course of Action Taken				
	Undergo Recompression	Self-treatment	Seek Medical Advice	Do Nothing	No Reply
A 37	1	2	0	3	4
B 26	6	2	2	13	4
C 13	0	0	1	9	3
D 7	1	1	1	5	0
Totals	8	5	4	57	11

Table 7
Frequency with Which Other Aches and Pains
Are Said to Occur

Group and Numbers	Once	Few Times	Several Times	With Most Decompressions	With Every Decompression	Don't Know
A (37)	5	21	7	1	1	2
B (26)	3	8	7	7	0	2
C (13)	2	7	4	0	0	0
D (7)	0	6	1	0	0	0
Totals	10	42	19	8	1	4

pains. Divers were asked to state how long their other aches and pains lasted after a dive, and the responses are presented in Fig. 4 in histogram form.

ARE THE PAINS SIGNIFICANT?

We have determined that divers report the occurrence of pains unlike

Table 8
Characteristics of the Other Aches and Pains

Group Letter and Number	Dull	Sharp	Gnawing	Burning	Don't Know
A (37)	24	7	8	2	1
B (26)	9	1	2	0	17
C (13)	10	3	3	0	0
D (7)	3	0	0	0	4
Totals	46	11	13	2	22

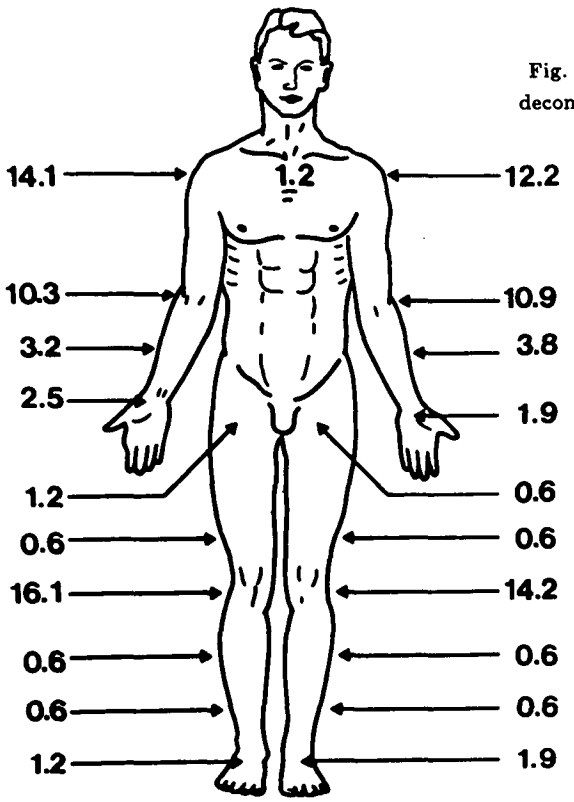


Fig. 1. Sites of pain reported to be Type I decompression sickness (percentages).

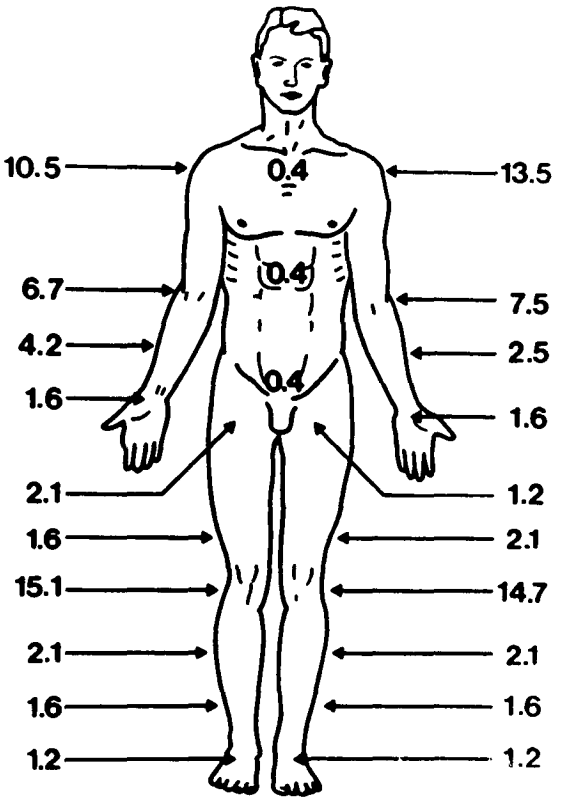
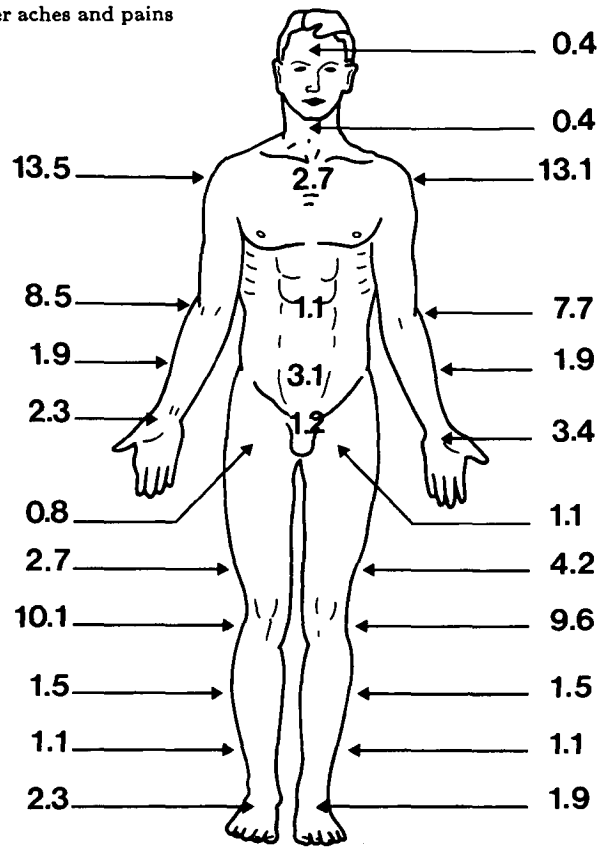


Fig. 2. Sites of pain reported to be niggles (percentages).

those of bends quite frequently. Pains occur during decompression and after surfacing and usually the divers do nothing about them. The presence of pain at a site after diving is associated with bone necrosis, and a 1985 retrospective analysis of bone necrosis in the studied population confirmed that the association between the occurrence of bone necrosis and other pain was quite high, as Table 9 demonstrates.

The incidence of bone necrosis in the men who had suffered no decompression sickness and who reported no other pain was low; of the 88 men in this group only 5 had aseptic bone necrosis when checked retrospectively (5.7%). Formal statistical analysis shows $P < 0.001$ using a chi-square test, where chi-square value in 21.57 with 3 *df*. These results may be expressed differently in Table 10.

Fig. 3. Distribution of other aches and pains (percentages).



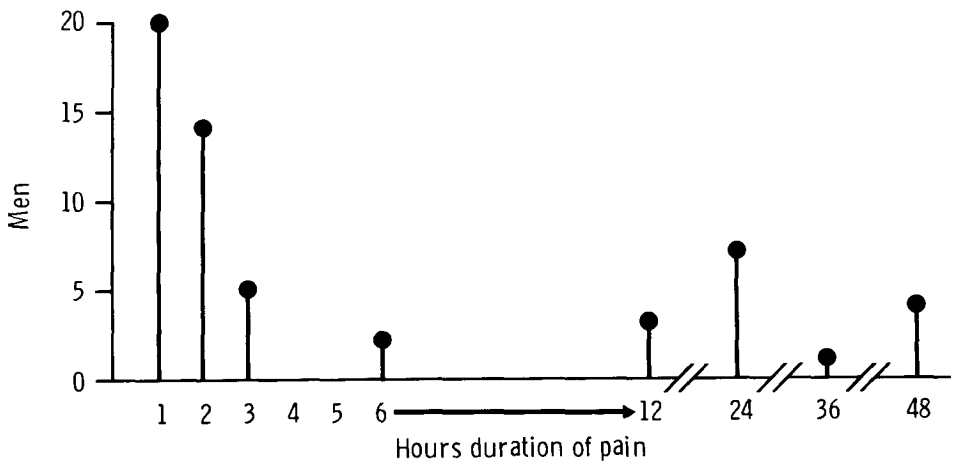


Fig. 4. Duration of other aches and pains.

Table 9
Association Between Other Pain and Bone Necrosis

DCS history plus other pain	43 men	No. with bone necrosis: 14 (32.6%)
Men with DCS history but no other pain	11 men	No. with bone necrosis: 1 (9.1%)
Men with no DCS history but other pain	29 men	No. with bone necrosis: 10 (34.5%)

Table 10
Decompression Sickness and Bone Necrosis

	Suffered DCS	No DCS History
With bone necrosis	15 men (27.8%)	15 men (12.8%)
No bone necrosis	39	102

$P < 0.02$

The correlation between bend pain and bone necrosis is positive but not highly significant (Table 11).

Table 11
Men Reporting Other Pain and Subsequent Bone Necrosis

	Suffered Other Pain	No Other Pain
With bone necrosis	24 men (33.5%)	6 men (6.1%)
No bone necrosis	48 men	93 men

$P < 0.001$

The correlation between reporting other pain and bone necrosis is highly significant.

Extending the analysis to determine if there is a correlation between bone *site* and pain *site*, brings the surprising result that the group numbers are too small for formal statistical analysis: a) 18 of 24 men reported Type I bend pain at a site also had necrosis at that site (75%); b) 12 of 15 men reporting other pain at a particular site had a lesion develop at that site (80%); and c) 11 out of 24 men who reported both bend pain and other pain at a particular site had a lesion at that location (78.6%).

DISCUSSION

This study suggests that the process of bone necrosis in diving is painful, although the pain may be identical to that of a bend or dissimilar to it; much depends on the diver's perception of pain. The large numbers who had both Type I decompression sickness and other aches and pains and who admit to doing nothing about the latter seem to suggest that the pain is a true clinical entity.

In sickle cell anemia, bone infarcts are found that are radiologically similar to those seen in divers. Bone pain is a common feature of sickle cell anemia and the pains have been shown to be the result of a localized, inflammatory response, confined by the bone cortex and with an associated periosteal elevation (2). The pain is relieved to a considerable extent by the application of warmth and a high fluid intake, either orally or parenterally (3). In tropical areas where sickle cell disease is prevalent, attacks are provoked particularly by cold (4) a situation not unlike diving where the incidence of bends also rises with cold.

The pain of bone infarction in sickle cell disease lasts for up to several days, but dull aches in the bones may on occasions last for many weeks. The data in Fig. 4 indicate that in diving, periods of up to 24 h of pain are not unusual.

We cannot say for certain if we have identified a pain unique to aseptic bone necrosis in diving, however it is of considerable clinical importance that such a pain exists and can be recognized. At an annual medical examination, the question "Have you had any bends?" is too cursory; a more detailed history of pain should be sought. In everyday diving practice, the supervisor should be aware of the possibility of aches and pains that may be of more than trivial significance. It is unlikely that the ache or pain that we attribute to aseptic bone necrosis on the basis of this study would be relieved by recompression, because we suspect that more divers would have sought recompression had experience taught them that it would work in relation to this particular pain.

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9th International Symposium on Underwater and Hyperbaric Physiology
Undersea and Hyperbaric Medical Society, Bethesda, MD, 1987

VOLATILE COMPOUNDS PROBABLY RELEASED FROM PAINT AT 46 BAR HELIUM-OXYGEN ATMOSPHERE

K. Jakobsen

During a 450-msw (1 msw = 1/10 bar) simulated dive at NUTEC, divers complained of a peculiar hydrocarbon smell. Chambers 3 and 4 (Fig. 1) had been painted 1.5 mo. before start of compression and no smell had been present at the surface. The chambers had been painted according to the specifications of the paint and the primer used. The paint-primer contained aromatic hydrocarbons, glycol ether, and alcohol as solvents. We therefore analyzed chamber gas for the presence of hydrocarbons.

METHODS

Gas samples were taken from all chambers and adsorbed on activated charcoal (Charcoal tubes lot 120 NIOSH. Approved SKC Inc., Pennsylvania) at 1 bar (1). The gas flow was 10 to 20 liter/min and the total volume of gas flowing through the tube was 100 to 200 liters at 1 bar. The volume was measured using a gas volume meter (Dehm and Zinkeisen D74) downstream from the test tube. First gas samples from the chambers were taken on Day 1 at 450 msw.

The samples were analyzed by the headspace gas chromatographic method as described by White et al. (2). The analyses were performed at the Norwegian Labour Directorate Laboratory district 8 (Arbeidstilsynets Landsdelslaboratorium), Bergen. Ethylbenzene and xylene could be detected but not separated by this method. A qualitative analysis of the hydrocarbons was performed first (using a mass spectrometer), and then the major hydrocarbons found were analyzed quantitatively on a gas chromatograph. Check analysis of the back-up layer of the sampling tubes was undertaken, but the pollutants were not found in any significant amount. The white spirits (stoddard solvent) standard used for calibration and calculation contained a total of approximately

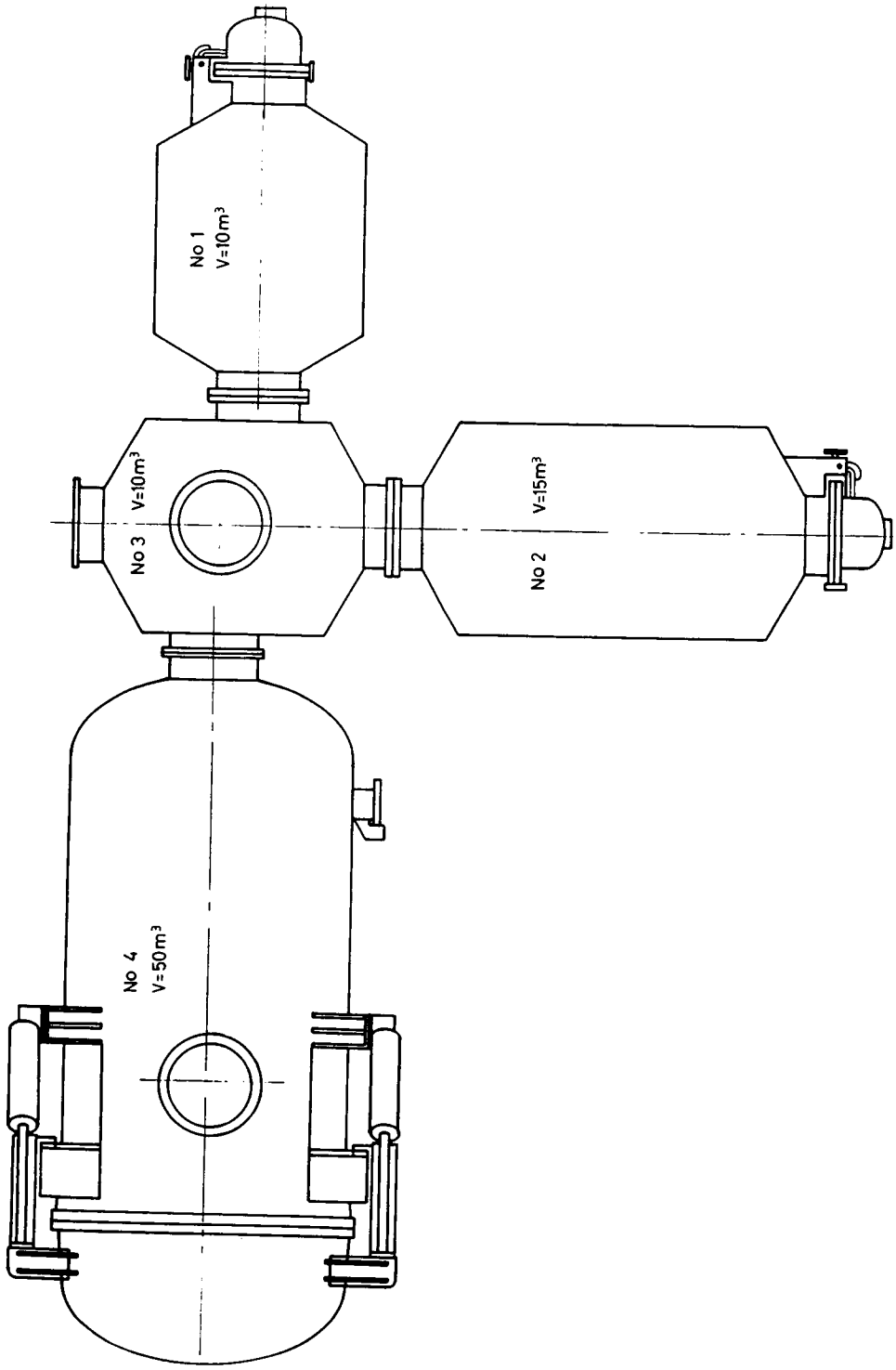

Fig. 1. NUTEC chamber complex.

17 vol% aromatic hydrocarbons. A sample of the paint-primer on the wall in one of the chambers was also analyzed.

RESULTS

The threshold limit values (TLV) used for ethylbenzene + xylene, toluene, C_6-C_7 aliphatics, butanol, and white spirits are given in Table 1. Table 2 shows the concentrations of these hydrocarbons in chambers 1, 2, 3, and 4 at 46 bar helium-oxygen. Significant amounts of ethylbenzene and xylene were found in chambers 1, 2, and 3 on Day 1 at 46 bar. Chamber 4 was not pressurized before Day 2. Chambers 3 and 4 always had the highest concentrations of ethylbenzene + xylene and butanol; chamber 3 having the higher concentrations. The toluene concentration was low the first day, but above 1.5 μbar in all chambers on Day 2. From Day 3, chambers 3 and 4 had the highest concentration of toluene. The C_6-C_7 aliphatics and white spirits did not show significantly higher concentrations in any of the chambers. The concentration of white spirit was on the detection limit.

Table 1
Surface TLV for Continuous and 8-h Exposure
for Some Hydrocarbons in Parts per Million*

Substance	8-h Exposure**	Continuous Exposure†
Ethylbenzene + xylene	75	20
Toluene	75	20
C_6-C_7 aliphatics	50	10††
Butanol	50	10††
White spirits	100	20††

* 1 ppm = 1 μbar at 1 bar. ** Eight-hour exposure limits from Norwegian standards (3). † Continuous exposure limits from NASA standards (4). †† Extrapolated.

At Days 8 and 9 a reduction in the concentration of butanol was observed. Some reduction of other contaminants was also found. In addition to the compounds given in Table 2, small, insignificant amounts of other solvents were found (less than 1 μbar).

Table 3 shows the composition of the volatile compounds from the paint-primer sample taken. The main compound was found to be ethylbenzene + xylene. The volatile compounds are probably mainly from the primer.

Table 2
Concentrations of the Main Hydrocarbons Found at 46 Bar Heliox Measured as Microbar

	Ethylbenzene + Xylene				Toluene				C_6-C_7 Aliphatics				Butanol				White Spirits			
Chamber No.	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Day 1	11.0	16.0	28	n.a.	<0.1	<0.1	<0.1	n.a.	0.3	0.3	0.3	n.a.	0.1	0.3	1.6	n.a.	0.2	< 0.1	0.1	n.a.
2	8.2	14.0	15	13	2.3	1.8	1.7	1.6	1.3	1.1	1.1	1.0	0.2	0.3	1.4	1.0	< 0.1	< 0.1	0.3	0.3
3	7.1	9.4	12	13	1.3	1.9	2.5	2.3	1.2	1.4	1.3	1.4	<0.1	0.1	1.0	1.3	n.d.	< 0.1	0.1	0.1
4	7.5	9.6	16	14	1.3	1.4	2.3	1.8	1.1	1.3	1.6	1.6	< 0.1	n.d.	1.1	1.0	0.1	n.d.	0.1	< 0.1
5	8.1	7.9	16	14	1.0	1.4	2.0	1.6	1.0	1.1	1.4	1.3	< 0.1	< 0.1	1.2	1.1	n.d.	n.d.	< 0.1	< 0.1
6	12.0	8.5	n.a.	n.a.	0.9	1.0	n.a.	n.a.	1.0	0.9	n.a.	n.a.	0.2	< 0.1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
8	5.7	5.7	15	14	0.6	0.8	1.2	1.2	0.7	0.7	0.9	0.8	n.d.	n.d.	0.3	0.1	n.a.	n.a.	n.a.	n.a.
9	4.8	6.1	12	12	0.7	1.4	1.6	1.6	0.7	0.8	0.8	0.7	n.d.	n.d.	0.2	0.1	n.a.	n.a.	n.a.	n.a.

n.a. = not analyzed; n.d. = not detected (below detection limit); 1 ppm = 1 μbar at 1 bar.

Table 3
Analysis of the Composition of the Volatile Compounds
from the Paint-Primer Sample

Substance	Portion
Ethylbenzene + xylene	main compound
n-Butanol	minor compound
2-Ethoxyethanol	minor compound
2-Ethoxyethyl acetate	trace compound
iso-Butanol	trace compound
White spirits (approx. 17 vol% aromatics)	trace compound

A test of contamination from the paint-primer was also performed 8 mo. after the chambers had been painted. Chambers 2 and 3 were both pressurized to 200 msw on helium-oxygen. No ethylbenzene + xylene was found in chamber 2, which had not been painted, but 2.4 μbar (11 mg/m^3) ethylbenzene + xylene was found in chamber 3. The total hydrocarbon concentration at pressure was < 2 mg/m^3 and 14 mg/m^3 in chamber 2 and 3, respectively.

The calculated total relative toxin levels in the chambers are given in Table 4. It is customary in toxicology to consider that similar toxins use the same pathologic mechanism. In the absence of information to the contrary, the effects of different chemicals are therefore considered as additive (5).

If the total relative exposure calculation in Table 4 exceeds unity, the total hydrocarbon limit is exceeded. The continuous total exposure limit was exceeded on Days 1, 2, 4, and 5 in chamber 3, and on Days 3, 4, and 5 in chamber 4. The 8-h total exposure levels were low in all chambers and did not exceed the limit.

DISCUSSION

Only compounds adsorbing to activated charcoal can be analyzed by headspace gas chromatography. Organic gases and vapors are adsorbed in preference to moisture, and sampling can be performed for long periods. The time between sampling and analysis was short, to avoid loss of sample compounds from the charcoal.

The main contaminants found in this study were ethylbenzene + xylene. The highest concentration of these compounds was found in chamber 3, but high levels were also observed in chamber 4. Chambers 3 and 4 had been painted 1.5 mo. before the dive. Chamber 3 had a bigger area painted relative to the total volume compared to chamber 4, which was partly filled with water. Inasmuch as chambers 3 and 4 both had high concentrations of ethylbenzene + xylene, chamber 3 having the higher, the compounds were probably

Table 4
Calculated Total Relative Toxin Exposure

	8-h Exposure				Continuous Exposure			
Chamber No.	1	2	3	4	1	2	3	4
Day No.1	0.16	0.23	0.41	-	0.61	0.87	1.61	-
2	0.17	0.24	0.27	0.23	0.68	0.93	1.11	0.95
3	0.14	0.18	0.24	0.26	0.54	0.72	0.97	1.05
4	0.14	0.17	0.30	0.26	0.56	0.68	1.20	1.05
5	0.14	0.15	0.28	0.25	0.56	0.58	1.16	1.02
6	0.19	0.14	-	-	0.77	0.57	-	-
8	0.10	0.10	0.24	0.22	0.39	0.40	0.93	0.85
9	0.09	0.12	0.20	0.20	0.35	0.46	0.78	0.76

Calculated by the equation $\frac{C_1}{T_1} + \frac{C_2}{T_2} \ldots + \frac{C_n}{T_n}$

C = measured toxin concentration; T = the corresponding threshold value.

emitted from the paint-primer in these chambers. Chambers 1 and 2 had not been painted before the dive, so the lower ethylbenzene + xylene levels found in these chambers were probably due to cross contamination from chamber 3. A sample from the paint-primer was analyzed, and confirmed that ethylbenzene and xylene were the main volatile compounds.

Butanol followed the same pattern as ethylbenzene and xylene, but at a lower concentration. Butanol was found as a volatile compound in the paint-primer used. The paint-primer also contained white spirits as a trace compound. The paint-primer could therefore be the source for the low concentrations of white spirits.

Toluene and C_6-C_7 aliphatics did not follow the same pattern as ethylbenzene and xylene, and were not detected in the paint-primer analysis. Therefore, other sources have to be considered for those contaminants. Sources producing volatile compounds are plastics, glues, petroleum products, wood, paper, cleaning agents, preservatives, foodstuffs, packaging materials, and the like. These items are commonly found in the chamber. The divers also excrete some organic materials (endogens) to the atmosphere.

A high activity alumina, impregnated with potassium permanganate (Purafil), was used to remove the volatile compounds. Between Days 1 and 2, Purafil was placed in the chamber's life support unit (LSU). At Day 7 the amount of Purafil was doubled. The amount of butanol decreased considerably at Days 8 and 9 compared to earlier in the dive. The concentration of

ethylbenzene + xylene, toluene, and C_6-C_7 aliphatics decreased slowly when the amount of Purafil was increased. Activated charcoal would probably have been a more suitable catalyst.

The limit proposed for continuous exposure was exceeded 4 times in chamber 3 and 3 times in chamber 4. However, the divers did not stay in chamber 3 and 4 continuously because chamber 3 was used as a transfer and sanitary chamber and chamber 4 as a bell for wet diving. The 8-h exposure limit, which is the most suitable limit for chambers 3 and 4, was never exceeded.

Even 8 mo. after chamber 3 was painted, small amounts of ethylbenzene + xylene were found in the helium-oxygen atmosphere at pressure. No ethylbenzene + xylene was found in chamber 2, which had not been painted. The release of xylene from the paint-primer is probably due to helium penetration into the paint during compression.

CONCLUSION

These results show that a survey of materials containing volatile compounds is important when used at hyperbaric pressure. One and one-half months after painting was finished and no smell of hydrocarbon was detected at the surface, significant amounts of such compounds were found at 46 bar heliox atmosphere, and even 8 mo. after painting, hydrocarbons from the paint-primer could be measured at 21 bar heliox atmosphere. Helium penetrates into the different materials, and thereby might release volatile compounds into the atmosphere. Because a hyperbaric chamber complex is a closed environmental system, a more suitable catalyst for removal of contaminants would be beneficial.

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Acknowledgment

I acknowledge Norske Shell for giving permission to present this data, collected during the Troll 450-m onshore dive at NUTEC in September 1985. I also thank R. Flo and O. Brunborg, at Arbeidstilsynets Landsdelslaboratorium, Bergen, for cooperation and analysis of gas samples, and E. Jacobsen for typing the manuscript.

APPENDIX

9th International Symposium on Underwater and Hyperbaric Physiology
Undersea and Hyperbaric Medical Society, Bethesda, MD, 1987

CONSTRAINTS AND CONSIDERATIONS FOR OPERATIONAL SCIENTIFIC DIVING FROM A SATURATION HABITAT USING AIR AND/OR NITROX[1]

W. S. Busch

The National Oceanic and Atmospheric Administration's (NOAA) Office of Undersea Research (OUR) is charged with developing an undersea research program that is consistent with and responsive to needs of the scientific community in the United States. The office provides the marine science community with an ensemble of undersea systems and capabilities to be used in conducting marine research. These cover a broad range of technological capabilities including scuba, saturation, and surface-supported air and mixed-gas systems. A detailed discussion of these capabilities is provided in OUR's latest annual report (1).

A recurring need of undersea scientists is to increase both their allowable bottom times and maximum working depths. NOAA is addressing this need by developing new saturation systems with greater depth capabilities and new diving and decompression schedules to be used with these new facilities. A long-term Caribbean-wide undersea research program, which will feature the new *George F. Bond* saturation habitat (Table 1) as its key element, is currently underway. This system, which allows 6 scientists to remain on the seafloor for up to 30 d at a maximum depth of 120 fsw, has replaced NOAA's *Hydrolab* habitat at St. Croix, U.S. Virgin Islands (2). *Hydrolab* was the only saturation habitat available to the "free world" marine science community, and while in use more than 66,000 h of scientist saturation time were logged. The

[1]The decompression, diving excursion, saturation data, and operational scenarios presented or referred to herein are preliminary and unverified. They are not to be used in any way until the final tables are officially released by NOAA.

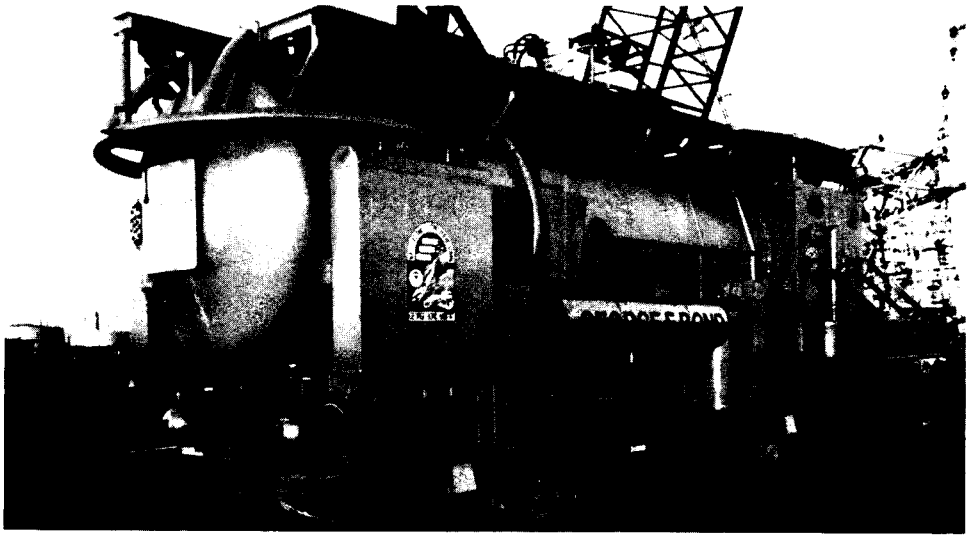


Table 1
George F. Bond -- Technical Specifications

GENERAL DATA		
Overall envelope and dimensions:		
Length:	43 ft	
Breadth:	20 ft (with surge tanks removed 12.5 ft)	
Height:	16.5 ft	
Internal lengths and volume:		
Main lock (ML)	21.5 ft	1400 ft^3
Entrance lock (EL)	8.5 ft	500 ft^3
Wet porch (WP)	8.0 ft	700 ft^3
Internal diameter:	9.0 ft	
Wet porch		
Length:	8.0 ft	
Breadth:	12.0 ft	
Height:	7.0 ft	
Weights		
Dry in air:	161,661 lb	
Ballast and trim:	12,725	
Total	174,386	
Displaced:	174,386	

Table 1 (Cont.)

Buoyancy:	
WP blown dry:	44,000
Wp flooded (with 17 in. bubble)	6,000
Surface buoyancy (ballast tanks blown)	11,410
Payload (mission weight including: personnel, provisions, and equipment)	5,000
Baseplate	TBD*
Surfaced freeboard	3 ft
Surfaced draft	12 to 13 ft
Personnel accommodations:	
6 scientists (aquanauts)	
Mission duration:	
Average:	10 d
Maximum:	30 d
Hyperbaric capability:	
Maximum external depth:	120 fsw (54 psi)
Maximum internal depth:	232 fsw (103 psi)
Emergency life support:	72 h for 6 persons + 1 and a complete decompression from maximum depth
Environmental ranges:	
External water temperature:	4-29 °C (39-85 °F)
External air temperature:	4-38 °C (39-100 °F)
Internal temperature:	20-35 °C (68-95 °F)
Internal humidity:	50-60% relative humidity
Design conditions:	Water current - 3.0 knots
	Wave height - 20 feet
	Wave period - up to 15 s
Electrical power:	
Connected load:	100 KVA
(1 phase, 220 V to habitat)	
120 V a.c., 24 V d.c. in habitat	
Normal system total operating load	60 KVA
Normal habitat (umbilical) load	20 KVA

Table 1 (Cont.)

External access to habitat:	
Manway size:	24 in. W × 60 in. H
Hatch size:	26 in. i.d.
Provisions/medical lock:	12 in. i.d.
Viewports:	23.5 in. (2 each)
	10 in. (5 each)
Diving systems:	
Scuba (air)	
Tether (air)	
Way station wet bell (nitrox)	

* To be determined

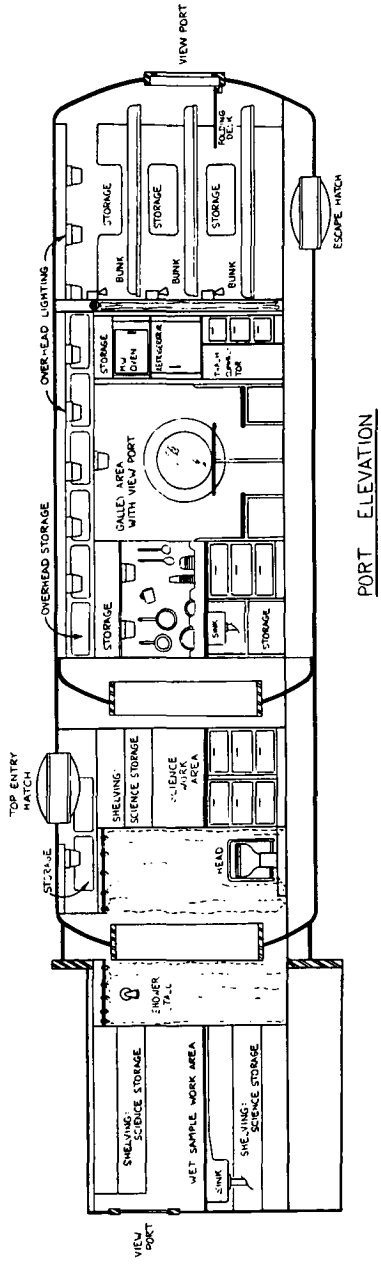
old habitat used air in an open-circuit, overboard dump mode and had a maximum saturation depth of 47 fsw (3).

The accommodate the *Bond*'s increased depth capability, new saturation, decompression, and excursion diving tables, using a nitrogen-oxygen (nitrox) mixture had to be developed. Although these new tables were developed specifically for the *Bond* habitat, they may also be applied to other diving systems. Commercial divers have used nitrox mixtures for many years, but their diving tables and scenarios are not appropriate for the needs of scientists. In developing both new tables and procedures for use with the *Bond*, NOAA has worked very closely with both industry representatives and members of the scientific community.

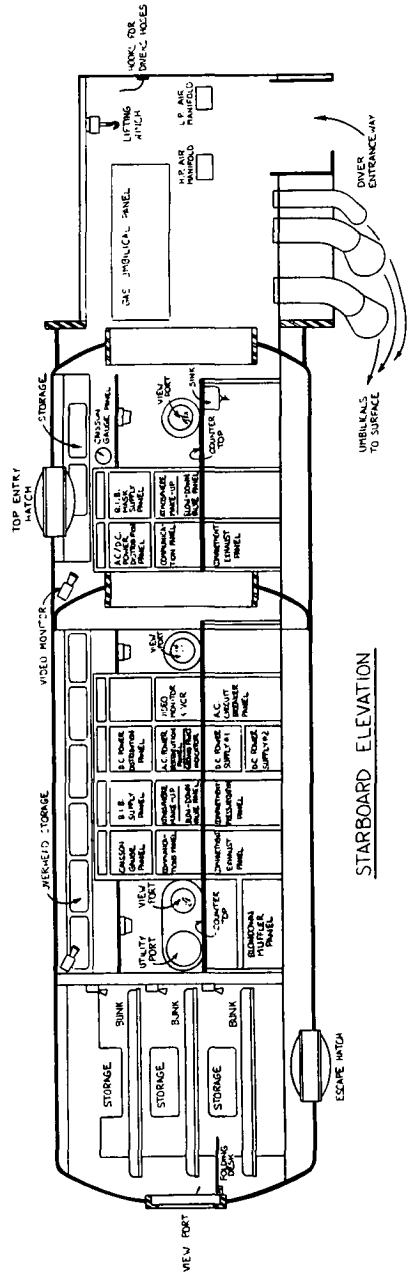
HYDROLAB OPERATIONS

Since 1978, when *Hydrolab* was first placed in operation in the Salt River Canyon, one-half mile offshore from the coast of St. Croix, an impressive number of operational diving statistics have been accumulated (Table 2). The habitat could support 4 aquanauts for 7 d (the time period for a standard mission) at a saturation depth of 47 fsw. Detailed technical specifications are provided in Table 3. Over the 8 yr of *Hydrolab* operations, extremely safe and efficient diving procedures and operational scenarios were developed for the scientist-aquanauts using the habitat.

NOAA's Diving Regulations, which are promulgated by NOAA's Diving Office, are the basis for all of NOAA's diving activities, including those from *Hydrolab* (4). For all dives, bottom time was measured as the interval between departure from the habitat depth and return to habitat depth. If both upward and downward excursions were planned for the same dive profile, it was considered safer to make the upward excursion first and the downward excursion last, and to make the deeper excursion before the shallower one.



PORT ELEVATION



STARBOARD ELEVATION

Table 2
Operational Statistics—Hydrolab
1978-1985

Total number of aquanauts:	331 (scientific staff) + 21 (administrative staff)
Total excursion man hours:	11,251 h (scientific only)
Total saturation hours:	55,056 h (scientific only)
Total number of scientific missions:	85
Total number of administrative missions to date:	7
Total number of scientific studies (reports):	96
Number of participating U.S. institutions:	94
Number of participating foreign institutions:	14
Number of foreign nations:	9

Table 3
Hydrolab—Technical Specifications
(System operations terminated in July 1985. Unit now on display in Smithsonian Natural History Museum, Washington, DC)

Table 3 (Cont.)

Habitat

Operational depth:	47 fsw
Cylindrical chamber	16 ft length, 8 ft diameter
Double lock entrance lock	4 ft length, 30 in. diameter, 24 in. hatch diam.

6 External viewports (one of which is 3 ft dia.)

Internal equipment

Sink with cold, hot, and ultra hot fresh water
Trash compactor
Single-burner hot plate
Air conditioner/heater
Shower - fresh water
Three 6 ft 4 in. × 24 in. bunks
Radio communications (with hard wire emergency line to shore base)
GFI protected 110 A.C. 15 amp electrical outlets

Life support

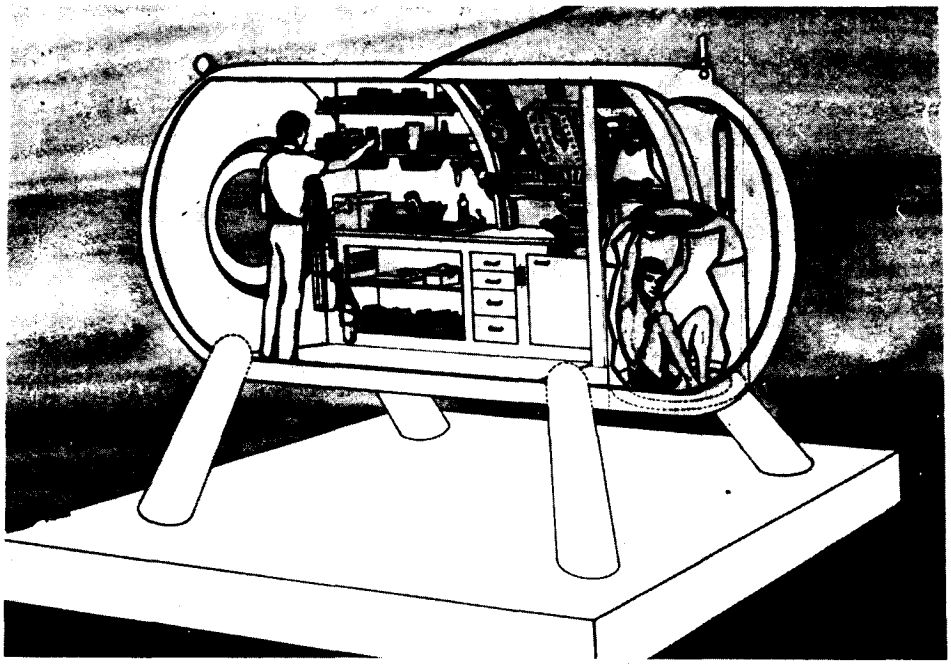
Air, electrical power, water supplied by umbilical from Life Support Barge (LSB)
Breathing air is a continuously ventilated open system
Emergency battery, CO_2 removal scrubber, and lights

External Equipment

External Air Station (PUTS): continuously ventilated enclosed roof structure with an air pocket.

1. continuous air
2. 12 VAC light
3. communications link to habitat
4. scuba charging line

Way stations:	6 transparent plexiglas hemispherical open bell type structures available—two with air supply located permanently
Transfer pots:	pressure-resistant containers used for transferring items to and from habitat
large:	16 in. dia × 24 in. deep
medium:	8 in. dia × 37 in. deep
small:	9 in. dia × 20 in. deep
small:	6 in. dia × 20 in. deep



Short downward excursions close to the habitat were determined to have only negligible effects on subsequent excursions. In almost all cases, accepted *Hydrolab* diving scenarios involved no-decompression excursions for the storage depth (Table 4) (5).

Unlimited bottom time was allowed at depths within ± 3 fsw of the saturation depth of 47 fsw. At depths between saturation and 55 fsw, a 6-h maximum bottom time was allowed. To provide a safety margin, the no-decompression time limits for all excursions were decreased by 5 min during actual dive operations. In addition, a minimum of 12 h was required for the surface interval (at saturation depth) for repetitive dives using the normal allowable excursion table times. In accordance with NOAA regulations, the maximum excursion depth was 130 fsw. This maximum excursion depth could be extended to 150 fsw with special approval of NOAA's Diving Coordinator.

With the maximum depth limited to 150 fsw, the seafloor contours or topography determined the maximum excursion distance (3). Additional limiting factors were the swimming time (20 min for 1000 yd) and the stamina of the aquanauts. During these extended excursions, extra air tanks were supplied by surface divers at predetermined undersea locations. Although *Hydrolab* was located at a depth of only 50 fsw, the habitat provided researchers with opportunities to work on the seafloor at depths and for time periods not previously available with conventional diving equipment.

Table 4
Downward No-Decompression Excursion Limits,
Air Saturation Depth = 47.0 fsw

Excursion Depth, fsw	Maximum Allowable Time, min
95	360*
100	323
105	253
110	210
115	181
120	137
125	108
130	91
135	69
140	56
145	48
150	42

* Use 360 min when calculating continuation or repetitive excursions.

Enhanced Scientific Needs

Marine scientists have repeatedly emphasized their need to work deeper and for longer periods of time than permitted by the capabilities of *Hydrolab*. In most near-shore waters, fishing pressures over the last 2 decades have caused marine stock populations (e.g., conch, lobsters, and large food fishes) to decline dramatically in shallow waters. Although scientists have studied the biology of these important species rather extensively in shallow water using facilities such as *Hydrolab*, almost nothing is known about the deeper populations of these stocks. For example, scientists do not understand the mechanisms that limit these populations or the amount of fishing pressure they can sustain. Scientific study of these deep-water populations is imperative in the immediate future if these resources are to be managed and utilized for long-term benefit. Saturation facilities at greater depths (e.g., 120 fsw) are likely to provide the best scientific results and the most cost-effective means of accomplishing this goal. Because of the depths and time limitations involved, the complexity of the experiments, and the rigorous monitoring and data collection required, longer in situ work periods are needed. A minimum of 2 or more wk of continual in situ scientific activity at a given research site is essential (M. Reaka, personal communication, July 1986).

The outer vertical walls of Caribbean fringing reefs below 130 fsw have the largest portion of undescribed fish species of any ecosystem in the United States, and yet are probably the least explored habitats in the world. Since these are regions of complex and heterogeneous topography, submersibles and remotely operated vehicles (ROVs) cannot be used. Saturated diving from a

Table 5

Maximum Ascending No-Decompression Excursions from Nitrogen-Oxygen Saturation

(Applies only to excursions done in 24 h after any prior descending excursion)

	Absolute Depth Measured From Sea Surface																		
Habitat Depth, fsw	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90
35	30	37	48	60	*	*	*	*											
40	24	31	40	52	60	*	*	*	*										
45	17	24	31	40	52	60	*	*	*	*									
50	12	18	25	32	42	60	*	*	*	*	*								
55	7	13	18	25	32	42	60	*	*	*	*	*							
60	0	7	13	18	25	32	42	60	*	*	*	*	*						
65	0	0	8	14	20	27	34	44	60	*	*	*	*	*					
70	0	0	0	8	14	20	27	34	44	60	*	*	*	*	*				
75	0	0	0	0	9	15	21	28	36	47	60	*	*	*	*	*			
80	0	0	0	0	0	9	15	21	28	36	47	60	*	*	*	*	*		
85	0	0	0	0	0	5	10	16	23	30	37	48	60	*	*	*	*	*	*
90	0	0	0	0	0	0	5	10	16	23	30	37	48	60	*	*	*	*	*
95	0	0	0	0	0	0	0	6	12	18	24	31	40	52	60	*	*	*	*
100	0	0	0	0	0	0	0	0	6	12	18	24	31	40	52	60	*	*	*
105	0	0	0	0	0	0	0	0	0	7	13	18	25	32	42	60	*	*	*
110	0	0	0	0	0	0	0	0	0	0	7	13	18	25	32	42	60	*	*
115	0	0	0	0	0	0	0	0	0	0	0	7	13	18	25	32	42	60	*
120	0	0	0	0	0	0	0	0	0	0	0	0	7	13	18	25	32	42	60

Values are minutes; asterisks indicate no time limit for that depth-time combination.

habitat offers the only meaningful method for studying this fauna. Excursions to 200 fsw (and ultimately deeper) for periods of at least 2 h are needed for effective observation and collection of specimens. Since most specimens of interest have a length of less than 1 in., submersibles cannot be used for observation. Long-distance excursions are of less importance than the ability to spend substantial periods in one location (L. Smith, personal communication, July 1985).

DIVING SCENARIOS FOR THE *GEORGE F. BOND* HABITAT USING NITROX

Initially, the new habitat will be placed into operation in Salt River Canyon at the original site and depth of *Hydrolab*. It will be operated at a saturation depth of 47 fsw on air in an open-circuit, overboard dump mode. This will allow the same dive procedures and constraints as were used with *Hydrolab*, which will minimize the impact to the operational staff and scientists during initial check-out and training phases. Once the system has been checked out, all modifications for tropical water operations have been made, and the operational staff has become completely familiar with the *Bond*'s operations, the habitat will be relocated to a deeper site. At this point, new diving procedures and operational scenarios will have to be introduced; these will be based on the new tables developed and verified by Hamilton Research, Ltd.

The new nitrox diving tables and procedures referred to as REPEX tables are designed particularly for diving scientists working from seafloor habitats in a saturated mode (6). Their objectives are: a) to provide a means of performing repetitive no-decompression stop excursions and longer excursions that use midwater stops; b) to present improved means of dealing with oxygen exposures; and c) to develop new saturation decompression and other ascent techniques. Using a nitrox mixture with an oxygen partial pressure of 0.3 to 0.35 atmospheres (atm), the REPEX tables address a saturation range from 30 to 120 fsw, with diving excursions from 65 to 200 fsw.

The new no-stop, repetitive excursion tables provide increased operational capabilities. Although the saturation is on nitrox, the excursions are performed on air which minimizes the effect of nitrogen narcosis on the diver. Being saturated on nitrox and then excursing on air permits the diver to accommodate the higher percent of nitrogen in the mixture. However, narcosis still limits the maximum excursion depth of these dives (7).

In the future, the use of trimix rather than air may permit the excursion depth to be extended. In trimix, the partial pressure of nitrogen would be lowered by replacing a portion of the nitrogen in the mixture with helium; however, changing over to trimix is operationally difficult and extremely critical. Because of the different diffusion rates of nitrogen and helium, the total inert gas partial pressure increases at the time the change is made, which could cause decompression sickness symptoms (M. Wells, personal communication, August 1986). In the *Bond* habitat program, air will be used for

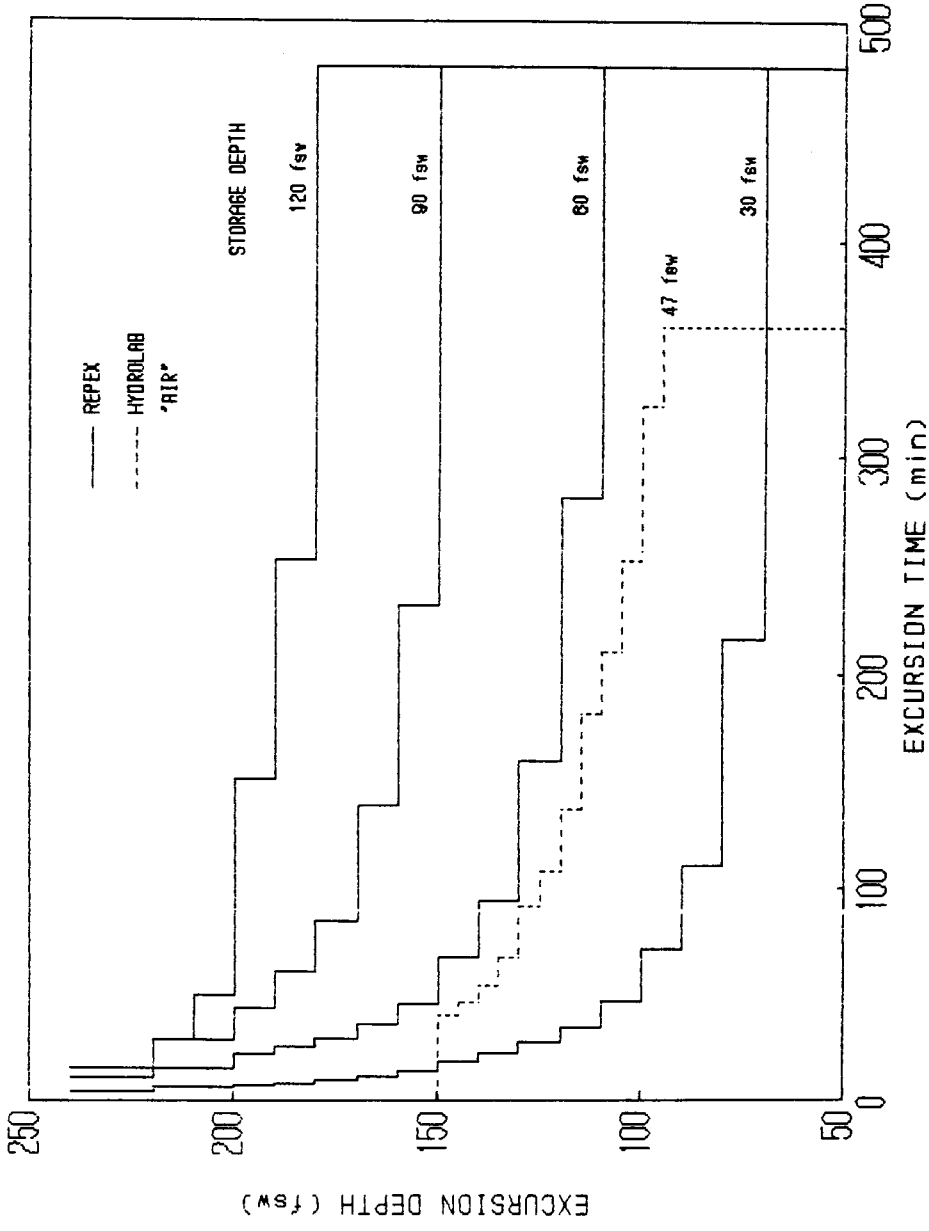


Fig. 1. No-stop excursion times for different storage depths.

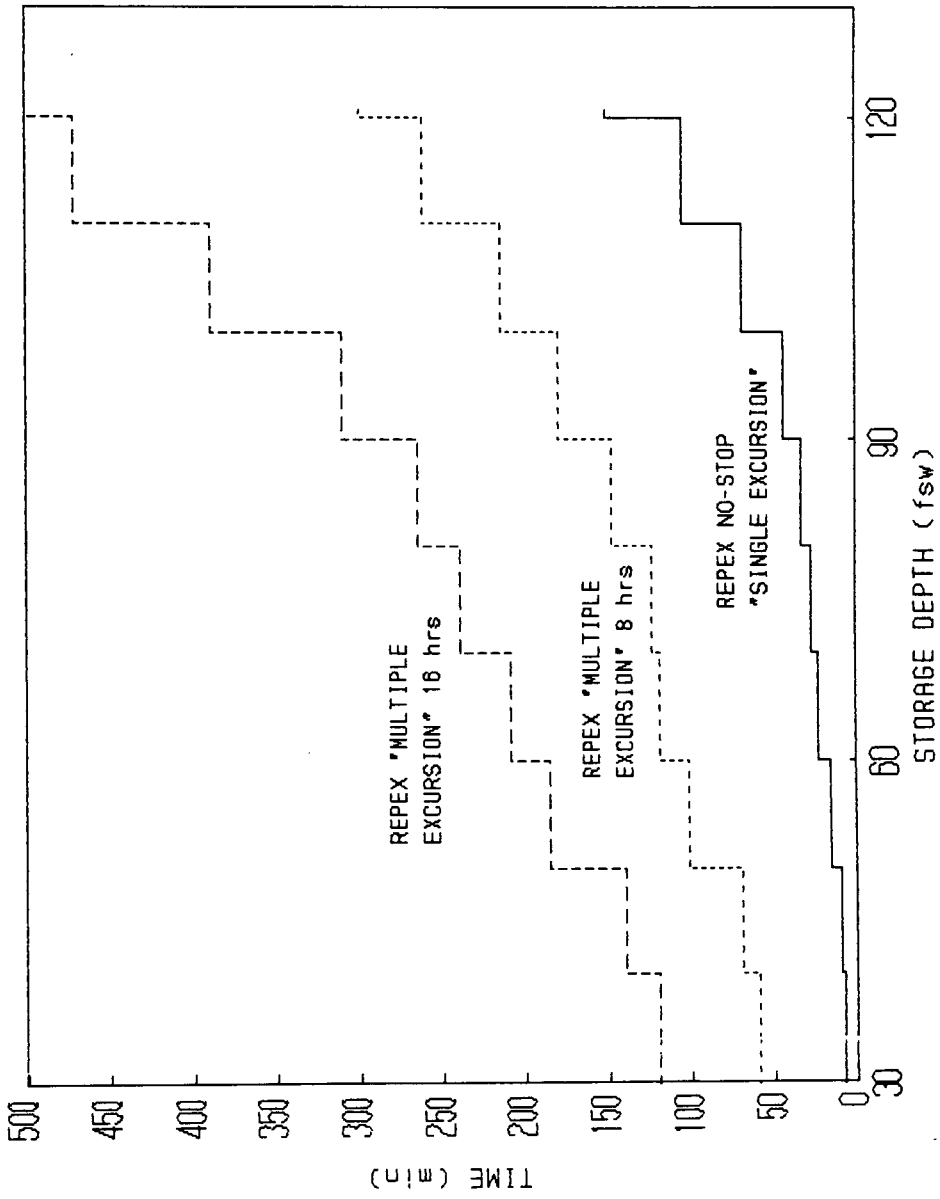


Fig. 2. Maximum excursion time for 1 d. Excursions to 200 fsw.

excursions to eliminate these problems.

The use of nitrox extends the maximum allowable saturation storage depth for the *Bond* habitat to 120 fsw, compared to a maximum of 50 fsw for air (as in *Hydrolab*). Figure 1 provides a comparison of these no-stop excursion times from four nitrox storage depths for *Bond* and *Hydrolab*. As Fig. 1 shows, the use of nitrox instead of air greatly increases the allowable bottom time even at 47 fsw.

With the new REPEX tables, divers can make repetitive dives after intervals back at storage depth ranging from 0.5 to 16 h by using adjustments in the allowable excursion times. After a surface interval of 16 h, they can assume they are totally clean (i.e., free of any excess nitrogen) (Fig. 2). The sequential number of the particular excursion dive and the interval since the last dive determine the repetitive adjustment required (7).

For a given maximum excursion depth, the REPEX maximum allowable no-decompression bottom times greatly exceed those of the original conservative oxygen limit portion of the NOAA tables. For a given maximum depth, in this case 200 fsw, the number of excursion dives allowable under the no-decompression scenario depends on the surface interval at saturation depth and the total period for the repetitive dives. In Fig. 2, an interval of 1 h was chosen arbitrarily with total diving periods of 8 and 16 h, respectively. The intervals and overall periods are dependent on the diver's capability and work scenario.

It must be noted that these vertical excursions include both upward and downward movements from the saturation depth. Extreme care must be taken by the diver in making upward excursions. Table 5 provides the maximum allowed times for a range of depths above given habitat saturation depths. Note that the depths are given as absolute depth measured from the sea surface (5). These data have been incorporated into the new REPEX tables.

The NOAA Diving Manual (5) provides a set of nitrox saturation tables used previously by other habitat programs. The REPEX tables provide longer bottom times than those of the NOAA OPS tables (Fig. 3) and are generally more conservative for individual excursions than the original NOAA tables. They also allow longer times for upper limits, 480 min instead of 360. The NOAA Habitat Nitrogen Diving Procedures (8) provide a detailed description of these new tables and procedures.

FUTURE RESEARCH DIRECTIONS

The implementation of these tables and the physiologic data obtained during their verification provide new research opportunities for both marine scientists and hyperbaric physiologists. As the aquanauts are able to saturate at deeper depths and to excurse even further, new concerns and areas requiring scientific investigation become apparent. Effects of nitrogen narcosis, oxygen toxicity, emergency decompressions and treatments, and long-term exposure at depth are just a few of the areas that must be addressed from an

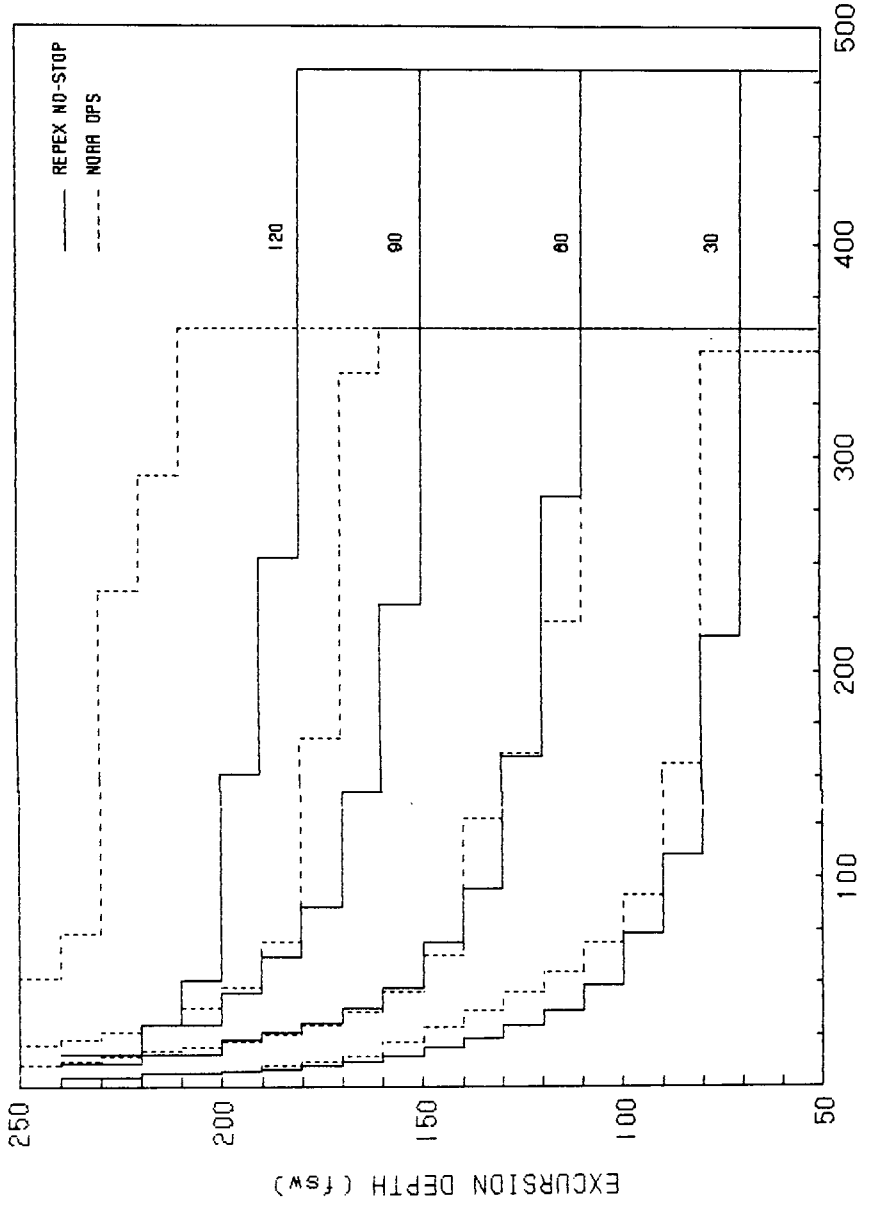


Fig. 3. No-stop excursion times.

operational-scientific diving standpoint.

Previous research efforts must now be translated into operational procedures. In addition, new efforts must be undertaken to allow scientists to excurse even further and for longer periods of time from these deeper saturation depths. Research should be started addressing the long-term detrimental physiologic effects on the scientists now working at these new depths. These would include cold, exercise, fatigue, carbon dioxide, and individual diver characteristics.

In addition to the physiologic aspects, technologic adjuncts such as new equipment, diver tools, and instrumentation should be addressed in making scientific diving operations safer and more productive.

SUMMARY

The National Oceanic and Atmospheric Administration is presently conducting extensive verification tests of the new REPEX tables and procedures. Three independent teams (2 all male and 1 all female) of 4 divers each will undergo 3 saturation dives each to test all aspects and combinations of excursion times and depths, procedures, and techniques. During these verification tests, more than 200 excursion dives will be done. Upon successful completion of these tables, all data and results will be reviewed and evaluated in detail. The final outcome will be a new set of diving tables that allow the marine science community to achieve increased access to the seafloor and undersea ecosystems. These tables, when used in conjunction with new technologies, such as the *George F. Bond* habitat and advanced surface-supported diving systems, will enable marine scientists to remain on the seafloor for periods of time not previously attainable.

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AUTHOR LIST

A. **Afifi**, University of California, Los Angeles, CA
C. **Akiba**, Tokyo Medical and Dental University, Japan
C. B. **Alexander**, University of Alabama at Birmingham, Birmingham, AL
E. C. **Alvord**, University of Washington, Seattle, WA
T. A. **Anderson**, Royal Australian Navy, Balmoral, NSW 2091, Australia
B. **Anderson Jr.**, Duke University Medical Center, Durham, NC
R. **Araki**, Saitama Medical School, Moohongo 38, Moroyama-machi, Iruma-gum, Saitama 350-04, Japan
J. A. **Ask**, Årstadveien 19, Bergen, Norway
S. **Awaya**, Nagoya University School of Medicine, Nagoya, Japan
A. J. **Bachrach**, Naval Medical Research, Bethesda, MD
D. J. **Bakker**, University Hospital, Amsterdam, The Netherlands
P. B. **Bennett**, Duke Medical Center, Durham, NC
R. G. **Beren**, Royal Adelaide Hospital, Adelaide, Australia
G. W. **Bergø**, University of Bergen, Årstadveien 19, Bergen, Norway
J. E. **Bevilacqua**, University of Pennsylvania Medical Center, Philadelphia, PA
G. **Bolstad**, NUTEC Ytre Laksevåg, Norway
C. H. **Bonney**, University of Texas Health Science Center, San Antonio, TX
A. A. **Bove**, Mayo Clinic, Rochester, Minnesota
M. E. **Bradley**, Naval Medical Research Institute, Bethesda, MD
R. W. **Brauer**, University of North Carolina, Wilmington, NC
D. M. **Browning**, Royal Adelaide Hospital, Adelaide, South Australia, Australia
A. O. **Brubakk**, University of Trondheim, Trondheim, Norway
W. S. **Busch**, National Oceanic and Atmospheric Administration, Washington, D.C.
E. M. **Camporesi**, Duke University Medical Center, Durham, NC
D. B. Chandler, University of Alabama at Birmingham, Birmingham, AL
L. P. **Chang**, Naval General Hospital, Taiwan, The Republic of China
S. H. **Chen**, Naval General Hospital, Taiwan, The Republic of China
S.-H. **Cho**, Maryland Insititute for Emergency Medical Services Systems, Baltimore, MD
J. K. **Choi**, Ocean and Underwater Medical Research Center, Chinhae, Korea
P. E. **Cianci**, Brookside Hospital, San Pablo, CA
J. B. **Clark**, Naval Medical Research Institute, Bethesda, MD
J. M. **Clark**, University of Pennsylvania Medical Center, Philadelphia, PA
J. R. **Clarke**, Naval Medical Research Institute, Bethesda, MD
J. R. **Claybaugh**, Tripler Army Medical Center, Honolulu, HI
W. H. **Cobb**, University of Pennsylvania Medical Center, Philadelphia, PA
R. W. **Coen**, University of California, San Diego, CA
G. H. **Cohn**, Geisinger Medical Center, Danville, PA
J. D. **Crapo**, Duke University Medical Center, Durham, NC
M. R. **Cross**, University of Newcastle upon Tyne, Newcastle, England
Y. I. **Daskalovic**, Israeli Naval Hyperbaric Institute, Haifa, Israel

H. G. Delauze, COMEX S.A. Marseille, France
P. Denoble, Institut za pomorsku medicinu, Split, Yugoslavia
T. A. Dillon, Royal Australian Navy, Naval Post Office, Balmoral, NSW, Australia
W. G. Djang, Duke University Medical Center, Durham, NC
N. Doi, Tokyo Medical and Dental University, Toyko, Japan
S. Drager, Brookside Hospital, San Pablo, CA
J. Dutcher, University of North Carolina, Wilmington, NC
A. J. Dutka, Naval Medical Research Institute, Bethesda, MD
B. C. Eatock, Defence and Civil Institute of Environmental Medicine, Downsview, Ontario, Canada 3B9
C. W. Edmonds, Diving Medical Centre, Sydney, NSW, Australia
W. J. Ehler, U.S. Air Force Medical Center, Lackland AFB, Texas
E. Eilender, Israeli Naval Hyperbaric Institute, Haifa, Israel
R. W. Emery, Maryland Institute for Emergency Services Systems, Baltimore, MD
C. W. Erwin, Duke University Medical Center, Durham, NC
B. Fischer, LVA Hospital Blausenbach, Nordrach, West Germany
D. E. Fletcher, University of Pennsylvania Medical Center, Philadelphia, PA N.
D. Flores, University of Pennsylvania Medical Center, Philadelphia, PA
E. T. Flynn, Naval Medical Research Institute, Bethesda, MD
C. Forni, CNRS, Marseille, France
B. Fowler, York University, North York, Ontario, Canada
T. J. R. Francis, Naval Medical Research Institute, Bethesda, MD
W. D. Fraser, University of Toronto, Toronto, Canada
B. A. Freeman, University of Alabama at Birmingham, Birmingham, AL
G. Frey, Federal Armed Forces Hospital, Ulm, West Germany
X. Fructus, COMEX S.A., Marseille, France
Y. Fujiwara, Kosei General Hospical, Hiroshima, Japan
J. D. Fulmer, University of Alabama at Birmingham, Birmingham, AL
H. Furuhashi, Nihon University, Tokyo, Japan
B. Gardette, COMEX S.A., Marseille, France
M. C. Gardette-Chauffour, CNRS, Marseille, France
R. Gelfand, University of Pennsylvania Medical Center, Philadelphia, PA
I. B. Gensemer, Geisinger Medical Center, Danville, PA 17822
R. Goad, Duke University Medical Center, Durham, NC
D. F. Gorman, Royal Adelaide Hospital, Adelaide, South Australia, Australia
S. Gosovic, Institut za pomorsku medicinu, Split, Yugoslavia
Y. Gotoh, Saitama Medical School, Saitama, Japan
R. D. Green, Royal Australian Navy, Balmoral, NSW, Australia
G. Hajduczok, State University of New York, Buffalo, NY
J.-P. Halonen, University Central Hospital, Turku, Finland
H. Hamilton, York University, North York, Ontario, Canada
R. W. Hamilton, Hamilton Reserch Ltd., Tarrytown, NY
M. Z. Hao, China Medical University, Shenyang, China
G. B. Hart, Memorial Medical Center, Long Beach, CA

H. Hayase, University of Nagoya School of Medicine, Nagoya, Japan
K. Hayashi, Kyushu Rosai Hospital, Kitakyushu-shi, Japan
D. J. Hei, University of Wisconsin, Madison, Wisconsin
W. Hinson, University of North Carolina, Wilmington, NC
M. P. Hlastala, University of Washington, Seattle, WA
M. Z. Hoa, China Medical University, Shenyang, China
I. Holloway, University of Newcastle upon Tyne, Newcastle, England
J. Holthaus, GKSS/Forschungszentrum, Geesthacht, Federal Republic of Germany
Y. Honda, Chiba University, Chiba city, Japan
S. K. Hong, State University of New York, Buffalo, New York
A. Hope, NUTEC, Ytre Laksevåg, Norway
M. Horiguchi, Nagoya University School of Medicine, Nagoya, Japan
S. Hruby, Institute of Applied Physiology and Medicine, Seattle, WA
M. Hugon, CNRS-GIS, Marseille, France
A. Hyagaji, Mitsui Toatsu Chemicals Inc., Japan
T. Ikeda, Japan Maritime Self-Defense Force Undersea Medical Center, Kanagawa, Japan
T. Ishikawa, University of Occupational and Environmental Health, Kitakyushu Japan
M. Ito, Nagoya University School of Medicine, Nagoya, Japan
A. Itoh, Japan Maritime Self-Defense Force Undersea Medical Center, Kanagawa, Japan
T. Iwa, Kanazawa University Hospital, Kanazawa, Japan
R. M. Jackson, University of Alabama at Birmingham, Birmingham, AL
I. Jacoby, University of California, San Diego, CA
K. Jakobsen, NUTEC, Ytre Laksevaag, Norway
H. M. Jansen, University of Amsterdam, Amsterdam, The Netherlands
P. C. Johnson, University of Pennsylvania Medical Center, Philadelphia, PA
K. Kamiyama, Ebara Metropolitan Hospital, Tokyo, Japan
T. Kaneda, Nagoya University, Nagoya, Japan
M. Kawashima, Kyushu Rosai Hospital, Kitakyushu, Japan
D. J. Kenyon, Abionics, Inc., Mounte Arlington, NJ
D. Kerem, Israel Oceanographic and Limnological Research, Haifa, Isreal
H. J. Kim, Ocean and Underwater Medical Research Center, Chinhae, Korea
M. Kitano, Saitama Cancer Center, Saitama-ken, Japan
V. Kizer, Institute of Applied Physiology and Medicine, Seattle, WA
H. Kløve, NUTEC, Ytre Laksevåg, Norway
D. R. Knight, Naval Submarine Base New London, Groton, CT
G. Knudsen, NUTEC, Ytre Laksevåg, Norway
K. Kobayashi, Saitama Medical School, Saitama, Japan
S. Kobayashi, Nagoya University School of Medicine, Nagoya, Japan
N. Konda, University of Occupational and Environmental Health, Kitakyushu, Japan
M. Kono, NM Group Application Lab, Japan
S. Kosugi, University of Waseda, Tokyo, Japan
J. A. Krasney, State University of New York, Buffalo, NY

K-W. **Lam**, University of Texas Health Science Center, San Antonio, TX
C. J. **Lambertsen**, University of Pennsylvania Medical Center, Philadelphia, PA
E. H. **Lanphier**, University of Wisconsin, Madison, WI
C. S. **Leach**, University of Pennsylvania Medical Center, Philadelphia, PA
H. C. **Lee**, Naval General Hospital, Taiwan, The Republic of China
H. **Lee**, Brookside Hospital, San Pablo, CA
C. E. **Lehner**, University of Wisconsin, Madison, WI
C. **Lemaire**, OCTARES, Marseille, France
H. **Lenders**, Federal Armed Forces Hospital, Ulm, West Germany
E. N. **Lightfoot**, University of Wisconsin, Madison, WI
Y-C **Lin**, University of Hawaii, Honolulu, Hawaii 96822
A. G. **Lindrup**, NUTEC Ytre Laksevåag, Norway
M. C. **Liou**, Harbor Branch Foundation, Ft. Pierce, FL
Z. Q. **Liu**, China Medical University, Shenyang, China
P. **Longobardi**, Instituto di Studie Ricerche ed Iperbariche, Naples, Italy
M. J. **Loxton**, Royal Australian Navy, Balmoral, NSW, Australia
H. W. **Lueders**, Brookside Hospital, San Pablo, CA
S. M. **Luria**, Naval Submarine Base New London, Groton, CT
F. P. **Lynch**, University of California, San Diego, CA
J. R. **Magel**, Queens College of the City of New York, Flushing, NY
K. **Makino**, Kyoto Institute of Technology, Japan
Y. **Mano**, Tokyo Medical and Dental University, Japan
T. **Marikawa**, Chiba University, Chiba City, Japan
J. **Martin**, University of Washingon, Seattle, WA
S. **Martini**, University of Trondheim, Trondheim, Norway
E. W. **Massey**, Duke University Medical Center, Durham, NC
R. **Mastroianni**, Istituto di Studi e Ricerche Subacques ed Iperbariche, Naples, Italy
M. **Matsuda**, Japan Marine Science and Technology Center, Yokosukashi, Japan
S. **Matsuoka**, University of Occupational and Environmental Health, Kitakyushu Japan
M. **Matsuura**, Nagoya University School of Medicine, Nagoya, Japan
W. D. **McArdle**, Queens College of the City of New York, Flushing, NY
D. **McCullough**, University of Toronto, Toronto, Canada
Y. **Mebane**, Duke University Medical Center, Durham, NC
G. **Medolla**, Istituto di Studi e Ricerche Subacques ed Iperbariche dell', Napoli, Italy
R. R. **Mercer**, Duke Medical Center, Durham, NC
K. **Miki**, State University of New York, Buffalo, NY
P. **Mitchell**, Duke University Medical Center, Durham, NC
Y. **Miyake**, Nagoya University School of Medicine, Nagoya, Japan
T. **Mizuno**, Tokyo Medical & Dental University, Tokyo, Japan
D. J. **Montabana**, University of Pennsylvania Medical Center, Philadelphia, PA
R. E. **Moon**, Duke University Medical Center, Durham, NC
M. **Mori**, Japan Marine Science and Technology Center, Yokosuka, Japan
T. **Morikawa**, Chiba University, Chiba, Japan

S. R. Muza, US Army Research Institute of Environmental Medicine, Natick, MA
L. W. Myers, UCLA Multiple Sclerosis Research Clinic, Los Angeles, CA
R. A. M. Myers, Maryland Institute for Emergency Services Systems, Baltimore, MD
E. Myrseth, NUTEC, Ytre Laksevåg, Norway
W. Nakamura, Chiba University, Chiba City, Japan
T. Nakashima, Nagoya University School of Medicine, Nagoya, Japan
H. Nakayama, Japan Marine Science and Technology Center, Yokosukashi, Japan
T. Nakayama, Tokyo Medical and Dental University, Japan
Y. Nanbo, Kosei General Hospital, Hiroshima, Japan
R. Naquet, CNRS, Gif sur Yvette, France
I. Nashimoto, Saitama Medical School, Saitama, Japan
T. S. Neuman, University of California, San Diego, CA
R. Y. Nishi, Defence and Civil Institute of Environmental Medicine, Downsview, Ontario, Canada
A. K. C. Niu, Naval General Hospital, Taiwan, The Republic of China
M. Nodera, Saitama Medical School, Saitama, Japan
E. H. Padbury, NUTEC, Ytre Laksevåg, Norway
J. Ohlsson, University of Washington, Seattle, WA
H. Oiwa, Japan Maritime Self-Defense Force Undersea Medical Center, Kanagawa, Japan
K. Okafuji, Kawashima Orthopedic Hospital, Ohita, Japan
J. Okubo, Tokyo Medical and Dental University, Tokyo, Japan
S. Okuda, University of Occupational and Environmental Health, Kitakyushu, Japan
H. Ohiwa, Japan Maritime Self-Defense Force, Kanagawa, Japan
J. Ohlsson, University of Washington, Seattle, WA
R. M. A. Osti, National Health Service ULSS, Padua, Italy
K. Ozawa, National Defense Medical College, Saitama, Japan
J. A. Paciorek, University of Bergen, Bergen, Norway
E. H. Padbury, NUTEC, Bergen, Norway
C. V. Paganelli, State University of New York, Buffalo, NY
F. M. Pallotta, Amministrazione Provinciale di Napoli, Istituto di Studi e Ricerche Subacques ed Iperbariche, Naples, Italy
M. Palta, University of Wisconsin, Madison, WI
K. Pandolf, US Army Research Institute of Environmental Medicine, Natick, MA
Y. H. Park, Kosin Medical College, Pusan, Korea
Y. S. Park, Kosin Medical College, Pusan, Korea
D. W. Parsons, Royal Adelaide Hospital, Adelaide, Australia
R. E. Peterson, Peterson Technical Service, West Chester, PA
G. J. Petrone, Brookside Hospital, San Pablo, CA
M. Pilgramm, Federal Armed Forces Hospital, Ulm, West Germany
J. K. Pimlott, Houlder Diving Research Facility, London, England
K. E. Pinkerton, Duke Medical Center, Durham, NC

J. B. Pisarello, University of Pennsylvania Medical Center, Philadelphia, PA
G. Porlier, York University, North York, Ontario, Canada
M. R. Powell, Institute of Applied Physiology and Medicine, Seattle, WA
A. T. Powers, University of California, San Diego, CA
S. Radic, Institut za pomorsku medicinu, Split, Yugoslavia
H. Rahn, State University of New York at Buffalo, Buffalo, NY
D. L. Reeves, Naval Aerospace Medical Researcgh Laboratory, Pensacola, FL
D. W. Rennie, State University of New York at Buffalo, Buffalo, NY
T. Robertson, University of Washington, Seattle, WA
W. Rogers, Naval Submarine Base New London, Groton, CT
I. Rønnestad, NUTEC, Bergen, Norway
C. M. Roos, University of Amsterdam, Amsterdam, The Netherlands
J. Ross, Brookside Hospital, San Pablo, CA
J. C. Rostain, CNRS, Marseille, France
M. J. Rowe, Memorial Medical Center, Long Beach, CA
F. Rusca, National Health Service ULSS, Padua, Italy
I. Rønnestad, NUTEC, Ytre Laksevåg, Norway
S. Sagawa, University of Occupational and Environmental Health, Kitakyushu, Japan
K. Sakakibara, University of Nagoya, Nagoya, Japan
Y. Sakakibara, Chiba University, Chiba city, Japan
H. Saltzman, Duke University Medical Center, Durham, NC
M. Sato, Nagoya University, Nagoya, Japan
M. N. Sawka, U.S. Army Research Institute of Environmental Medicine, Natick, MA
H. G. Schafstall, GKSS/Forschungszentrum, Geethacht, Federal Republic of Germany
M. Schiavon, National Health Service ULSS, Padova, Italy
C. Schiraldi, National Health Service ULSS, Padua, Italy
W. Schnegelsberg, GKSS/Forschungszentrum, Geethacht, Federal Republic of Germany
J. J. Schreuder, University of Amsterdam, Amsterdam, The Netherlands
D. M. Schwartz, University of Pennsylvania Medical Center, Philadelphia, PA
K. Seki, Japan Marine Science and Technology Center, Yokosukashi, Japan
R. L. Shapiro, Brookside Hospital, San Pablo, CA
D. L. Shelton, Duke University Medical Center, Durham, NC
M. Shibayama, Tokyo Medical and Dental University, Japan
K. Shiraki, University of Occupational and Environmental Health, Kitakyushu, Japan
A. Sipinen, University Central Hospital, Turku, Finland
S. K. Snyder-White, Maryland Institute for Emergency Services Systems, Baltimore, MD
J. F. Socks, Naval Submarine Base New London, Groton, CT
W. Sterk, DADCODAT, Zuidwolde, The Netherlands
B. Stolp, Duke University Medical Center, Durham, NC
M. B. Strauss, Memorial Medical Center, Long Beach, CA

H. **Sugiyama**, Ebara Metropolitan Hospital, Tokyo, Japan
D. J. **Suh**, Ocean and Underwater Medical Research Center, Armed Forces, Chinhae, Korea
H. **Takahashi**, University of Nagoya, Nagoya, Japan
N. **Takano**, Tokyo Medical and Dental University, Japan
K. **Takao**, Kawashima Orthopedic Hospital, Ohita, Japan
H. **Tamura**, Kawashima Orthopedic Hospital, Ohita, Japan
Y. **Tanaka**, Chiba University, Chiba City, Japan
J. **Tatsuno**, National Defense Medical College, Saitama, Japan
S. **Tokufuji**, Kyushu Rosai Hospital, Kitakyushu-shi, Japan
M. M. **Toner**, Queens College of the City of New York, Flushing, NY
D. **Torbati**, University of Pennsylvania, Philadelphia, PA
I. **Tyssebotn**, University of Bergen, Bergen, Norway
K. **Uchida**, Kawashima Orthopedic Hospital, Ohita, Japan
M. **Ueda**, Nagoya University, Nagoya, Japan
H. **Urayama**, Kanazawa University Hospital, Ishikawa, Japan
H. **Ursin**, NUTEC, Ytre Laksevåg, Norway
H. D. **Van Liew**, State University of New York, Buffalo, NY
R. D. **Vann**, Duke University Medical Center, Durham, NC
C. F. **Veal, Jr.**, University of Alabama, Birmingham, AL
R. **Værnes**, NUTEC, Ytre Laksevåg, Norway
D. N. **Walder**, University of Newcastle upon Tyne, Newcastle, England
C. A. **Ward**, University of Toronto, Toronto, Canada,
Y. **Watanabe**, Nagoya University School of Medicine, Nagoya, Japan
Y. **Watanabe**, Kanazawa University Hospital, Ishikawa, Japan
P. K. **Weathersby**, University of Toronto, Toronto, Canada
M. M. **Winsborough**, Naval Medical Research Institute, Bethesda, MD
H. **Wissler**, The University of Texas at Austin, Austin, TX
A. **Yamaguchi**, Kyushu Rehabilitation College, Kitakyushu-shi, Japan
S. **Yamamoto**, University of Occupational and Environmental Health, Kitakyushu, Japan
N. **Yanagita**, Nagoya University School of Medicine, Nagoya, Japan
C. **Yang**, Naval General Hospital, Taiwan, The Republic of China
D. S. **Yeon**, Ocean and Underwater Medical Research Center, Chinhae, Korea
H. **Yokoi**, Nagoya University School of Medicine, Nagoya, Japan
T. **Yokoyama**, Keio University, Shinjuku-Shinanomachi, Tokyo, Japan
H. **Yoshino**, University of Occupational and Environmental Health, Kitakyushu Japan
A. J. **Young**, U.S. Army Research Institute of Environmental Medicine, Natick, MA
C. K. **Yu**, Perry Companies International Ltd., Tokyo, Japan
T. **Yusa**, University of the Ryukyus, Okinawa, Japan
D. **Zannini**, Corso Europa, Genova, Italy
M. **Zivkovic**, Institut za pomorsku medicinu, Split, Yugoslavia
W. W. A. **Zuurmond**, University of Amsterdam, Amsterdam, The Netherlands

AUTHOR INDEX