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UNDERWATER PHYSIOLOGY V

PROCEEDINGS OF THE FIFTH SYMPOSIUM
ON UNDERWATER PHYSIOLOGY

Sponsored by

Institute for Environmental Medicine,
University of Pennsylvania Medical Center

Undersea Medical Society

U.S. Office of Naval Research

U.S. National Oceanic and Atmospheric Administration

Edited by C. J. Lambertsen

FASEB, Bethesda, Maryland, 1976

FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY
9650 Rockville Pike, Bethesda, Maryland 20014

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 76-11404

PRINTED IN THE UNITED STATES OF AMERICA BY PUBLICATION PRESS, INC., BALTIMORE, MARYLAND

63 cited in DB

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LIST OF AUTHORS AND PANELISTS

K. N. ACKLES, Defence and Civil Institute of Environmental Medicine, Box 2000, Downsview, Ontario, Canada

G. M. ADAMS, Naval Submarine Medical Research Laboratory, Naval Submarine Base, Groton, Connecticut 06340

J. A. ADOLFSON, Försvarets Forskningsanstalt, National Defence Research Institute, Naval Medicin Division, Naval Diving Office, S-100 14 Stockholm, Sweden

C. AGARATE, Hyperbaric Research Center, COMEX, Marseille, France

T. K. AKERS, Department of Physiology and Pharmacology, University of North Dakota, Grand Forks, North Dakota 58201

G. ALBANO, Clinica Neurologica, Via la Loggia, Palermo, Italy.

J. M. ALEXANDER, 22525 Lassen Street, Chatsworth, California 91311

N. R. ANTHONISEN, Respiratory Division, Royal Victoria Hospital and McGill University, Montreal 112, Quebec, Canada

C. ASMUNDSON, Department of Kinesiology, Simon Fraser University, Burnaby 2, B.C., Canada

A. J. BACHRACH, Behavioral Sciences Department, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland 20014

G. BALOUET, Marine Nationale, Groupe d'Études et de Recherches Sous-Marines, Laboratorie de Physiologie, 83800 Toulon Naval, France

E. W. BANISTER, Department of Kinesiology, Simon Fraser University, Burnaby 2, B.C., Canada

A. BARET, C.E.R.B., H.I.A. Sainte-Anne, 83800 Toulon Naval, France

E. E. P. BARNARD, Department of the Medical Director-General of the Navy, Ministry of Defence, London SW6 7TR, England

A. BARRANX, C.E.R.B., H.I.A. Sainte-Anne, 83800 Toulon Naval, France

L. BARTHÉLEMY, Service de Physiologie, Faculté de Médecine, B. P. 815, 29279 Brest, France

A. BATTESTI, C.E.R.B., H.I.A. Sainte-Anne, 83800 Toulon Naval, France

E. L. BECKMAN, Moody Marine Institute, Texas A and M University, Fort Crockett 311, Galveston, Texas 77550

A. R. BEHNKE, 2241 Sacramento Street, San Francisco, California 94115

P. B. BENNETT, Professor of Anesthesiology, and Associate Professor of Physiology, Duke University Medical Center, Durham, North Carolina 27710

N. M. G. BHAKTAN, Department of Kinesiology, Simon Fraser University, Burnaby 2, B.C., Canada

G. D. BLENKARN, Department of Anesthesiology, Duke University Medical Center, Durham, North Carolina 27710

T. P. BOND, Department of Physiology, University of Texas Medical Branch, Galveston, Texas 77550

M. BONFIGLIO, Professor, Department of Orthopaedic Surgery, University of Iowa, Iowa City, Iowa 52242

R. C. BORNMANN, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland 20014

C. BOUTIER, C.E.R.B., H.I.A. Sainte-Anne, 83800 Toulon Naval, France

A. A. BOVE, Department of Medicine, Health Sciences Center, Temple University, Philadelphia, Pennsylvania 19140

M. E. BRADLEY, Naval Medical Research Institute, Bethesda, Maryland 20014

B. BROUSSOLLE, C.E.R.B., H.I.A. Sainte-Anne, 83800 Toulon Naval, France

A. A. BÜHLMANN, Department of Internal Medicine, University of Zürich, Kantonsspital, Zürich, Switzerland

H. BURNET, C.E.R.B., H.I.A. Sainte-Anne, 83800 Toulon Naval, France

S. D. CAMPBELL, Institute of Applied Physiology and Medicine, Seattle, Washington 98122

L. C. CARLSON, Department of Physiology, University of North Dakota, Grand Forks, North Dakota 58201 (in care of T. K. Akers)

J. CHOUTEAU, Professeur, Faculté des Sciences, Université de Provence, Place Victor Hugo, 13 Marseille, France

A. T. K. COCKETT, Division of Urology, University of Rochester, School of Medicine and Dentistry, Rochester, New York 14620

D. B. COLTMAN, Department of Surgery, Royal Victoria Infirmary, The University of Newcastle upon Tyne, Newcastle upon Tyne, England

M. COLUMBA, Istituto di Fisica Tecnica, University of Palermo, Palermo, Italy

P. T. COX, North Staffordshire, Royal Infirmary, Accident Department, Princes Road, Hartshill, Stoke-on-Trent, England ST4 7LN

A. B. Craig, Department of Physiology, University of Rochester, School of Medicine and Dentistry, Rochester, New York 14620

D. Cresson, C.E.R.B., H.I.A. Sainte-Anne, 83800 Toulon Naval, France

W. D. Currie, Division of Radiology, Duke University Medical Center, Durham, North Carolina 27710

J. K. Davidson, Department of Diagnostic Radiology, Western Infirmary, Glasgow, G11 6NT, Scotland

A. J. Davison, Department of Kinesiology, Simon Fraser University, Burnaby 2, B.C., Canada

J. G. Dickson, Jr., Department of Anesthesia, University of Pennsylvania, Philadelphia, Pennsylvania 19104

D. Doell, Respiratory Division, Royal Victoria Hospital and McGill University Montreal, Quebec, Canada

M. Dvorak, Department of Physiology, University of Rochester, School of Medicine and Dentistry, Rochester, New York 14620

W. J. Eaton, Royal Naval Physiological Laboratory, Alverstoke, Hants, England

E. I. Eger II, Department of Anesthesia, University of California Medical Center, San Francisco, California 94143

D. H. Elliott, Royal Naval Physiological Laboratory, Alverstoke, Hants, England

C. J. Fagan, Department of Radiology, University of Texas Medical Branch, Galveston, Texas 77550

L. Fagraeus, Department of Anesthesiology, Duke University Medical Center, Durham, North Carolina 27710

L. Farhi, Department of Physiology, SUNY at Buffalo School of Medicine, Buffalo, New York 14214

S. H. Feinstein, Institute for Advanced Study of the Communication Processes, University of Florida, Gainesville, Florida 32611

E. T. Flynn, Jr., Navy School of Diving Salvage, Washington Navy Yard, Washington, D.C. 20374

X. R. Fructus, Hyperbaric Research Center, COMEX, Marseille, France

H. Gehring, Department of Internal Medicine, University of Zürich, Kantonsspital, Zürich, Switzerland

R. S. Gelein, Division of Radiobiology, Duke University Medical Center, Durham, North Carolina 27710

R. Gelfand, Institute for Environmental Medicine, Medical Center, University of Pennsylvania, Philadelphia, Pennsylvania 19174

H. Gilder, Departments of Surgery and Biochemistry, The New York Hospital–Cornell Medical Center, 1300 York Avenue, New York, New York 10021

S. F. GOTTLIEB, Biological Sciences Division, Purdue University, Fort Wayne Campus, 2101 Coliseum Boulevard East, Fort Wayne, Indiana 46805

D. J. GRAVES, Department of Chemical and Biochemical Engineering, University of Pennsylvania, Philadelphia 19104

H. GUENARD, Laboratoire de Médicine Aerospatiale du Centre d'Essais en Vol, 91-Bretigny, COMEX, 13-Marseille, France

M. M. GUEST, Shriner's Burn Institute, University of Texas Medical Branch, Galveston, Texas 77550

J. M. HALLENBECK, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland 20014

M. J. HALSEY, Division of Anaesthesia, Clinical Research Centre, Harrow, Middlesex, England

R. W. HAMILTON, JR., Tarrytown Labs, Ltd., 2 Hudson Street, Tarrytown, New York 10591

M. HANSEN, Institute of Medical Physiology C, University of Copenhagen, Raadmansgaade 71, DK2200, Copenhagen N, Denmark

H. V. HEMPLEMAN, Royal Naval Physiological Laboratory, Fort Road, Alverstoke, Hants, PO12 2 DU, England

P. L. HENDRICKS, Veterans Administration Hospital, 3350 La Jolla Village Drive, San Diego, California 92161

J. M. HERRON, Baptist Medical Center System, 9600 West Twelfth, Little Rock, Arkansas 72201

C. M. HESSER, Department of Naval Medicine, Karolinska Institute, S-104 01, Stockholm 60, Sweden

B. A. HILLS, Marine Biomedical Institute, University of Texas, Galveston, Texas 77550

B. HOKE, Department of Psychiatry, U.S. Naval Hospital, Oakland, California 94627

H. HOLLIEN, Communication Sciences Laboratory, University of Florida, Gainesville, Florida 32601

P. HOLLIEN, Communication Sciences Laboratory, University of Florida, Gainesville, Florida 32601

D. E. HOLNESS, Defence and Civil Institute of Environmental Medicine, Box 2000, Downsview, Ontario, Canada

S. K. HONG, Department of Physiology SUNY at Buffalo School of Medicine, Buffalo, New York 14214

J. L' HUILLIER, Laboratoire de Médecine Aerospatiale du Centre d'Essais en Vol, 91-Bretigny, COMEX, 13-Marseille, France

R. HYACINTHE, C.E.R.B., H.I.A. Sainte-Anne, 83800 Toulon Naval, France

J. IDICULA, Surg. Lcdr., INS VIRBAHU, c/o Fleet Mail Office, Vishakhapatnam 530014, India

G. IMBERT, Laboratoire de Psychophysiologie, Centre St. Jerome, Université de Provence, Rue Henri Poincaré 13397, Marseille Cedex 4, France

D. L. JACKSON, Environmental Stress Division, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland 20014

D. C. JOHANSON, Institute of Applied Physiology and Medicine, Seattle, Washington 98122

J. P. JONES, JR., Transit Compressed Air Medical Center, San Francisco, California.

G. KARREMAN, Bockus Research Institute, Institute for Environmental Medicine and Department of Physiology, University of Pennsylvania, Philadelphia, Pennsylvania 19174

R. S. KENNEDY, Head, Human Factors Engineering Branch, Code 1242, Pacific Missile Center, Point Mugu, California 93042

D. W. KENT, Memorial Hospital Medical Center, Long Beach, California 90801

G. J. KOEHLER, Biological Sciences Division, Purdue University, Fort Wayne Campus, Fort Wayne, Indiana 46805

H. J. KREKELER, F.R.G. 51 Bonn-Bad Godesberg, Zanderstrasse 1A, Germany

S. KRONHEIM, Office of Naval Research, 800 N. Quincy Street, Arlington, Virginia 22217

J. A. KYLSTRA, F. G. Hall Laboratory for Environmental Research, Duke University Medical Center, Durham, North Carolina 27710

D. A. LALLY, University of Kentucky Medical Center, Lexington, Kentucky 40506

C. J. LAMBERTSEN, Institute for Environmental Medicine, Medical Center, University of Pennsylvania, Philadelphia, Pennsylvania 19174

T. D. LANGLEY, Ocean Systems, Inc., Union Carbide Technical Center, Tarrytown, New York 10591

E. H. LANPHIER, Nashotah House, Nashotah, Wisconsin 53058

J. LE CHUITON, Marine Nationale, Groupe d'Études et de Recherches Sous-Marines, Laboratorie de Physiologie, 83800 Toulon Naval, France

J. C. LE PECHON, Société Doris, Paris, France

D. LINNARSSON, Departments of Aviation and Naval Medicine, Faculty of Medicine, Karolinska Institutet, S-10401 Stockholm 60, Sweden

C. E. G. LUNDGREN, Laboratory of Aviation and Naval Physiology, Institute of Physiology, University of Lund, Solvegatan 19, S-223 62 Lund, Sweden

A. G. MACDONALD, Physiology Department, Marischal College, University of Aberdeen, Aberdeen AB9, 1AS, Scotland

J. MADSEN, Institute of Medical Physiology C, University of Copenhagen, Raadmansgaade 71, DK 2200 Copenhagen N, Denmark

C. K. McSherry, Departments of Surgery and Biochemistry, The New York Hospital-Cornell Medical Center, 1300 York Ave., New York, New York 10021

A. Michaud, Marine Nationale, Groupe d'Études et de Recherches Sous-Marines, Laboratorie de Physiologie, 83800 Toulon Naval, France

A. Michel, Marine Nationale, Groupe d'Études et de Recherches Sous-Marines, Laboratorie de Physiologie, 83800 Toulon Naval, France

T. O. Moore, Department of Physiology, University of Hawaii School of Medicine, Honolulu, Hawaii 96822

J. F. Morlock, Department of Physiology, University of Hawaii School of Medicine, Honolulu, Hawaii 96822

J. B. Morrison, Department of Kinesiology, Simon Fraser University, Burnaby 2, B.C., Canada

K. Muysers, Laboratorium für Atmung und Kreislauf, 413 Moers, Krankenhaus Bethanien, Germany

R. Naquet, Laboratory of Neurophysiology, C.N.R.S., Gif-sur-Yvette, France

C. Nichols, Scheie Eye Institute, Philadelphia, Pennsylvania 19104

G. von Nieding, F.R.G.-1 Berlin 21, Essenerstrasse #19, Germany

S. J. Norton, Department of Chemistry, North Texas State University, Denton, Texas 76203

G. Odaglia, Instituto di Medicina del Lavoro dell'Universita di Genova, Viale Benedetto XV, 10-16132, Genova, Italy

H. C. Ornhagen, Department of Physiology, SUNY at Buffalo School of Medicine, Buffalo, New York 14214

J. Parc, Marine Nationale, Groupe d'Études et de Recherches Sous-Marines (G.E.R.S.), Laboratorie de Physiologie, 83800 Toulon Naval, France

W. D. M. Paton, Department of Pharmacology, Oxford University, South Parks Road, Oxford, England

S. M. Pauley, 124 Riviera Way, Laguna Beach, California 92651

R. Peterson, Institute for Environmental Medicine, Medical Center, University of Pennsylvania, Philadelphia, Pennsylvania 19174

A. Pilmanis, University of Southern California, Catalina Marine Science Center, P.O. Box 398, Avalon, California 90704

S. Plouvier, C.E.R.B., H.I.A. Sainte-Anne, 83800 Toulon Naval, France

J. A. Quinn, Department of Chemical and Biochemical Engineering, University of Pennsylvania, Philadelphia, Pennsylvania 19104

M. W. Radomski, Defence and Civil Institute of Environmental Medicine, Box 2000, Downsview, Ontario, Canada

L. V. G. Rhodes, Biological Sciences Division, Purdue University, Fort Wayne Campus, Fort Wayne, Indiana 46805

R. RICCI, C.E.R.B., H.I.A. Sainte-Anne, 83800 Toulon Naval, France

A. P. ROBERTS, Division of Urology, University of Rochester School of Medicine, Rochester, New York 14642

J. C. ROSTAIN, Laboratory of Applied Neurophysiology, INP, C.N.R.S., Marseille, France

S. H. ROTH, Faculty of Medicine, Division of Pharmacology and Therapeutics, University of Calgary, 2920 24 Avenue, N.W., Calgary, Alberta, Canada T2N 1N4

H. ROTHMAN, Institute for Advanced Study of the Communication Process, University of Florida, Gainesville, Florida 32611

J. ROWE, Defence and Civil Institute of Environmental Medicine, Box 2000, Downsview, Ontario, Canada

H. A. SALTZMAN, F. G. Hall Laboratory for Environmental Research, Duke University Medical Center, Durham, North Carolina 27710

A. P. SANDERS, Division of Radiobiology, Duke University Medical Center, Durham, North Carolina 27710

K. E. SCHAEFER, Biomedical Sciences Division, Naval Submarine Medical Center, Naval Submarine Base, Groton, Connecticut 06340

W. H. SCHOENFISCH, Department of Biology, The American University, Washington, D.C. 20016

H. R. SCHREINER, Manager, Corporate Research, Union Carbide Corporation, Tarrytown, New York 10591

C. A. SCOTT, Defence and Civil Institute of Environmental Medicine, Box 2000, Downsview, Ontario, Canada

F. SICARDI, Centre Experimental Hyperbare de la COMEX, Marseille Cedex 2, France

U. SMIDT, Laboratorium für Atmung und Kreislauf, 413 Moers, Krankenhaus Bethanien, Germany

E. B. SMITH, Physical Chemistry Laboratory, Oxford University, South Parks Road, Oxford OX1 3OZ, England

K. H. SMITH, Virginia Mason Research Center, 1000 Seneca Street, Seattle, Washington 98101

R. A. SMITH, Department of Anesthesiology, University of California School of Medicine, San Francisco, California 94143

J. SMITH-SIVERTSEN, Royal Norwegian Navy Diving School, 5070 Haakonsvern Naval Base, Mathopen, Norway

M. P. SPENCER, Institute of Applied Physiology and Medicine, 556-18th Avenue, Seattle, Washington 98122

G. SPERATI, Divisione di Otorinolaringoiatria, Ospedali Civili, Viale Benedetto XV, 10-16132 Genova, Italy

P. J. STEGALL, Virginia Mason Research Center, 1000 Seneca Street, Seattle, Washington 98101

R. H. STRAUSS, 100 Walker Street, Cambridge, Massachusetts 02138

J. R. THOMAS, Behavioral Sciences Department, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland 20014

D. R. THORNE, Experimental Psychophysiology Department, Walter Reed Army Medical Center, Washington, D.C. 20012

J. TIMBAL, Laboratoire de Médecine Aerospatiale du Centre d'Essais en Vol, 91-Bretigny, COMEX, 13 Marseille, France

P. VARÈNE, Laboratoire de Physiologie, U.E.R. des Sciences Médicales III, Université de Bordeaux II, 146 Rue Leó-Saignat, 33076 Bordeaux-Cedex, France

H. VIEILLEFOND, Laboratoire de Médicine Aerospatiale du Centre d'Essais en Vol, 91-Bretigny, COMEX, 13 Marseille, France

J. J. VOROSMARTI, JR., Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland 20014

G. P. VOSE, Research Institute, Texas Woman's University, Denton, Texas 76204

D. N. WALDER, Department of Surgery, The Royal Victoria Infirmary, The University of Newcastle-upon-Tyne, Newcastle-upon-Tyne NE1 4LP, England

J. M. WALSH, Behavioral Sciences Department, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Maryland 20014

W. J. WATSON, Defence and Civil Institute of Environmental Medicine, Box 2000, Downsview, Ontario, Canada

P. WEBB, Webb Associates, P.O. Box 308, Yellow Springs, Ohio 45387

C. H. WELLS, Shriner's Burn Institute, University of Texas Medical Branch, Galveston, Texas 77550

J. M. WELLS, JR., Manned Undersea Science and Technology Office, National Oceanic and Atmospheric Administration, 6010 Executive Boulevard, Rockville, Maryland 20852

J. D. WOOD, Department of Biochemistry, University of Saskatchewan, Saskatoon, Canada

H. WORTH, Laboratorium für Atmung und Kreislauf, 413 Moers, Krankenhaus Bethanien, Germany

W. B. WRIGHT, Fairfax Hospital, Department of Emergency Services, 3300 Gallows Road, Falls Church, Virginia 23346

D. ZANNINI, Istituto di Medicina del Lavoro dell'Universita di Genova, Viale Benedetto XV 10, 16132 Genova, Italy

A. M. ZIMMERMAN, Department of Zoology, University of Toronto, Toronto, Ontario, Canada M5S 1A1

S. ZIMMERMAN, Glendon College, York University, Toronto, Ontario, Canada

M. ZUTTER, Respiratory Division, Royal Victoria Hospital and McGill University, Montreal, Quebec, Canada

PREFACE*

Over the recent period of massive technical accomplishment in lunar exploration we have simultaneously embarked upon a new phase in undersea research and operations. While less evident than the universally known events of the aerospace programs, this has been a phase of challenge, rapid advance in method, and consolidation of scientific information. Until the present time, and in spite of intensive investigation, practical open sea diving has progressed only slowly to the full depths of the continental shelves. Only now has active investigative and practical effort begun to extend work to the slopes beyond the depths of these continental shelves.

Advancement of diving has for many decades been prevented or, at the least, discouraged by lack of far-sighted interest by industrial and military leaders around the world. However, stimulated by a peculiar episode of history, and not by steady development of understanding among industrial and scientific leaders, a period has at last been reached in which scientific, operational, engineering, commercial and military goals are beginning to coalesce toward major extension of undersea capability. The critical episode was an embargo on oil shipments from the Middle East to more industrialized countries—an embargo imposed by oil-producing nations to influence political support patterns in an Israeli-Arab war. Now, with little or no experience in work at depths greater than about 500 feet of sea water, oil producers have been stimulated to explore for (and produce) oil at depths to 1500 feet of sea water in 1975, with hopes of reaching 3000 feet within a few more years and 6000 feet by 1980.

Thus, in a single step of awakened interest, industrial and naval leaders have begun to accept the already obvious physiological feasibility of diving effectively to depths of at least 1000 feet of sea water and are aware of the need to know the "limits" of human performance. By the time of this Symposium, man had been exposed to pressures equivalent to 2000 feet of sea water. He had also been found able to breathe well at rest and in moderate work, even when the density of respired gas was equivalent to that to be expected with helium at pressures of more than 5000 feet of sea water. Clearly the highly advanced undersea physiological research of the past several years can guide determination of effective means for accomplishing the desired work at intermediate depths.

What is now important is that, with demonstrated practicality of undersea work beyond any earlier vision of such practicality, several important paths must be followed. First, the detailed physiological studies and engineering developments which will allow practical diving to these depths must be continued, and those should be coordinated. This is the time for the industrial users to invest in obtaining the critically important information, sought after in step-by-step investigations of immense technical difficulty. The desired next phases of research and application must be carried out with continuous investment by industry, aiding itself by this means. New and prominent supporting roles for the health-related agencies must emerge, to assure continuity of basic and applied investigations, and to include long-term as well as acute consequences of extreme exposure. At one time it was

*From "The Changing Face of Manned Undersea Activity", Institute for Environmental Medicine, University of Pennsylvania.

enough to have naval support from one or a few countries carry the investigative burden for the entire slow evolution of diving. Now, the traditional unilateral supporting role of the naval organizations has been caused to change by the gross expansion of industrial undersea operations. The need now is for a coalition of scientific and technical leaders—university, industrial and federal—to carry effective manned undersea activity to its varied limits. These leaders must concentrate upon assuring progressive and imaginative research, development of means for its application, and means for consolidating information concerning capability and safety limitations. Advances to be sought must include gross improvement in the methods of shallow and intermediate depth diving as well as methods for deep extensions. They must also include the development of working systems so engineered as to contain and protect man *against* actual exposure to pressure, for work so deep in the sea that he could not tolerate direct exposure to the pressures involved. It is now necessary in this way to bring together the background of experience in basic and applied science, operations, economics and engineering.

Of special present importance to all concerned is that this field has emerged from a period in which the facilities available were in part responsible for limiting progress and consolidation of gains. This is no longer the case. In fact, relatively numerous pressure research systems already exist or are under completion to carry studies of men and animals beyond even the 2000-foot exposures accomplished, to 3500- and even 5000-foot pressure equivalents. Now, as actually at all times in the past, the real limitations are two-fold. Primary is the extreme shortage of dedicated and skilled investigators to man the existing highly specialized facilities, willing to accept the discomfort and hazard of such work. The second limitation lies in the distressfully slow and incomplete rate of transfer of existing and emerging information to those groups and agencies which do not generally and readily recognize the existence or significance of the large advances currently being made. Toward this end it now becomes necessary to assure that the ultimate user of information becomes himself a part of the advance, thereby aiding technological transfer. This must be the case whether the user is an industrial organization (as in offshore construction or oil exploration) or a federal agency (concerned with military functions or with national resources).

The advances from this Symposium onward, then, can be expected to further extend depth and duration of diving, to speed attainment of depth, and to accomplish the desired acceleration of decompression. As these occur it can also be expected that the many gains already accomplished will be consolidated. New approaches will provide means of tracking subtle or residual effects, and detailed attention to the application and training will permit full realization of the opportunities deriving year by year from research itself. By the time of the next Symposium the place of undersea research and operations should be firmly established.

C. J. LAMBERTSEN

Previously published symposia in this series (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, Publication 377, Washington, D.C., 1955; *Proceedings of the Second Symposium on Underwater Physiology,* National Academy of Sciences-National Research Council, Publication 1181, Washington, D.C., 1963; *Underwater Physiology: Proceedings of the Third Symposium on Underwater Physiology,* Williams & Wilkins, Baltimore, Maryland, 1967; *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.

ACKNOWLEDGMENTS

In this fifth step in a planned documentation of international progress and purpose in undersea physiology the Sponsors and Planning Group acknowledge the extensive contributions of many dedicated individuals to the success of the Symposium and the publication of its Proceedings.

Exceptional contributions were made by Mrs. Mary Eichhorn Fletcher who carried out the direct and difficult technical editorial and communications functions, bringing together the purposes and data of authors from many countries. Dr. Leonard Libber and Miss Suzanne Kronheim of the Physiology Branch, Office of Naval Research, continued to provide the purpose and support that has permitted this series to proceed without interruption over now many years. Dr. James Miller of the Office of Manned Undersea Science and Technology joined in planning and support for the program. Dr. Peter Bennett undertook arrangements for the accommodation and general interest of all attendees. Assistance with conducting the meetings was provided by Mrs. Eleanor Hopkin and Miss Nancy Struble. The graphic skills of Mrs. Mary Rosowski, Mrs. Betty Hanley and Mr. Ladislav Medved adapted many of the diagrams and illustrations upon which ready understanding of these chapters depends. Mrs. Judith H. Bianchi aided considerably in preliminary preparation and editing of the manuscripts. The exceptional, long service of these contributors is greatly appreciated by the editor who was so directly supported by their efforts, as well as by the Planning Group.

Part I. COMPREHENSIVE STUDIES OF PRESSURE AND DECOMPRESSION

PHYSIOLOGICAL STUDIES DURING A DEEP, SIMULATED OXYGEN-HELIUM DIVE TO 1500 FEET

J. B. Morrison, P. B. Bennett, E. E. P. Barnard and W. J. Eaton

Early in 1965, experiments were carried out at the Royal Naval Physiological Laboratory by Bennett (2), in which men were exposed to oxygen-helium at 600 feet for 4 hours and 800 feet for 1 hour during which tests were made of mental and manual efficiency. Decreases of 18% in the mental test of arithmetic ability and 25% in the Ball Bearing Test of fine manual dexterity were found at 600 feet which were twice as severe at 800 feet. These decrements were accompanied by dizziness, nausea, vomiting and a marked tremor of the hands, arms and even whole body which came to be called "helium tremors." However, in about 1½ hours the subject's condition returned to normal. Much speculation arose as to the cause, including a hypercapnia, hypocapnia and the noise, heat and rate of compression. It was considered on the basis of these results that man would probably be severely incapacitated at 1000 feet.

In the United States in 1965, the first simulated saturation dive with oxygen-helium was made to 650 feet for 48 hours (17) which was followed by an increasing number of simulated dives which gradually extended the depth limit. Among the notable saturation exposures in the range 600–825 feet were dives by Cabarrou et al. (12); Waldvogel and Bühlmann (31); Weybrew and Parker (32) and Schaefer et al. (29); Kelley et al. (18) and Bradley et al. (8). The first saturation dive to 1000 feet was achieved when a U.S. Navy dive was carried out at Duke University in December, 1968 (28).

The many detailed physiological, medical and psychological measurements that were made during these dives established that man could both live and work at these high pressures without significant disturbance. Problems such as helium tremors and nausea did occur but were found to be remarkably reduced by slowing the rate of compression. Thus in the 1000-foot dive at Duke University with a compression time of 24 hours, there were no tremors in any of the five subjects. This was considerably slower than the 100 ft/minute of the earlier British dives (2) or even the 60 minutes to 650 feet by Ocean Systems (17) in which mild tremors and dizziness were reported.

However, in June 1968, Brauer and Veyrunes carried out a record deep dive in a series by the COMEX organization of Marseille which was terminated after only 4 minutes at 1189 feet. The dive was abandoned, following a 2-hour compression, due to the onset at depths greater than 1000 feet, of a marked increase in slow wave theta activity (4–8 cycles/second) in the electroencephalogram (EEG), accompanied by helium tremors and "micro-sleep" involving an imperceptible transition between brief periods of somnolence and wakefulness

with right-left disorientation and difficulty in reading instrument dials (9). In earlier monkey experiments by Brauer, Johnson, Pessotti and Redding (10), a similar but more serious sequence of events was seen during deep oxygen-helium diving, entailing tremors, a period of somnolence and finally convulsions, and it was inferred on the basis of these studies and the June, 1968 experiments that convulsions could well be expected at about 1200 feet.

These signs and symptoms were labelled the "High Pressure Nervous Syndrome" (HPNS) and it was inferred that this constituted a "helium barrier" at about 1200 feet. Yet MacInnis, Dickson and Lambertsen (24) and Dossett and Hempleman (15) were able to expose mice and rats to over 4000 feet with oxygen-helium without convulsions occurring and concluded that the trembling and hypersensitivity could be controlled by very slow compression. Thus use of a very slow rate of compression should permit man to dive deeper than 1200 feet without serious difficulty or risk of convulsions.

Of even greater significance to the theory that the "helium barrier" may not prevent man from diving to very great depths was the human experiment in February 1969 carried out by a Swiss-British team in the Deep Trials Unit at RNPL which established, for three Swiss subjects, a world simulated wet diving record of 3 days at 1000 feet with 5 hours of underwater excursions to 1150 feet (11). Intensive physiological and psychological investigations were made from which it was concluded that there were no serious limitations to man performing effectively at this depth.

The present experiment was therefore planned and in March 1970 men were exposed to 1500 feet under very carefully controlled conditions (4). With thorough physiological, psychological and medical monitoring and special emphasis being given to EEG and tremor measurements, it was hoped to determine the etiology and importance of the HPNS when diving both very deep and at continental shelf depths in a saturated state.

Lanphier (21) and others (25, 26, 30, 33) have demonstrated the decrease in maximum voluntary ventilation (MVV) with increasing depth and have related the observed changes to relative gas density. From the available experimental data, Lanphier extended the predictive curve for oxygen-helium breathing to depths hitherto unattained. The curve suggests that MVV in the region of 80 liters/minute should be possible at 1500 feet. Maximum ventilation sustained at exercise, however, may be considerably less. Few experimental measurements have been made of the effects of deep saturation diving on respiratory functions. Dougherty and Schaefer (16) show a decrement of maximum inspiratory and expiratory flow rates on compression to 600, 800 and 1000 feet. Partial recovery of these parameters by some 23–44% was seen with increasing time at depth. This finding complicates any prediction of respiratory parameters in deep saturation diving. Their findings did not, however, indicate any respiratory limitations for the performance of moderate work at these depths (29).

At 600 feet Bradley et al. (8) showed respiratory responses at rest under moderate workload to be essentially unaltered. At a workload of 900 kg·m/minute, however, one of the two subjects showed a marked increase in oxygen consumption, carbon dioxide production and $P_{A_{CO_2}}$. Salzano et al. (28) made a detailed study of cardiorespiratory responses at a saturation depth of 1000 feet. Three subjects were investigated at workloads of up to 735 kg·m/min. Results showed a greater oxygen consumption than at surface, larger tidal volume, together with lower respiratory rate and ventilatory equivalent ($\dot{V}_E/\dot{V}_{O_2}$). Two of the subjects showed abnormally high $P_{a_{CO_2}}$ levels when performing the heaviest workload at a ventilation of up to 55 liters/minute and an oxygen consumption of just under 2 liters/minute.

As the present dive aimed at an eventual depth 30% greater than any previous saturation dive and in view of the possible problem of HPNS a workload of 300 kg·m/min, considered to be substantially below the expected maximum load, was used. This was found to represent an oxygen consumption of 1.1 liters/minute at a ventilation of 25 to 27 liters/minute at surface. It was intended that a quantitative analysis of respiration at rest and a moderate workload would establish a sound basis on which to examine higher workloads in subsequent experiments.

Preliminary experiments were made involving the exposure of subjects in the closed chamber without pressurization followed by exposure for 3 days to 40 feet and 24 hours at 100 feet, 300 feet and 450 feet to test the many vital life support systems and to familiarize both the subjects and scientific staff with the various techniques, equipment and measurements to be made. Since the 300-foot and 450-foot dives were carried out by the same subjects as the 1500-foot dive this enabled comparison of the physiological and medical data over a wide range of depths (4).

Life Support System and Dive Procedure

The dive took place in the dry chamber complex (Fig. 1) of 5.5 feet internal diameter consisting of a 260 cubic feet main compartment and a 105 cubic feet end lock (4). Maximum working pressure of the chamber is 69 atmospheres (2250 fsw). Chamber temperature was controlled by means of an electrical heating system consisting of tapes wound around the shell of the vessel and covered by an insulating fiber glass jacket. This enabled a comfortable temperature of 30°C to be maintained except during compression when 35°C was reached.

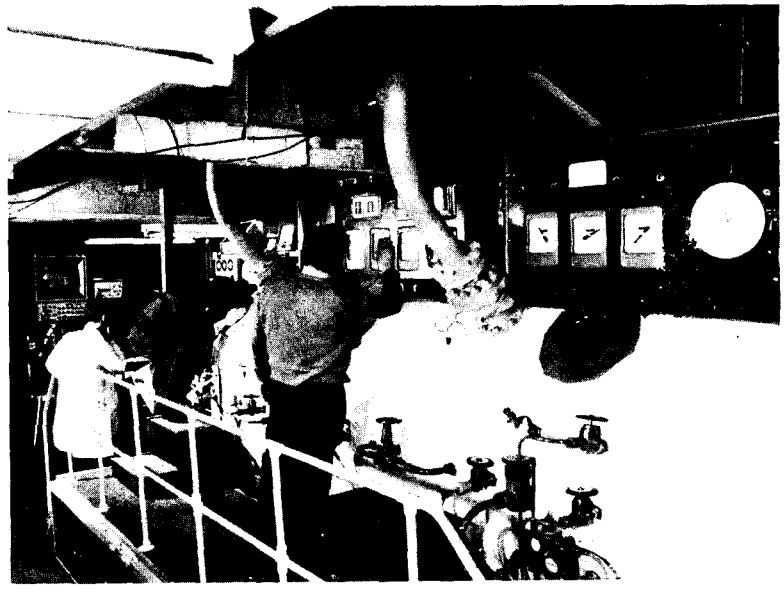

FIG. 1. The pressure chamber complex at the Royal Naval Physiological Laboratory, shown during the record dive to 1500 feet in March, 1970.

FIG. 2. The two subjects perform tests of manual dexterity. Subject PS (left) is carrying out the Peg Board Test and subject JB the Ball Bearing Test. EEG and ECG electrodes may be seen together with the umbilicals carrying the data to the recording apparatus.

Each compartment was fitted with a gland through which electrical communications were made. The glands had a total capacity of 14 screened and 48 unscreened leads which carried EEG, ECG, various transducer and communication signals to the instrumentation situated outside the chamber.

To remove carbon dioxide, the chamber atmosphere was drawn through soda lime canisters by means of two impeller fan motors driven by compressed air. Water vapor, ammonia and hydrogen sulphide were removed in a similar manner using canisters of silica gel and carbon granules. The carbon dioxide level in the chamber was maintained at less than 0.5% of 1 atmosphere at all times and relative humidity was held in the region of 70%–90%. The gas mixture specified for the dive was 45% of 1 atmosphere of oxygen (tolerance ±5%), less than 2% of 1 atmosphere nitrogen, and the remainder of the gas mixture, helium. The correct oxygen level was maintained by means of an oxygen injection system controlled by polarographic sensors. Gas composition was checked by gas chromatograph each hour and a continuous measure of oxygen percentage was available from the polarographic oxygen sensors. At no time during the dive was there any problem in connection with the life support system.

The two divers selected—J. B. and P. S. (Fig. 2), aged 26 and 20 respectively—were on the scientific staff of the laboratory and had previous simulated and actual diving experience. The dive profile is shown in Fig. 3. The dive took place in stages with major stops of 24 hours at 600 and 1000 feet, 22 hours at 1300 feet and 10 hours at 1500 feet—a depth 300 feet beyond man's previous experience. This pattern allowed extensive experimental data to be collected at intermediate and final depths. Compression to 600 feet and hence to 1000 feet was direct at 6 minutes/100 feet; from 1000 feet onward further short stages were intro-

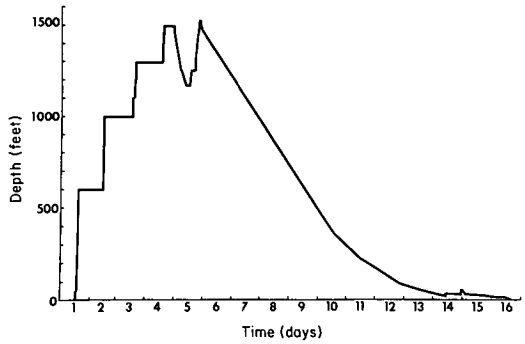

FIG. 3. Dive profile showing compression stages to 1500 feet for 10 hours and decompression. (March 3–18, 1970.)

duced with 1-hour stops at every 100-foot increment. During these stages basic physiological tests were made including EEG, ECG, respiratory rate, end-tidal CO_2 and performance tests.

A total of 3.5 days were spent in excess of 1200 feet, the depth at which the physiological barrier had been predicted. In the course of the dive helium tremors, frequency of the electroencephalogram, auditory-evoked responses, and mechanical and mental performance tests were measured for 22 hours. In addition urine was collected every 24 hours for electrolyte, stress hormone, and acidity analysis. A further 20 hours of measurements were made of respiratory function.

Psychological, Neurophysiological and Biochemical Tests

A battery of psychological, neurophysiological and biochemical tests (3, 6, 7) was applied at regular intervals.

The spontaneous electroencephalogram was recorded from stick-on electrodes at the vertex and occiput using a method of application which permitted noise-free recordings from the continuously attached electrodes over 15 days (6). On-line frequency analysis in the bands delta (2–4 hz), theta (4–8 hz), alpha (8–13 hz), beta 1 (13–20 hz) and beta 2 (20–30 hz) was achieved with a Nihon Kohden MAF 5 analyzer. Averaged auditory induced evoked cortical potentials from 64 "clicks" at 1/1.5 seconds were recorded with a Biomac 1000 computer and all EEG data recorded for later analysis both on paper and on FM tape.

Tremor of the hands was quantified by coupling the frequency analyzer to an SLE TREM 1 transducer attached to the middle finger of the right hand (7). Additional evidence for the presence of psychomotor involvement was obtained from the Ball Bearing Test in which the subject picks up ball bearings one at a time with forceps and places them in a tube; and also from the Purdue Peg Board Test which requires the assembly of small pegs and washers on a board. The Touch Test required the subjects, while blindfolded, to sort two sizes and textures of ball bearings. In the Wechsler Bellevue Digit Symbol Test (Visual Analogies Test) the subjects related symbols to sets of numbers as a measure of cognition; finally, the Arithmetic Test was used with two figure by one figure multiplication.

All urine voided each 24 hours was collected and analyzed for volume, corticosteroids, calcium, sodium, potassium, magnesium, phosphorus, chloride and net renal acid (3). Venous blood samples from the brachial vein were analyzed for hemoglobin, white cell

count and differential, hematocrit and platelets together with serum electrolytes and enzymes before and after the dive.

During compression the EEG alpha, beta 1 and beta 2 activities decreased while the theta and delta increased. This was more prevalent in subject P. S. than subject J. B. but was only detected from the frequency analyzer results: the raw EEG gave little or no indication of an increased theta activity (6).

On reaching stable pressure, analysis of the percentage change in the five common frequency bands of the EEG (delta, theta, alpha, beta 1 and beta 2) showed significant changes in the two subjects (6). Subject P. S. with eyes open or shut showed a dramatic increase in theta activity. At 600 feet, with eyes closed, there was in 6 hours a 25% peak increase in theta which returned gradually to normal (Fig. 4). Compression to 1000 feet again increased the theta activity to 75% above controls over the first 6 hours. After 12 hours this had decreased to only 20–35%. Compression to 1300 feet resulted—in a further 5–6 hours—in a maximum theta increase of 90%. After 12 hours this peak had returned to lower values. On compression to 1500 feet there was another increase in theta but the change was not as severe as at 1000 feet and 1300 feet. Other changes included a decrement in the amount of delta activity (2–4 c/s) until, during decompression, a vestibular syndrome occurred with dizziness and vomiting which resulted on the third day in a marked increase in delta activity. Other frequencies showed a reduction by over 30% at 1300 feet and 1500 feet. This reduction may be seen in Fig. 5 which compares the EEG and analysis at 50 feet with that of 1500 feet. During the vestibular syndrome this decrement was even worse, but on return to the surface the delta activity had returned to normal and there was a significant recovery of all activity other than theta which remained high.

In subject J. B. the percentage changes in slow activity were not as clear due to the presence of large amounts of delta and theta activity in the controls. However, the total theta activity was in fact greater in subject J. B. than in P. S. (Fig. 6).

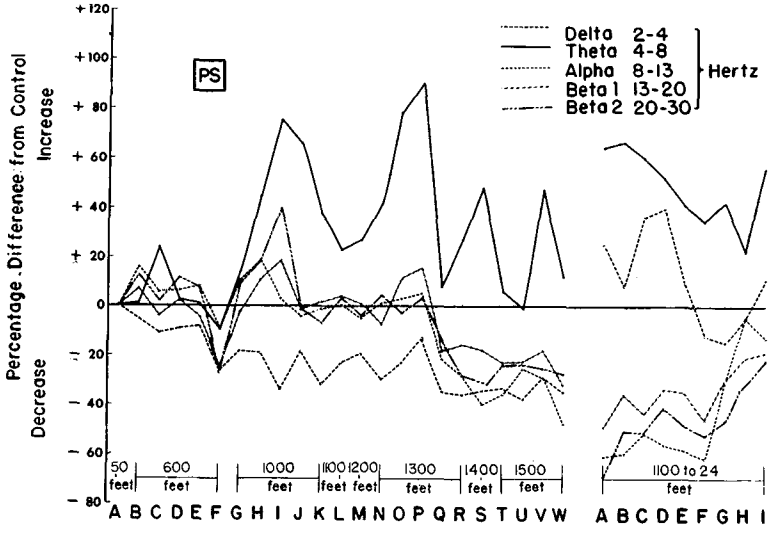

FIG. 4. Subject PS eyes closed; analysis of the percentage change in the spontaneous EEG activity bands at various stable stages of the dive, as compared with control measurement at 50 feet.

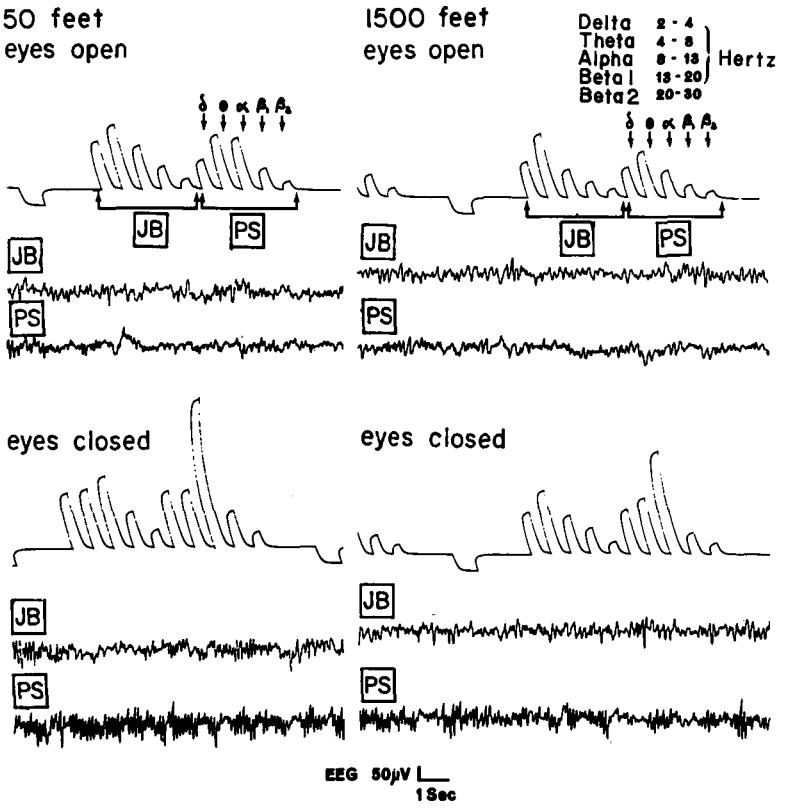

FIG. 5. Examples of the EEG and activity analyses in subjects JB and PS with eyes open and closed while breathing oxygen-helium at simulated depths of 50 feet and 1500 feet.

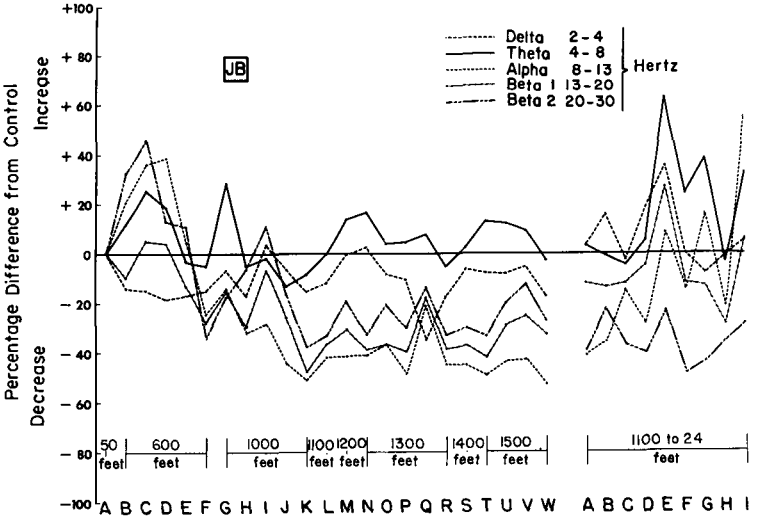

FIG. 6. Subject JB eyes closed; the analysis is the same as for Fig. 4.

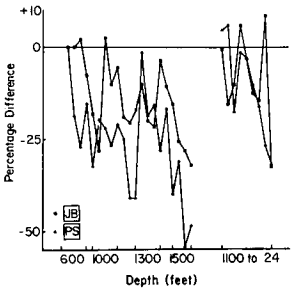

FIG. 7. Percentage change in the size of the N_1P_2 phase of the averaged auditory evoked cortical potentials in subjects PS and JB.

The auditory cortical evoked response (6) showed an average 15% decrement for J. B. and 25% for P. S. during most of the dive. At 1500, however, both men showed a progressive depression of the spike height of the N_1P_2 wave of the cortical evoked response (Fig. 7). After 10 hours at 1500 feet this resulted in a decrement of 30% for J. B. and 50%–55% for P. S. At the first decompression measurements 2–3 days later at 1100 feet, the evoked response had returned to normal.

The degree of postural tremor recorded by the transducer on the finger indicated significant tremors for J. B. but not P. S. (Fig. 8). The tremors were enhanced by each of the compression phases and were more prominent on waking each morning (7). The changes were in amplitude rather than frequency as the analyzer indicated similar changes in all activity bands. Subject J. B. showed tremors up to 600% of predive values but these increases soon settled to lower levels. More stable values indicated a progressive increase in baseline tremor with pressure accompanied by an intention tremor. Thus at 1500 feet considerable tremor and muscle jerk were evident when assembling the bicycle ergometer although the symptoms did not prevent ability to complete the work.

The results of the performance tests (7) indicated a decrement in the tests of manual dexterity, especially fine dexterity such as with the Ball Bearing Test and Peg Board Test. The worst impairment occurred immediately after compression and could be correlated with the incidence of tremors. Thus J. B. showed the greatest decrement, the peak reductions in the Ball Bearing Test (Fig. 9) coinciding with the peaks of tremor activity (Fig. 8). Subject P. S. in general showed less decrement in motor tasks. Tests of intellectual ability such as the Arithmetic and Visual Analogies Tests indicated no significant decrement in mental

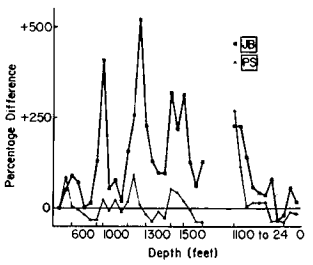

FIG. 8. Percentage change in the mean middle finger tremor during stage compression to 1500 feet with oxygen-helium for both subjects.

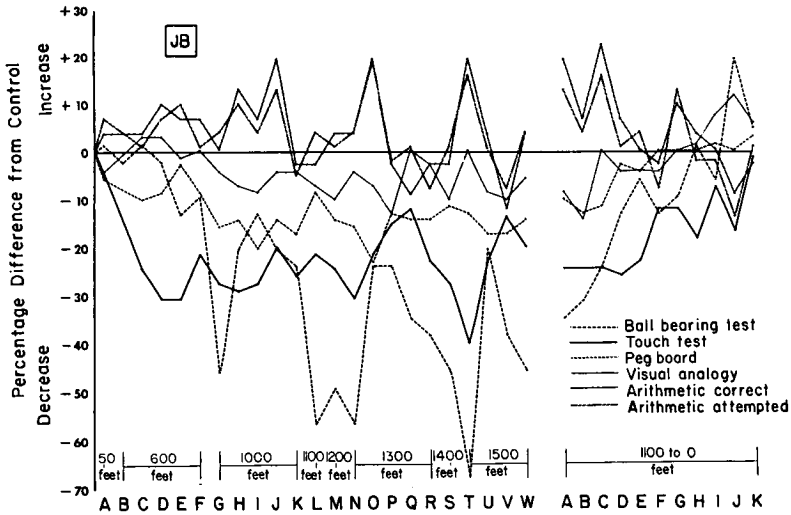

FIG. 9. Performance efficiency in subject JB during stage compression and exposure to 1500 feet with oxygen-helium and subsequent decompression.

tasks. Subjectively the men noted dizziness and nausea during the first 2 hours at 600 feet and again at 1000 feet.

The changes in urine electrolytes were similar to those found by Bühlmann et al. (11) during an earlier Swiss-British dive. Thus between 600 feet and 1500 feet there was retention of sodium (Fig. 10), calcium, chloride and, to a lesser degree, magnesium with a diuresis of potassium (Fig. 10) and phosphorus (3). The urine volumes (Fig. 10) did not change radically except for a low predive value for subject P. S. During decompression electrolyte equilibrium was reestablished after a rebound effect. Corticosteroid excretion was high in J. B. throughout the experiment and in P. S. during the vestibular syndrome in particular.

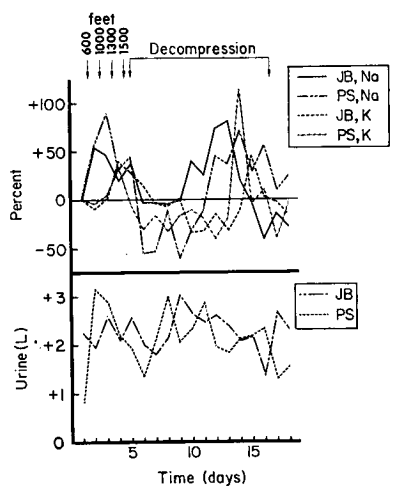

FIG. 10. Changes in 24-hour urine volume in subjects JB and PS and percentage change in their urine sodium (Na) and potassium (K) during the dive.

After the dive there were no significant changes in blood except for an increase in white cell count by 3000–4000 and a fall of platelets of 78,000 and 135,000 for P. S. and J. B., respectively.

The use of an on-line EEG frequency analyzer for the first time in such deep oxygen–helium dives has permitted classification of the EEG changes. They are of two types: one the increase in theta activity elicited by the compression, the other a reduction of overall electrical activity of the brain. The theta increase once started by compression continues for 6 hours but by 20 hours has returned to near normal levels. However, the overall electrical activity remains depressed as do the auditory evoked potentials and this merits further study.

The performance results support earlier hypotheses based on the electroshock work of Carpenter (13), and in-vivo studies of the penetration of lipid monolayers by inert gases, done by Bennett, Papahadjopoulos and Bangham (5), that helium will not adsorp to lipid membranes and is not therefore a narcotic like nitrogen and argon. The mental deterioration reported in the early British dives to 600 feet and 800 feet (2) was no doubt due to the rapid compression rate of 100 feet/minute as compared with the present 16–17 feet/minute. Zaltsman (34) has reported that rates of 30–60 feet/minute to 220–630 feet with oxygen–helium also caused impaired mental efficiency, memory and motor reflex without EEG changes.

It is relevant that confusion and tendency to somnolence occurred in J. B. at 1535 feet during the recompression to try to ameliorate the signs and symptoms of the vestibular decompression sickness in P. S. The pressure was increased in a series of short compressions and stages over about 8½ hours from 1160 feet to 1535 feet.

When it is considered that 20 hours at stable pressure are required for the EEG changes to resolve, failure to permit such adaptation may well result in severe disruption of the function of the brain, leading to confusion, "microsleep" or possibly even convulsions. Thus it would seem advisable to incorporate 24-hour stages in the compression phase of very deep oxygen–helium dives as well as a relatively slow rate of compression. Alternatively a very slow rate of compression indeed may be sufficient to permit enough adaptation for most practical purposes. A further alternative, based on work by Brauer (9, 10) and Zaltsman (34), is to add small amounts of a narcotic inert gas such as nitrogen or argon.

The most likely cause of HPNS and related psychomotor effects would seem to be the pressure itself. This is difficult to prove in mammals. However, work on newts and mice by Lever, Miller, Paton and Smith (23) together with earlier experiments by Kylstra, Nantz, Crowe, Wagner and Saltzman (20) and MacInnis, Dickson and Lambertsen (24) involving such experiments as hydraulically compressed fluorocarbon-breathing mice, appear to confirm the role of high pressure per se as the cause of the HPNS and not any pharmacological action of helium.

Finally, the reason for the retention or diuresis effect of deep oxygen–helium dives on urine electrolytes is not clear. Among possible factors considered are prolonged lack of exercise resulting in a retention of sodium, calcium etc. as reported by Cabarrou et al. (12) during a simulated 800-foot dive, hypercapnia (11), and stress or pharmacological action of helium (3). Schaefer, Carey and Dougherty (29) during dives to 800 feet and 1000 feet reported a rise in bicarbonate and urinary CO_2 which was believed to be due to hyperventilation with fluid shifts as a result of osmotic gradients of dissolved gases. In the present experiment there was a similar tendency to hyperventilation and decreased resting alveolar carbon dioxide. Further work is required, however, to determine the true cause.

Respiratory Function and Exercise

Measurements of respiration were made by Morrison and Florio (27) at rest and at a moderate workload of 300 kg·m/minute using a bicycle ergometer. Inspiratory gas flows were measured by a pneumotachograph and pressure transducer. The analog signal of inspiratory gas flow was recorded on magnetic tape and subsequently analyzed by computer methods to give mean tidal volume, V_T, inspired minute volume, $\dot{V}_I$, (BTPS), and respiration rate (4). The carbon dioxide content of mixed expired, end-tidal and chamber atmosphere gas samples was measured by gas chromatograph (Beckman GC2A). Heart rate of the subjects was also monitored. All measurements were taken with the diver positioned on the ergometer. The experiment was conducted at 600, 1000, 1300 and 1500 feet and also at surface breathing air.

When resting, both divers showed an increase in minute ventilation of about 2 liters at depths relative to surface measurements (Fig. 11). Values of ventilation measured at the various depths, however, did not show any significant variation. The corresponding mea-

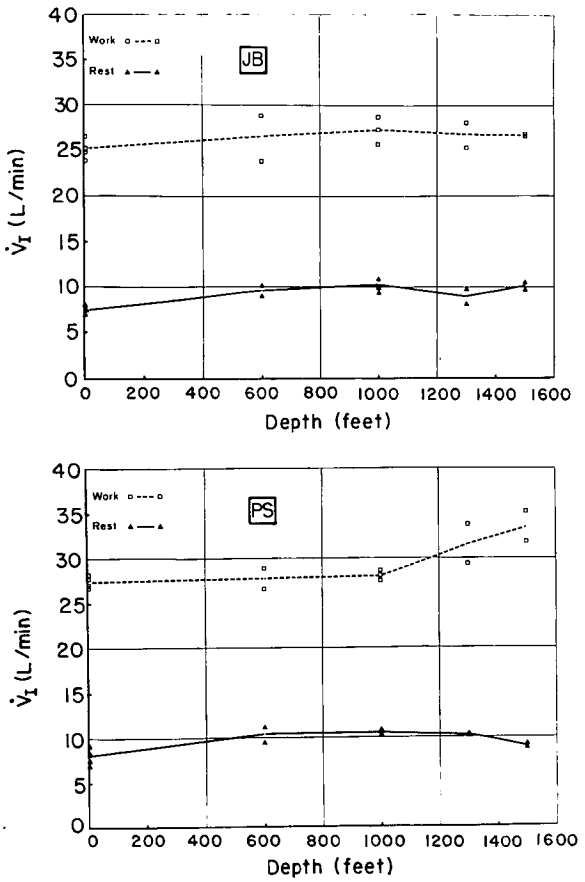

FIG. 11. Relationship of minute ventilation, $\dot{V}_I$, of the two divers, measured at rest and at a workload of 300 kg·m/min, to depth.

surements of end-tidal carbon dioxide (P_{ACO_2}) were as much as 10% lower than surface values. These results shown in Fig. 12 indicate that the divers may have been hyperventilating slightly when at rest. Several factors of the environment were altered from surface control conditions and could contribute to these changes: for example increase in oxygen partial pressure, temperature, thermal conductivity and degree of stress on the subjects.

When exercising, ventilations again showed a tendency to be increased under pressure but to a much lesser degree than at rest (Fig. 11). Corresponding end-tidal carbon dioxide measurements (Fig. 12) were unaltered or slightly greater than surface values. Only at 1500 feet was there any significant increase in either ventilation or end-tidal carbon dioxide partial pressure when exercising. There was no significant change in carbon dioxide production either at rest or exercise, except in one subject at 1500 feet where a marked increase was recorded during exercise. The relationship of ventilation to carbon dioxide production (27) indicated a slightly less efficient carbon dioxide elimination per liter of ventilation during the dive. This effect, however, could be largely accounted for by the greater inspired carbon dioxide (<0.5% of 1 atmosphere) in the chamber relative to atmospheric conditions. Calculations of alveolar ventilation and physiological dead space based on measurements of end-tidal and mixed expired gas samples, showed dead space to tidal volume ratio to be unaltered or increased under pressure despite higher tidal volumes. This suggests a possible alteration in the basic relationship of physiological dead space to tidal volume (27).

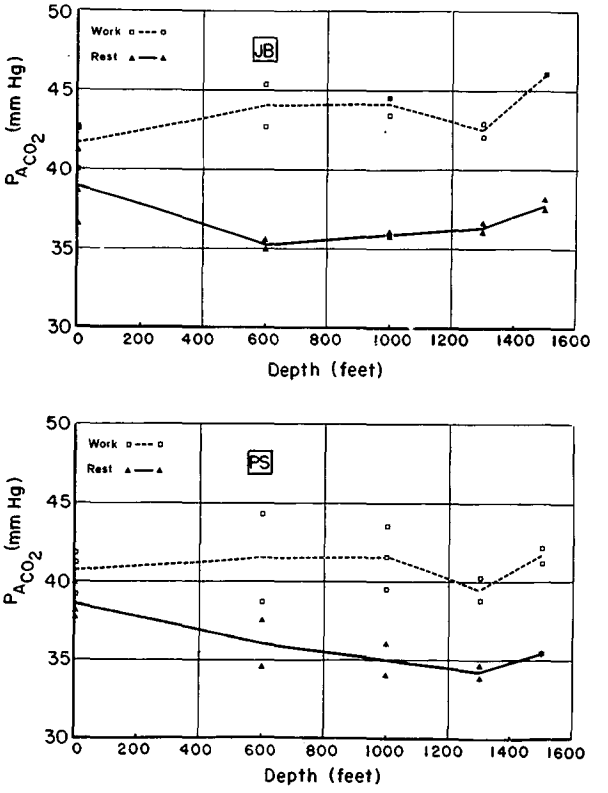

FIG. 12. Relationship of end-tidal carbon dioxide partial pressure, P_{ACO_2} of the two divers, measured at rest and at a workload of 300 kg·m/min, to depth.

In contrast to increases in ventilation, respiratory rates were on the average decreased during the dive, the effect being more significant when exercising and as depth increased. Corresponding tidal volumes were considerably increased under pressure (Fig. 13). When exercising at 1000 feet mean tidal volumes of both subjects were over 20% greater than at surface, compared with a 4%–10% increase in ventilation. The alteration in the relationship of minute ventilation to respiratory rate and tidal volume was expected and similar effects were shown in previous work by Salzano et al. (28) and Schaefer et al. (29). The changes most probably relate to mechanics of breathing a dense gas medium and are brought about to reduce the breathing effort, in particular when exercising. In Fig. 13 the slope of the relationship suggests a possible correlation with increasing gas density in the case of J. B. The data for subject P. S. shows a similar trend but is more scattered.

Throughout the dive there was evidence of mild bradycardia when resting, and a much greater effect during exercise. The bradycardia effect shown in Fig. 14 did not appear to increase, however, beyond 600 feet, suggesting that if bradycardia is a function of gas density or pressure, then the effect is of a nonlinear nature.

At higher pressures it became necessary to breathe through the mouth rather than the

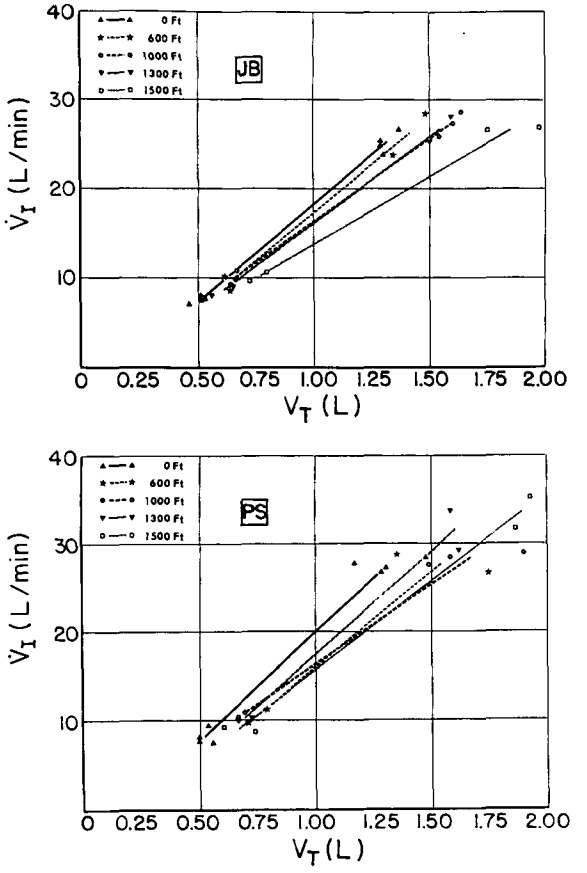

FIG. 13. Relationship of ventilation, $\dot{V}_I$, to tidal volume, V_T, at surface and under pressure.

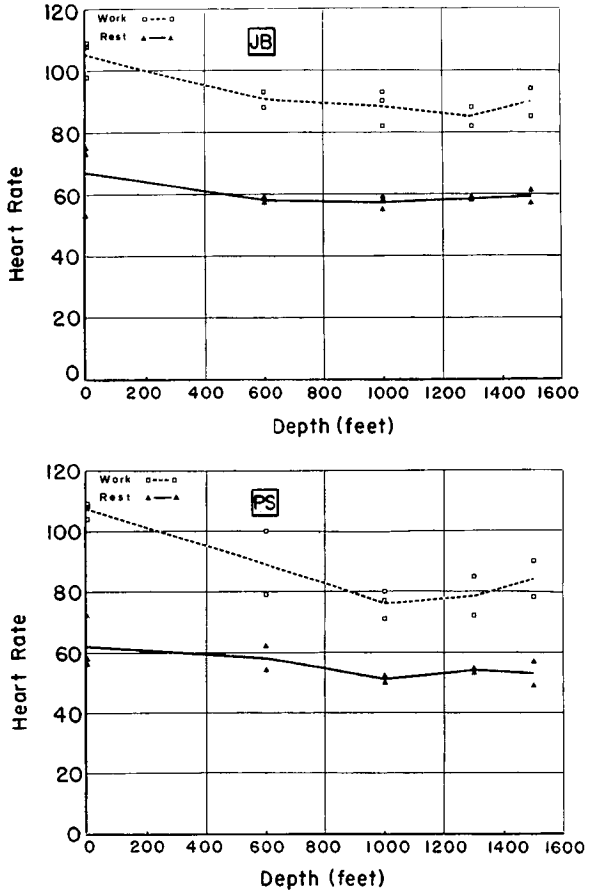

FIG. 14. Relationship of heart rate of the two divers, measured at rest and at a workload of 300 kg·m/min, to depth.

nose and temperature regulation became difficult—small variations from 30°C producing comments of too hot or too cold. It was the opinion of the subjects that the workload was well within their capabilities at all depths. At 1500 feet both subjects were conscious of an increased resistance to breathing and a considerable overheating effect during the exercise period. It was considered that although a higher workload could be achieved without undue stress, heavy workloads could not have been comfortably maintained.

Some clicks were noted at the knee joint by J. B. when pedalling the ergometer at 1500 feet. Similar clicks have been noted during oxygen-nitrogen dives to 4 ata (Morrison unpublished) but only at higher workloads, and the effect may indicate a change in the lubricant properties of the synovial fluid of the joint caused by fluid shifts due to compression (19).

In general the changes in respiratory function at rest and exercise were of a minor nature with little variation between 600 and 1500 feet. Furthermore, the respiratory responses measured at 1500 feet were of a similar nature to those established at 800–1000 feet by previous oxygen-helium saturation dives (28, 29).

Decompression

A treatment schedule for decompression sickness following oxygen-helium dives produced by Barnard (1) used a single exponential decompression. Its relative success, even after long and complicated dives which could be considered "saturation" dives, made it a natural starting point for the development of a schedule for 1500 feet.

The basic model was as follows: decompression was considered to lead to phase-separation of excess gas. If the rate of expansion of this gas exceeded its rate of elimination then decompression sickness would occur. By inference, this outcome indicated that the rate of ascent was too rapid.

The decompression profile was computed from the relationship

$$y = y_0 e^{-k.t} \tag{1}$$

where:

y_0 = maximum depth in feet
t = duration of decompression in hours
y = depth after time t
k = time constant.

Previous experience from as deep as 455 feet had shown a value for $k = 0.052$ to be satisfactory. In preliminary dives of this series this was again found to be so for an exposure of 24 hours at 100 feet, but unsatisfactory from 300 feet. Modification of the rate to $k = 0.04$ also failed to prevent mild decompression sickness following an exposure of 24 hours at 450 feet. The half-time of this decompression curve was 17.5 hours which was very close to that used by Chouteau et al. (14). Comparison of these results with the form of the decompression given by Bühlmann et al. (11) led to the adoption of $k = 0.028$ (half-time 24 hours) for the final dive to 1500 feet.

After an initial drop of 33 feet, decompression began at a rate of 40 feet/hour. At 1260 feet a state resembling Ménière's syndrome developed in one of the divers. The subsequent treatment of this case has been described by Leitch (22) but it is of interest to note that recompression was carried out to a depth of 1535 feet, the deepest known recompression treatment.

When decompression was begun again it was immediate from 1535 to 1500 feet and then at a constant linear rate of 10 feet/hour down to 300 feet where the original schedule was rejoined. Further symptoms were reported at 840 feet and again at 730 feet but these mild limb pains cleared by 645 feet. At 26 feet one diver admitted to pain in the right thigh, which he said had been present for 3 days and which subsequently became chronic and only cleared after surfacing. The planned decompression for this dive was to have taken 138 hours; in fact 260 hours were spent decompressing from 1535 feet.

The conclusions to be drawn from a single exposure are extremely limited but it would seem that the initial rate of decompression of 40 feet/hour from 1500 feet is too rapid for some individuals while the initial rate of 20 feet/hour employed by Bühlmann from 1000 feet was satisfactory, as was the subsequent rate of 10 feet/hour from 1535 feet. This latter rate would lead to a value of approximately 0.007 for the time constant of decompression using the model outlined. The disparity between the resulting decompression time at this

latter rate and the decompression in Physalie V (210 hours) as reported by Fructus (personal communication) leads to the conclusion that the relationship $y = y_0 e^{-kt}$ is not the optimum for the calculation of decompression from deep saturation exposures.

Conclusions

The physiological changes observed during the dive can be summarized as follows: There was no mental deterioration at depths as great as 1500 feet, but there was a decrement of psychomotor performance due to the presence of tremors. Helium does not produce an inert gas narcosis similar to that of nitrogen or argon. It is concluded that the high pressure nervous syndrome is an important hazard of deep oxygen-helium diving but that it may be ameliorated by slow compression and/or the use of staged compression. The cause of HPNS is apparently a combination of absolute pressure and rate of compression, and the use of compression stages of 24 hours permits some adaptation. During rest and performance of moderate exercise on a bicycle ergometer there were minor changes in respiratory function accompanied by a marked bradycardia effect but no significant respiratory problems. In general it can be concluded from the experiment that man can exist and work with reasonable comfort in an oxygen-helium environment down to depths of 1500 feet. That the basic philosophy of slow compression with stages as used in this experiment will permit extension of deep diving beyond the so-called "helium barrier" at 1200 feet has been endorsed since 1970 by further dives by the French to 1706 feet and 2000 feet.

Acknowledgments

The authors wish to acknowledge the assistance of A. Ferrier, D. Leitch, E. J. Towse, J. Florio and S. P. Gray who assisted with technical aspects of the dive, and also the two divers J. Bevan and P. Sharphouse. Administrative aspects were controlled by H. V. Hempleman, Superintendent R.N.P.L., under whose authority the dive took place.

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POSTPONING THE "HIGH PRESSURE NERVOUS SYNDROME" TO 1640 FEET AND BEYOND

X. Fructus, C. Agarate, R. Naquet and J. C. Rostain

In 1968, during an oxygen-helium dive carried out with a P_{O_2} of 0.6–0.7 ata and a compression speed of approximately 600 feet/hour, certain neurological and electroencephalographical disorders were observed. These disorders were given the name of High Pressure Nervous Syndrome (HPNS) (3, 5, 6).

This syndrome was characterized by the following: appearance of a tremor at about 700 feet, occurrence of drowsiness from 1000 feet onward, progressive onset of a dysmetria beyond 1050 feet, and EEG changes made up of theta activities occurring first over the temporo-occipital region in one diver (830 feet) and occurring over the anterior and mid-regions in both. The EEG also began to resemble a light sleep record rather than an "awake" recording (stage I).

Experiments with animals (4, 8) as well as with human beings (1, 2) have since confirmed the existence of the HPNS and its basic symptoms. These experiments have also enabled identification of certain conditions—notably the speed of compression—leading to its appearance.

Between November, 1970 and May, 1972 three experiments of very deep dives were performed (Physalie V, Sagittaire II, Physalie VI) using progressively improved compression curves.

Technique

Hyperbaric Complex

The dives were performed in the pressure chambers of the Hyperbaric Research Center at COMEX's headquarters in Marseilles, France. Three chambers were utilized. The first was a horizontal, cylindrical, lock-chamber having a volume of 4 cubic meters and equipped with sanitary facilities; the working pressure was 36 ata. The second was a vertical, cylindrical hydropneumatic chamber having a volume of 10.9 cubic meters; the working pressure was 36 ata. Only the upper portion of this chamber was used as a living room. The third was a horizontal, cylindrical chamber having a volume of 3.8 cubic meters which served as both sleeping quarters and test chamber between 1 and 36 ata. This was the only chamber utilized beyond 36 ata.

TABLE I

BREATHING MEDIA CHARACTERISTICS DURING THE DIFFERENT DIVES BETWEEN 1640 AND 2001 FEET

Dive	Physalie V	Sagittaire II	Physalie VI
Depth (meters)	520	500	610
P_{IO_2} (ata)	0.42	0.40	0.40
P_{IN_2} (ata)	<0.13	<0.13	<0.13
P_{ICO_2} (ata)	<0.003	<0.003	<0.003
H_2O% of saturation	75-85	40-60	30-50
Specific gravity at 31 °C (gm/L)	9.04	8.78	10.51

Each chamber was equipped with a medical lock enabling the passage of food and small articles, and with a control panel permitting the manipulation of gases.

The pressure in the chambers was monitored by a series of pressure gauges and by means of pressure sensors utilizing strain gauges. The reading was taken on a digital voltmeter (in meters).

Environmental control was ensured by a group of volumetric pumps which circulated the atmosphere through a series of filters and returned it to the chambers through a thermal exchanger which regulated the temperature.

The series of filters contained soda lime, silica gel, and activated charcoal: the first to eliminate CO_2, the second to eliminate humidity, and the third to eliminate hydrocarbons and heavy gases. By means of this system the various environmental parameters were maintained within the range of values given in Table I.

Oxygen was analyzed by means of a SERVOMEX "OA 137" or "OA 250" and galvanic oxygen partial pressure sensors. Carbon dioxide was monitored by means of an infrared absorption analyzer, the UNOR S2. In addition, these measurements were verified by gas chromatography (MICROTEK MT 150).

Hygrometry was measured by means of hair hygrometers and chromatography. Platinum temperature probes placed at various points inside the chambers permitted measurement of the temperature of the chambers.

COMPRESSION METHODS

The working hypothesis used for calculating the compression curves was based on the theory of osmotic dysbarism (7). This theory makes use of the existing disequilibrium between the saturation of inert gas in slow and fast tissues at the time of rapid variations in pressure. It was hypothesized that a limit of the differences of gaseous pressure may exist, below which osmotic dysbarism would cause difficulties related to HPNS.

If P is the depth, and T is the half-time of the tissue under consideration, and $G(P)$ is the gradient function chosen for this tissue, the compression speed is:

$$\frac{dP}{dT} = \frac{\log 2 \times G}{T(1 - G')} \tag{1}$$

where $G' = dG/dP$.

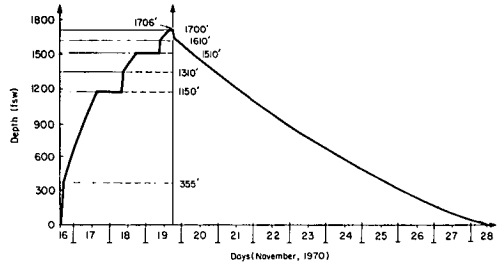

FIG. 1. Profile of the 1706-foot simulated dive: Physalie V.

For Physalie V (Fig. 1) the selected gradient was achieved by a fast compression phase of 200 feet/hour, down to 354 feet. Compression was subsequently slowed and interrupted by two 16-hour stops at 1150 and 1510 feet. To test their influence on the HPNS, two periods of rapid compression (200 feet/hour)—from 1150 to 1310 feet and from 1510 to 1607 feet—were conducted after the stops.

In the Sagittaire II experiment (Fig. 2) this theoretical compression curve was used uninterruptedly to permit a comparison of the two methods. The results obtained showed both the utility of stops at intermediary depths and the adverse effects of rapid compression at 200 feet/hour.

To verify these conclusions and to check the possibility of reaching greater depths, the following method was chosen for Physalie VI (Fig. 3): the permissible gradient at a given depth was reduced; all fast compression phases were suppressed, even the initial one from the surface; and a 2-day stop was effected at 1150 feet to reduce possible HPNS symptoms before proceeding deeper.

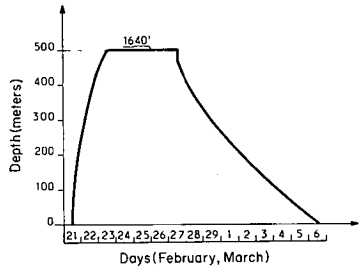

FIG. 2. Profile of the Sagittaire II dive (100 hours at 1640 feet).

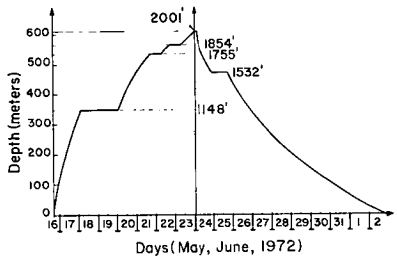

FIG. 3. Profile of the 2001-foot dive: Physalie VI.

TABLE II

DURATION OF THE VARIOUS PHASES OF THE THREE DIVES

	Compression	Stay	Decompression
Physalie V 1706 feet	74 hr 23 min	1 hr 17 min	210 hr
Sagittaire II 1640 feet	49 hr	100 hr	189 hr 30 min
Physalie VI 2001 feet	177 hr	1 hr 20 min	233 hr

In addition, two 14-hour stops were carried out at 1755 and 1854 feet to check the divers' condition at these hitherto unknown depths and to avoid compressing them during their sleep.

The duration of the different phases of the various dives is shown in Table II.

RECORDING TECHNIQUES

During these three dives, neurophysiological studies were carried out to measure the tremor, and to analyze the EEG activities during both sleep and wakefulness. The waking EEGs were recorded during both rest and intellectual and manual labor. Only the results obtained for the tremor and the waking EEG during rest will be given here.

The Tremor

The tremor was measured by means of a geophone placed on the middle finger of the right hand, the arm of the subject being stretched out in front of him (the "oath test"). To obtain the frequency and the average amplitude of the tremor, sequences of 17 seconds were recorded and processed by PDP-12 computer (Digital Equipment Corporation). For each test conducted during the dives, the amplitudes thus obtained were expressed in terms of percentage difference from the surface control values.

EEG Activities

The EEGs were recorded by means of Alvar capsulex electrodes stuck onto the scalp with Collodion (Physalie V) or by means of ECEM "fishhook" needles inserted into the scalp and held in place by a strip of gauze and Collodion (Sagittaire II and Physalie VI).

Four electrodes (frontal, central, mid-temporal and occipital) were used for Physalie V, and five for Sagittaire II and Physalie VI (the fifth in the mid-vertex region). The EEG could thus be recorded during the whole dive.

Three derivations were utilized for the EEG: fronto-polar/central, central/mid-temporal and mid-temporal/occipital. An additional derivation—the mid-vertex/occipital—was utilized for the visual evoked responses (Sagittaire II and Physalie VI).

To obtain the power spectra, sequences were recorded on magnetic tape during every test several times a day and subsequently processed on a PDP-12 computer. The values obtained by averaging several spectra at a given moment in each frequency band (delta, theta, alpha, beta) were expressed in terms of percentage difference from the average control values.

The average visual evoked responses were recorded by means of the mid-vertex/occipital derivation. They were averaged by means of an ENHANCETRON 1024 or Intertechnique DIDAC 800 computer. Light stimuli were sent out at a rate of one every 1.2 seconds by means of an Alvar stroboscope, the specially equipped lamp being placed inside the chamber.

Results

Clinical Analysis

The Tremor

Physalie V. During the Physalie V dive, the finger tremor appeared between 1000 and 1150 feet (Fig. 4) and reached 200% during the stop at 1150 feet. The tremor did not increase during compression from 1150 to 1510 feet but increased very rapidly after compression (200 feet/hour) from 1510 to 1607 feet. It attained values of 400–600% between 1640 and 1706 feet. During decompression the tremor decreased and above 1000 feet returned to almost normal. The frequency of this tremor ranged between 8 and 12 cps and was usually 10 cps.

Sagittaire II. During this dive finger tremor appeared toward 1000 feet (Fig. 5) and increased during compression to about 400% at maximum pressure. The tremor's average

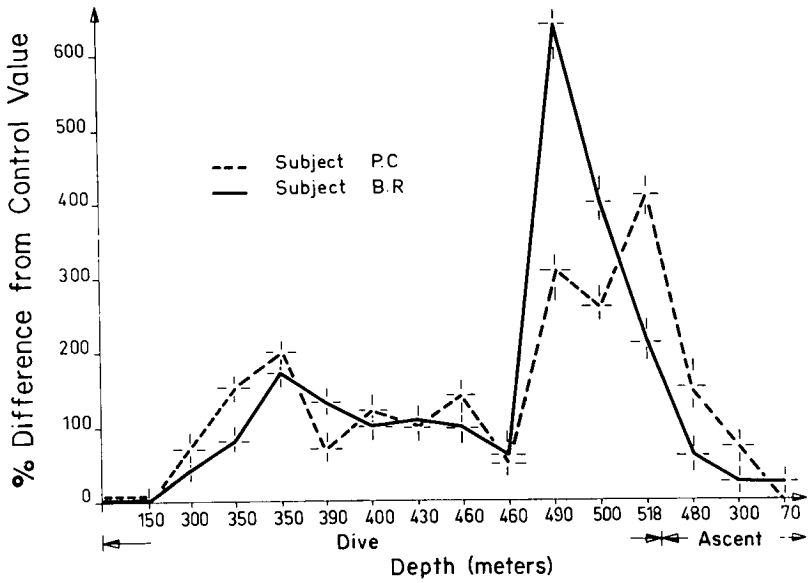

FIG. 4. Evolution of finger tremor as a function of the depth during the Physalie V dive.

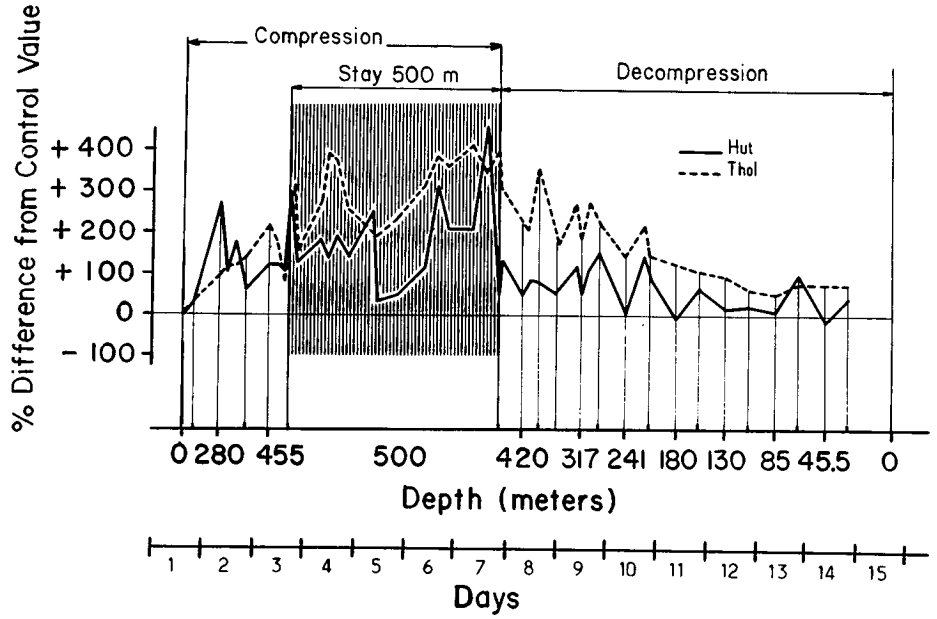

FIG. 5. Evolution of finger tremor as a function of the depth during the Sagittaire II dive. The shaded portion represents the stay at 1640 feet.

amplitude remained high during the first day at 1640 feet. It then decreased between the second and third day and increased again during the fourth day. During decompression, the tremor decreased more or less regularly in both divers.

Physalie VI. During the Physalie VI dive the finger tremor increased in both divers between 650 and 1000 feet from the second day (Fig. 6). During the stop at 1150 feet and during the morning of the first day it reached an amplitude of 150%. It then decreased and finally disappeared the evening of this same day to reappear on the divers' waking on the second day while still at the stop at 1150 feet. It disappeared once again toward the end of the day.

During compression from 1150 feet to 2000 feet, the tremor reappeared. Its amplitude was always more pronounced in the morning. On arriving at 2000 feet it was slightly greater than at 1150 feet until it reached approximately 250%. The tremor disappeared very rapidly during decompression. In each case the frequency of the tremor ranged from 8 to 12 cps.

Other Clinical Observations

During these three dives, various disorders were observed. Apart from the static tremor, spasmodic muscle jerks appeared. These were situated in the extremities of the limbs but tended to include the upper parts of the limbs with increased pressure (1640 feet).

Sleep was also disturbed and often interrupted by wakings, especially beyond 1500 feet. The divers reported having numerous dreams which also had particular characteristics, e.g., levitation.

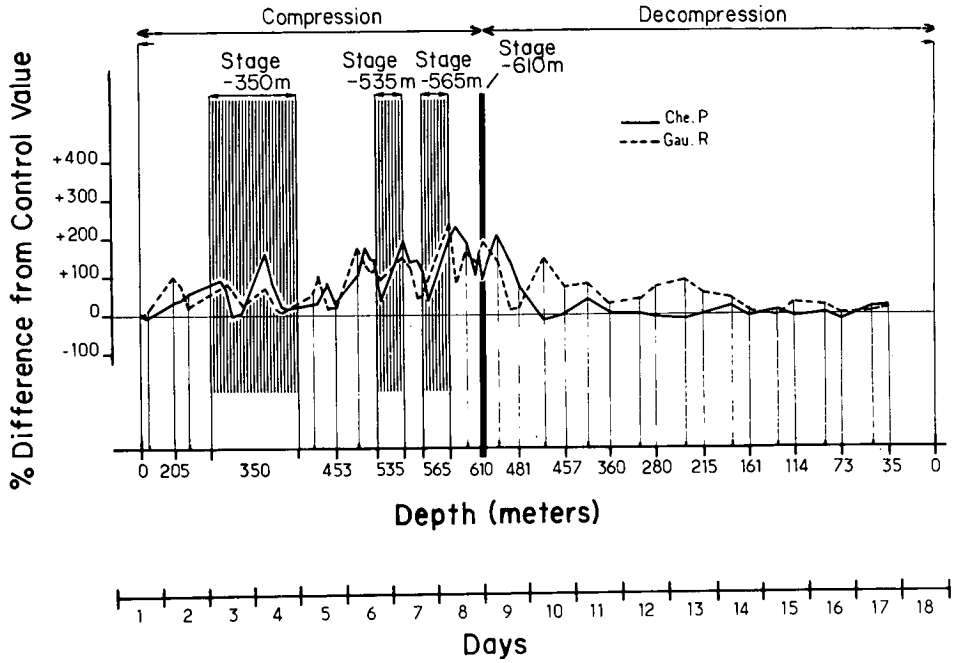

FIG. 6. Evolution of finger tremor with depth during Physalie VI dive.

Occasionally during the day, the subject became drowsy during the rest period; however, this was easily overcome by external stimulus. This phenomenon was particularly noticeable during the Sagittaire II dive beyond 1000 feet but was of little note or nonexistent during the Physalie V and VI dives.

ELECTROENCEPHALOGRAPHICAL ANALYSIS

Physalie V. The changes appeared between 1150 and 1300 feet, and in both divers they consisted of activities of theta frequency occurring over the anterior or mid-regions.

When the eyes were closed, the EEG recordings at the surface level showed alpha activity at 10 cps for diver A and 10.5 cps for diver B. In the latter, short bursts of theta activity (5-6 cps) were observed in the frontal region. During compression theta frequencies increased between 1150 and 1300 feet. These changes increased after rapid compression from 1500 to 1600 feet and became almost permanent in the anterior and central regions, although their amplitude remained low. At 1706 feet theta activity did not increase noticeably, while alpha activity was slightly less pronounced. These changes decreased during decompression in both divers.

The power spectra of the EEG activities confirmed, defined and quantified these results. Thus, in diver A, increased theta activities were observed from 1000 to 1150 feet in the anterior and central regions (Fig. 7).

This increase was accompanied by an increase in delta frequencies, also in the same areas. Maximum values of 800% were reached between 1510 and 1706 feet. Depending on the

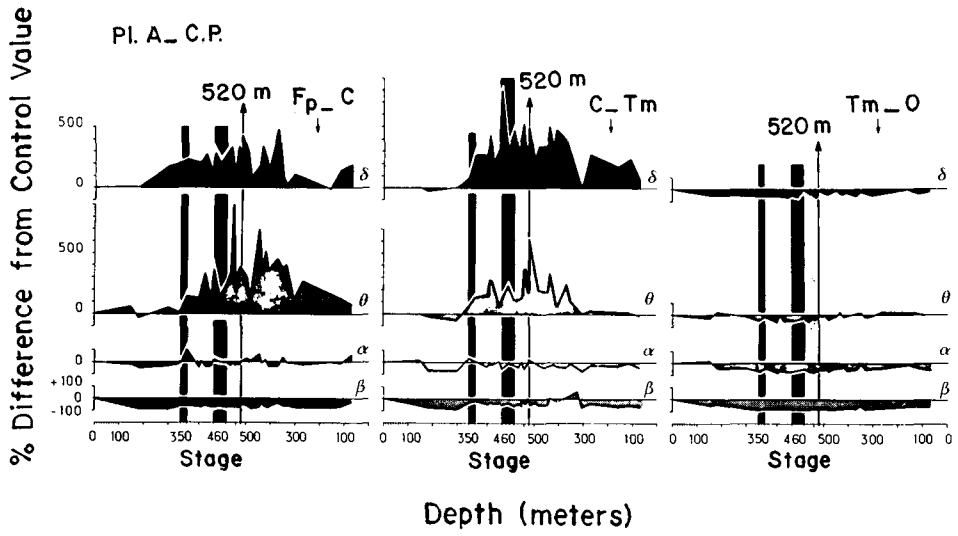

FIG. 7. Evolution of EEG frequencies power at increasing depths in diver A (Physalie V). Three derivations were utilized for the EEG analysis: *Left*: Fp-C, fronto-polar/central; *center*: C-Tm, central/mid-temporal; *right*: Tm-O, mid-temporal/occipital. The frequency bands were analyzed separately from top to bottom: 0-4 cps (delta); 4-7 cps (theta); 8-13 cps (alpha); 14-22 cps (beta). The evolution of the power is represented from left to right for each frequency band and for each derivation. The black vertical columns represent the stops. Increased delta frequencies occurred beyond 1000 feet and increased theta frequencies occurred beyond 1150 feet. These increases, shown in the anterior and middle derivations for both frequencies, reached maximum values between 1500 and 1706 feet. Depending on the derivation, the power of the other frequencies (alpha and beta) diminished slightly or remained unchanged. During decompression, the power values of the delta and theta frequencies remained great up to 1300 feet and then decreased. The power of the other frequencies remained low up to the end of decompression, when it started to return to normal.

derivations, the strength of the other frequencies (alpha and beta) decreased slightly or remained unchanged. In diver B an increase in the strength of the theta frequencies was observed in the centro-temporal region between 650 and 1000 feet and in the temporo-occipital region below 1000 feet. These phenomena did not decrease during the stops at 1150 and 1510 feet; on the contrary, they continued to increase and reached a maximum of 800-1000% between 1510 and 1706 feet. On the anterior derivation the increase remained slight (100%).

The modifications in the power of the other frequencies varied greatly: they either decreased (anterior derivation) or increased slightly (posterior derivation).

In both subjects the modifications decreased more or less regularly above 1300 feet. Only the alpha and beta frequencies remained slow until the end of decompression, when they started to return to normal.

Sagittaire II. The first EEG changes occurred between 1000 and 1050 feet. They resembled tracings of drowsiness and appeared very shortly after the subjects closed their eyes.

The EEG recordings done at surface level of both divers (A and B) were characterized by the appearance at eye closure of an abundant (50 μV), continuous alpha activity at 10 cps which was accompanied by a rhythmical activity at 9-10 cps in the frontal region.

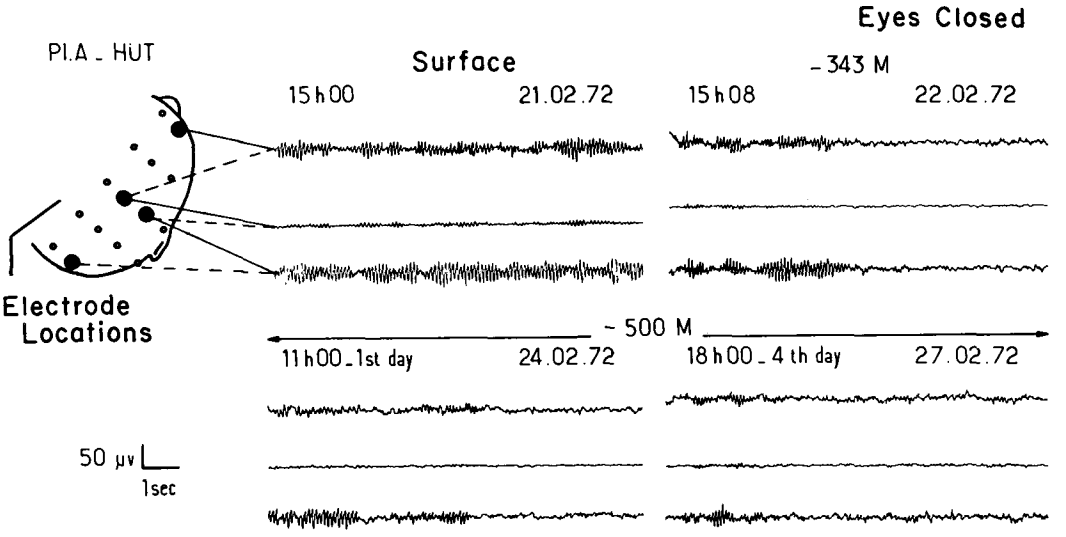

FIG. 8. Diver A's EEG recording (Sagittaire II). At surface level with eyes closed, the tracing showed continuous, abundant (50 μV) alpha activity at 10 cps, accompanied by a rhythmic activity at 10 cps. At 1640 feet on the first day, several bursts of 6 cps, low amplitude theta activity appeared, as seen especially in the central/mid-temporal derivation. At 1640 feet on the last day, the changes became more pronounced; the tracings resembling sleep recordings were more numerous and appeared more frequently. Anterior activities also increased. These are diver A's recordings; this subject showed less pronounced changes than diver B.

Beyond 1000 feet in diver B and 1050 feet in diver A (Fig. 8), the alpha activity, which appeared at eye closure, was replaced after a few seconds by changes resembling stage I of sleep recordings. This decreased level of vigilance remained unchanged during the entire compression in diver A, while it became more accentuated in diver B. In addition in diver B, the theta activities increased beyond 1300 feet, whether his eyes were open or closed. This increase in theta activities was scarcely noticeable in diver A at 1640 feet.

During the stay at maximum pressure, these disorders persisted and were always more pronounced in diver B. These modifications decreased during decompression, although the decreased level of vigilance persisted a long time. In diver B it only disappeared 12 hours after leaving the compression chamber.

Physalie VI. The EEG recordings at the surface level of the two divers were characterized by the appearance of extensive, continuous alpha activities at eye closure; (in diver A: 10 cps, 50 μV; in diver B: 10–11 cps, 50–75 μV). This activity was accompanied by a rhythmical activity at 10 cps in the frontal regions and in diver A was interrupted by a few bursts of theta activity at 6 cps.

The first EEG changes appeared at 900 feet. In diver A they were characterized by increased fronto-central theta activities at 6 cps and decreased amplitude of the alpha activities. In diver B only decreased alpha activity was observed. During the stop at 1150 feet, these modifications persisted the first day but became less pronounced on the second day. During compression from 1150 to 2000 feet, these changes increased again beyond 1300 feet, where the first bursts of theta activity occurred in the frontal region in diver B.

The middle and anterior theta waves reached a high level, particularly beyond 1850 feet. At the same time a slight decrease of vigilance (i.e., tendency for drowsiness to occur) was observed. These phenomena were more pronounced in diver A. In diver A anterior and middle theta activities were pronounced at 2001 feet and alpha activity was still present at eye closure but was much less abundant. In diver B, the anterior and middle theta activity remained slight compared with that observed in diver A. Although still present, the alpha activity was much less extensive and less stable than at the surface level. The decreased level of vigilance persisted in both divers.

These changes decreased during decompression: drowsiness disappeared at about 500 feet while the other modifications disappeared between 650 and 300 feet.

The power spectra of the EEG activities showed increased strength in theta activities. In diver A, this took place at 900 feet and occurred in the anterior and mid-regions (Fig. 9), while in diver B it took place at about 1000 feet and occurred primarily in the frontal region. This increase remained relatively slight and even tended to disappear during the stop at 1150 feet.

These changes increased once again during compression from 1150 feet to 2001 feet. The increased strength of the theta activities was accompanied by increased delta activities (ante-

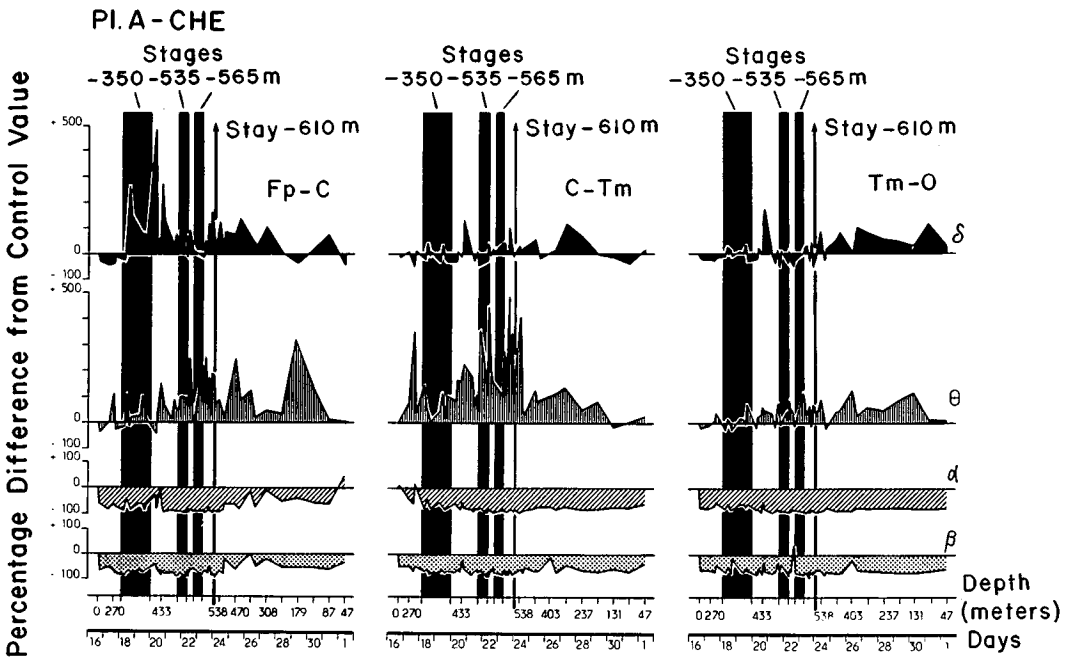

FIG. 9. Evolution of power spectra of EEG activities in relation to diving depths in diver A during the Physalie VI dive (same graphic presentation as for Fig. 7.) At 900 feet the power of the theta activities increased in the anterior and, especially, mid-(C-Tm) regions. This increase remained relatively slight during the stop at 1150 feet and even tended to disappear. At the same time, increased delta frequencies appeared in the anterior region. During compression from 1150 to 2001 feet, the power of the theta and delta activities increased and reached values of 400–500% for theta activities and of 100–300% for delta activities at 2001 feet. At the same time, alpha and beta activities decreased in all derivations. During decompression, the recordings began to return to normal at about 1300 feet and were normal at about 500 feet, except for the rapid activities which remained slightly decreased.

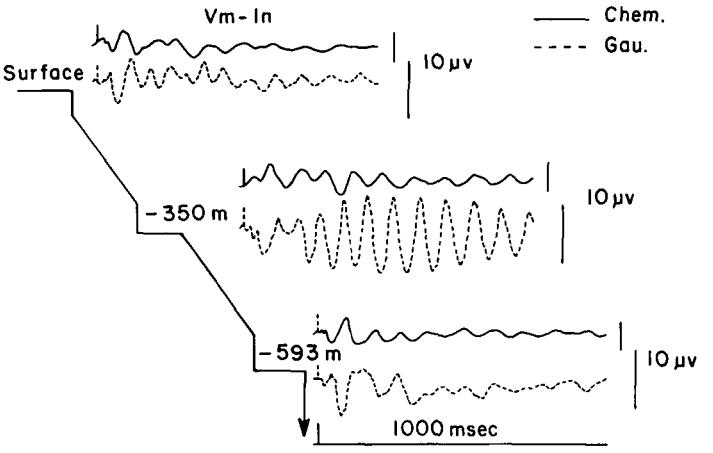

FIG. 10. Evolution of visual evoked responses during the Physalie VI dive.

rior region in subject A; central region in subject B) and by decreased strength of alpha and beta activities. It should be noted that beyond 1750 feet the changes were more pronounced in the morning upon waking than during the rest of the day.

Between 1850 and 2001 feet, differences of 400–500% for theta frequencies and of 100–300% for delta frequencies were observed. During decompression the high percentage of delta and theta waves and the low percentage of alpha and beta activities persisted until about 1300 feet; at this depth the activities began to return to normal.

Return to normal occurred in diver B at about 650 feet and at about 500 feet in diver A. Exceptionally, in diver A the high frequency bands remained slightly attenuated until he left the chamber.

EVOKED RESPONSES

The visually evoked responses recorded during Sagittaire II showed in both divers an increased amplitude for the majority of the components (especially waves III, IV and V).

This phenomenon was again observed during Physalie VI, but only beyond 1650 feet (Fig. 10). In fact, during the first phase (between the surface and 1650 feet), the amplitude of the various components of the evoked response decreased slightly and was accompanied by an increased rhythmical post-discharge. During the second phase (beyond 650 feet) the phenomena were reversed: the amplitude of the various components of the response increased, while the rhythmical post-discharge became less abundant and even disappeared. Its frequency decreased by 1 to 1.5 cps, as did the alpha frequency. The visually evoked responses tended to return to normal toward the end of decompression.

Discussion and Conclusion

Both clinically and electrophysiologically the changes generally attested to the considerable improvement of the compression method used for Physalie VI as compared with that used

for Physalie V. The results show that fast compression phases and no stops are equally important factors contributing to disorders.

Clinically, whatever the compression method used, the tremor always appeared at about 1000 feet and reached more or less similar values (100%) at 1150 feet. This seems to indicate that either the first compression phase should still be improved or that the appearance of tremor is independent of the speed of compression.

On the other hand, during the second compression phase, from 1150 feet to maximum pressure, the tremor values varied greatly and seemed to be more related to the method of compression than to the depth. Actually, due to slow compression with intermediate stops, the tremor was less important at 2001 feet than for dives carried out to 1640 and 1706 feet (200% as compared with 400% and 700%).

The EEG changes, which consisted of an increased number of slow frequencies and a decreased number of high frequencies (phenomena observed by Bennett and Towse [2]), also seemed to depend on the method of compression.

Thus, the EEG changes observed at 2001 feet were less extensive or, at the most, equal to those observed in Physalie V at 1706 feet. On the other hand, for Sagittaire II in which more rapid compression without stops was utilized, the disorders observed at 1640 feet were by far the most abundant. However, they did not reach the level attained during the Physalie III dive to 1200 feet (3, 5, 6).

Moreover, as observed in the EEG recordings, the decreased level of vigilance was much more noticeable in dives where continuous compression was conducted (Physalie III and Sagittaire II). However, the almost total absence of any decrease in disorders during the 100-hour stay at 1640 feet shows that the method of compression is not the only contributing factor.

These experiments have also shown the important role that individual factors play in the appearance of the HPNS. In addition, the changes in visually evoked responses differ in many ways from those observed in the auditory evoked responses (2).

It must be pointed out that, in spite of the various clinical and neurophysiological disorders present, the two subjects were able to perform their work and psychometrical tests equally well during the dives as at the surface level.

Two important conclusions can be drawn from the results of these experiments:

1) It is possible to delay or attenuate the appearance of the HPNS by using a carefully studied compression curve which enables the divers to adapt themselves progressively to high pressures.

2) The complexity of the phenomena leading to the appearance of the HPNS has been clearly demonstrated by the prolonged stay at 1640 feet and by the aggravation of the disorders from 1750 feet in spite of a slow compression interrupted by stops.

ACKNOWLEDGMENTS

This work was supported in part by the CNEXO and in part by the DRME (grant numbers 70-34-297 and 71-34-342).

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COLLABORATIVE INVESTIGATION OF LIMITS OF HUMAN TOLERANCE TO PRESSURIZATION WITH HELIUM, NEON AND NITROGEN. SIMULATION OF DENSITY EQUIVALENT TO HELIUM-OXYGEN RESPIRATION AT DEPTHS TO 2000, 3000, 4000 AND 5000 FEET OF SEA WATER.

*C. J. Lambertsen**

This report concerns the third in a series of "Predictive Studies", conducted by collaborating investigators of several disciplines and different laboratories, brought together in common purpose to explore the ultimate limits of human capability in the deep undersea environment (14, 21, 25, 28). It will provide the purposes and integrated scope of related investigations being reported separately in greater detail elsewhere in this volume (16, 19, 26, 31).

The Predictive Studies Series represents a planned effort to define and to examine quantitatively the influences of the major stresses imposed by undersea work at extreme depths. These include the *physical* limitations upon pulmonary ventilation, the *pharmacological* effects of narcotic inert gases, the *physiological* effects of altered body temperature, the *biophysical* consequences of increased hydrostatic pressures, and the *composite* changes induced by these different stresses in the systems for respiratory control, pulmonary function, purposeful exercise, and intellectual performance. In conducting the Predictive Studies it is conceived that these stresses and their effects upon fundamental life processes must all increase with progressive increases in ambient pressure, that each may be masked by compensatory processes or adaptations, and that each compensatory mechanism must have its own limits.

It is also recognized that no true limit for human tolerance to compression has yet been demonstrated. However, in searching for the ultimate limits of human tolerance to the undersea environment, it must be considered inevitable that, when using helium or any other respirable inert gases with oxygen at increasingly greater depths, pressures will indeed be reached which will eventually produce intolerable physiological decompensations. As pressure is increased, these decompensations will involve failures not only of respiratory-pulmonary function but also of mental ability, physical work capacity, neuromuscular coordination and other critical functions. In designing studies to search out such failures it must be anticipated that these several types of derangements and any adaptations to them

*The author, in presenting for this Symposium part of the results of a collaborative study, represents his direct co-investigators in the project.

will not occur at the same time or to the same degree at any particular high pressure. They must therefore be tracked individually and over continuous periods of time.

To determine the ultimate limits for effective human undersea activity requires decompression as well as the initial compression. This requirement for slow decompression from prolonged or saturation exposures to gases at high pressure imposes an exceptionally important handicap to the conduct of "limit" studies since, as physiological, performance or other difficulties are eventually encountered at great depth while breathing even a low density gas such as helium, the abnormalities will probably persist throughout the high pressure exposure, including the multiday periods required even for the initial stages of a "saturation decompression". This requirement for slow decompression means that it will not be possible to escape rapidly from or relieve the very stresses which generated a limiting dysfunction or even a damaging effect. Then, during such a period of continuous, unrelievable stress, additional deteriorations could occur to further aggravate changes initially induced by the original exposure to extreme pressure (25). Physiologically stressful exposures from which a requirement for slow decompression prevents ready escape could, therefore, have far more serious consequences than exposure to more severe, brief, acute stresses under more readily reversible circumstances.

The study to be described, "Predictive Studies III," uses different inert gases to separate major variables in undersea physiology. It has determined the influences of nitrogen, neon and helium, each alone and superimposed upon a high ambient hydrostatic pressure, and by use of dense gases in respiration has simulated exposure to the high density of a helium-oxygen atmosphere at extreme ambient pressures, beyond the limits of any existing environmental chambers. This intensive study of effects generated by the denser gas nitrogen at relatively low ambient pressures, and by neon at intermediate pressures, allowed investigation of the effects to be expected from influences of increased respiratory work at extreme depths. By use of dense gases at high ambient pressure, in an ambient environment of helium, effects of extreme density could be determined under conditions separable from the potentially hazardous influences of extreme increase in hydrostatic pressure itself, over an ambient pressure range which has already been demonstrated to be safe for extended periods of time, and where a safe escape from a decompensated state should be possible by simply returning to an ambient helium atmosphere on substituting it for nitrogen or neon.

"Dose-Response" Concept—Specific Conditions and Physiological Stresses

Design of the overall study was aimed at controlled, "dose-response" investigation of the dominant stresses conceived to be associated with exposure to nitrogen, neon or helium, and the combination of nitrogen or neon with helium at increased pressure. These stresses included:

1) Increased respiratory resistance and work of breathing, due to greater density of respired gas. Effects of changes in gas viscosity upon pulmonary function.

2) Diminished capacity for exercise, due to airway resistance, increased work of breathing and respiratory muscle fatigue, interference with alveolar ventilation or interference with intra-alveolar diffusion of oxygen or carbon dioxide.

3) Narcotic effects of inert gases, leading to decreases in sensory acuity, intellectual function, motor coordination and manual dexterity. Exaggeration of inert gas narcosis by carbon dioxide retention, expected in exercise at extreme gas density.

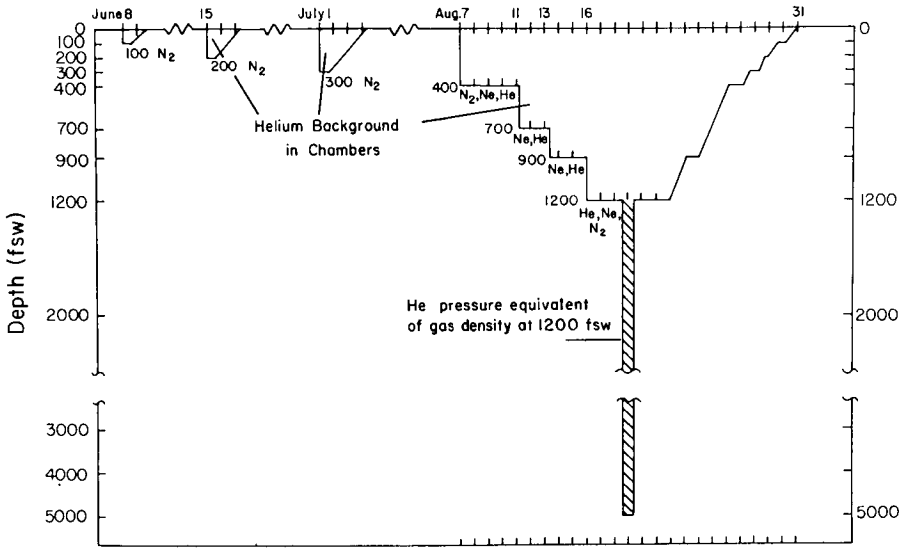

FIG. 1. Pressurization-exposure profile for series of dose-response exposures to N_2, Ne and He. Actual maximum pressure reached was equivalent to 1200-foot depth in sea. Shaded downward extension represents respiratory gas density equivalent to helium breathing at 2000, 3000, 4000 and 5000-foot depths, simulated by administration of N_2 or Ne with He at the stable 1200-foot pressure equivalent.

4) Alterations of body fluid volumes associated with increased ambient pressure.

5) Development of tremor or other neurophysiological effects associated with compression, and modifications of such neurophysiological effects during exposure to high partial pressures of inert gas.

The principal aims of the overall study were to provide for: quantitative measurement during exposure to each inert gas at each of a sequence of increasing pressures of the gas (the dose-response study); cross-related interdisciplinary investigations (respiratory-pulmonary, metabolic, neurophysiological, chemical, physical, cognitive, psychomotor); and direct, comparative study of the effects of three different inert gases (N_2, Ne, He) upon the same physiological functions under equivalent environmental conditions in the same subjects.

To accomplish the purposes of dose-response investigation, a group of normal men was systematically exposed to nitrogen with natural oxygen at ambient pressures equivalent to 0, 100, 200, 300 and 400 feet of sea water, and also exposed to helium and crude neon* at 0, 400, 700 and 900 feet of sea water (Fig. 1). The baseline ambient pressure was then increased additionally to a level equivalent to 1200 feet of sea water, again with a natural partial pressure of inspired oxygen. During a several-day residence at this "depth", the subjects were further exposed for limited periods to respiration of neon at a high partial pressure, which permitted study of acute physiological and performance limitations at respiratory gas densities equivalent to helium-oxygen breathing at depths to 2000, 3000, 4000, and 5000 feet of sea water. Table I shows the diving depth equivalents for helium, neon and nitrogen as these gases were administered with natural oxygen pressure. For special purposes related to investigation of the concept of hydrostatic pressure reversal of narcosis, approximately 10

*Crude neon used was analyzed as 76.8% neon, 23.2% helium.

TABLE I

MEASUREMENT CONDITIONS
DEPTHS FOR EQUIVALENT GAS DENSITY*

Nitrogen Series			Neon Series			Helium Series		
Dose-Response Condition	Density Equivalence		Dose-Response Condition	Density Equivalence		Dose-Response Condition	Density Equivalence	
	Ne	He		N_2	He		N_2	Ne
0	18	167	0			0		
			18	0	167			
100	199	900						
141	272	1200						
						167	0	18
200	359	1558						
			272	141	1200			
300	529	2259						
400	699	2960	400	233	1786	400	32	72
			699	400	2960			
			900	536	3789	900	100	199
1200:			1200	—	5025	1200	141	272
326 N_2								
7 O_2	747	3159						
900 He								

*All values for dose-response condition are expressed for practical comparative purposes as pressure equivalent of depth in "feet of sea water." 1 ata = approximately 33 feet of sea water. Therefore, use the relationship:

$$\text{ata} = \frac{\text{Depth in feet} + 33 \text{ feet}}{33 \text{ feet}}$$ for conversion to atmospheres absolute.

atmospheres of nitrogen were included with helium respired at the 37-atmosphere maximum saturation pressure.

On completion of each period of measurements during the composite nitrogen-helium or neon-helium exposures, the subjects returned to the ambient baseline helium-oxygen atmosphere simply by discontinuing the respiration of the other gases.

Each of the four young men selected as subjects had participated one year earlier in a 14-day exposure to elevated nitrogen pressures (4 ata) (25). As was the pattern for the previous Predictive Studies II, the subjects were selected for their high degree of physical, mental and technical competence, and for exceptional motivation. They were extensively trained both physically and technically during control phases at 1 atmosphere prior to and during the initial pressurization phases at 100-, 200- and 300-foot depth equivalents. Each subject-pair became an efficient, cooperative and competitive subject-technician team whose work inside the sealed chamber was tightly and purposefully linked to the functions performed by individuals outside the chamber system. These high standards were considered necessary features of the experiment design, since a major purpose of the overall study was to probe limits of human tolerance.

TABLE II

SCOPE OF PROGRAM

Observations	Subject Teams
Pulmonary function	A
Pulmonary mechanics	
Dynamic ventilation	
Gas exchange	
Lung volumes	
Respiratory functions	A
CO_2 reactivity	
Exercise tolerance	
Respiratory-blood gas exchange	
Temperature regulation	A+B
Blood chemistry and endocrine function	A+B
Hematological characteristics	A+B
Nervous system functions	
Central electric functions	A+B
Sensory functions	A+B
Cognitive functions	B
Tremor and coordination	B
Psychomotor performance	B
Voice analysis	B

Scope of Study

Table II indicates the scope of the series of investigations comprising the overall program. The four subjects were divided into two teams with a separate series of investigations for each team but with matching of gases, pressures, temperature and other conditions of the program.

Table III illustrates the individual components of each major study, along with the ambient pressure levels and the respired gas conditions under which specific measurements were made. Details of the measurement apparatus and methods are presented in separate reports of several study components (e.g. Respiratory Control, Pulmonary Mechanics, Psychomotor Performance, etc.). The sequence of workloads imposed for the study of exercise tolerance is specifically illustrated (Fig. 2) because there have been several indications elsewhere that

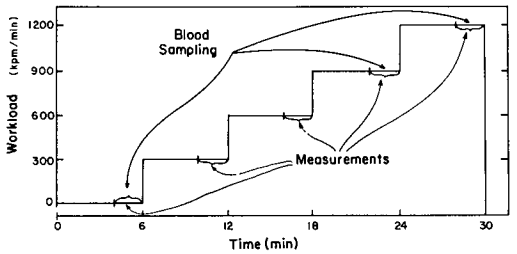

FIG. 2. Pattern of continuous, increasing workload performed by subject pair A, under conditions of increasing ambient pressure, narcosis and increasing respiratory gas density. Return to ambient respiratory gas environment followed 6-minute rest period after exercise.

TABLE III

SPECIFIC MEASUREMENTS

I. *PULMONARY FUNCTION*

- Pulmonary Mechanics
  - Airway resistance
    - Rest, exercise, forced ventilation
  - Flow resistive work of breathing
  - Respiratory pressures
    - Esophageal pressure, alveolar pressure
  - Compliance
  - Maximum expiratory pressure flow
- Dynamic Ventilation
  - Forced expiratory velocity
  - Forced inspiratory velocity
  - Maximum ventilatory volume
- Gas Exchange
  - Alveolar-arterial oxygen gradient
  - Ventilation-perfusion
- Lung Volumes-Exchangeable
  - Vital capacity
  - Dead space

II. *RESPIRATORY FUNCTION*

- Reactivity (CO_2 Response)
  - End-tidal P_{CO_2}
  - Respiratory frequency, depth and minute volume
  - Esophageal pressure
  - Work of breathing
- Exercise Tolerance
  - (6-min serial exposures to 300, 600, 900, 1200 kpm/min)
  - Physical performance
  - Metabolic characteristics
  - Pulmonary function in work
  - Ventilatory response in work
  - Alveolar-blood gas exchange

III. *BLOOD CHEMISTRY, CELLULAR AND ENDOCRINE FACTORS*

- Hematology-Cellular Characteristics
- Blood Chemical Composition
- Endocrine Studies

IV. *NEUROPHYSIOLOGICAL FUNCTION*

- Electroencephalogram
  - Rest
  - Exercise
- Evoked Brain Responses
  - Auditory
  - Somatosensory
  - Visual
- Tremor
  - Microtremor
  - Postural tremor
- Special Senses
  - Vision
    - Acuity
    - Color perception
    - Accommodation
    - Critical flicker fusion frequency
  - Taste
  - Hearing
  - Vestibular function

V. *PERFORMANCE*

- Psychomotor Performance
  - Gross coordination
    - Pursuit rotor
    - Bennett hand-tool dexterity test
    - Purdue pegboard
  - Fine coordination: small displacement tracking
  - Simple reaction time
  - Manual tapping rate
  - Muscle strength
    - Maximum grip strength
    - Strength estimation
- Cognitive Function
  - Paced arithmetic
  - Stroop test (rapid recognition task)
  - Productive time estimation
- Voice
  - Spectral analysis

physical work capacity at increased hydrostatic and gas pressures will be grossly diminished. The sequence used, involving uninterrupted periods of progressively increasing work, represented approximately 80% of the normal exercise capacity during air breathing at 1 atmosphere.

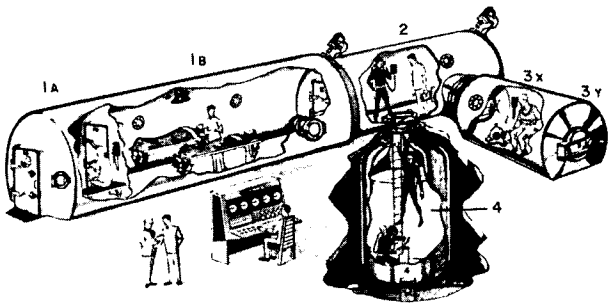

FIG. 3. Environmental chamber system employed as pressurizable laboratories in study: chamber 2 for exercise-pulmonary-respiratory-blood gas laboratory; chambers 3X and 3Y for neurological, sensory and performance laboratory. Compartments were pressurized with a mixture of He and natural (0.21 ata) O_2 at ambient pressures above 100-foot depth equivalent, where N_2 with 0.21 ata 0_2 was used. Both chamber 2 and 3 systems were used at night for living spaces. Shower and toilet facilities functioned at all pressures. Food, supplies and samples were transferred by means of small pressure locks through walls of each compartment.

Environmental Chamber Systems

The chambers in which the studies were performed were part of a six-compartment environmental simulator system, equipped both as laboratories and as living compartments (Fig. 3). Control and measurement of temperature, gas movement, humidity, oxygen and inert gas composition, and lighting, together with chemical removal of carbon dioxide, provided baseline life support requirements. Locks provided for transfer of equipment, samples, supplies and food, including large equipment for experiments. Means were provided for electronically converting the distorted voice sounds originating in the high pressure helium atmosphere to intelligible communication from subjects to external investigators. (Helium Speech Unscrambler, Helle Engineering, Inc., San Diego, California, Model WP-10H.) Compartments were equipped by the subjects for use as laboratories during the day and for residence at night. Shower and toilet facilities existed for personal hygiene.

One chamber, 8 feet in diameter and 12 feet long, was used by subjects II and X and as the laboratory for exercise, respiratory, pulmonary and blood measurements. Subjects III and V used an adjoining and connecting chamber 6 feet in diameter and 8 feet long for most of the electrophysiological and performance studies.

Decompression

Decompression was planned as for saturation exposure to helium with oxygen, but with a pre-arranged sequence of stops to permit investigations to continue during the days of decompression, to provide for separate investigation of the respiratory-pulmonary influences of density and viscosity of respired gases. Decompression itself was therefore not a subject of study. Details of the decompression procedure employed are provided in an ancillary publication (21) or are available from the Decompression Data Bank (5, 30).

Phasing of Measurements

Figure 4 indicates the pattern of experiment and measurement during a typical day in the

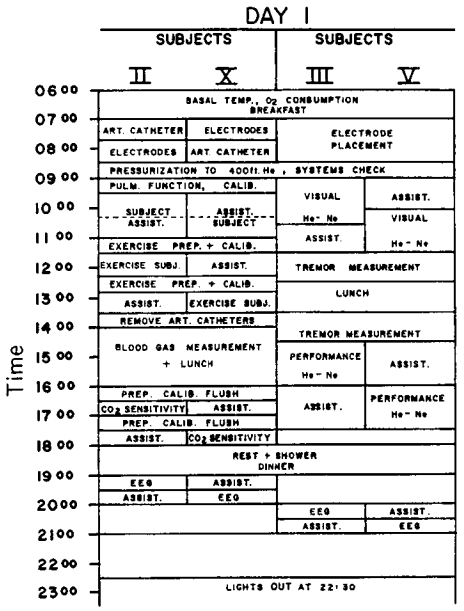

FIG. 4. Characteristic subject schedule of preparation and measurement: day one, 400-foot depth equivalent. Subjects alternated as subject and assistant throughout day, after insertion of vascular and esophageal catheters and application of electrodes. All electrodes and instrumentation were removed nightly except for sleep electroencephalograph electrodes.

series. Throughout the study the step-by-step increases in ambient pressure were accomplished overnight or on non-working days. Experiment periods, such as those shown for 400 feet of sea water in the figure, were therefore always at a stable pressure.

Composite Results

The summary which follows is to provide perspective and continuity for the overall program by integrating findings from several components of the study of pressure and inert gas effects. Details of methods and actual measurements are elaborated for individual projects in the specific reports, in which it is not possible to describe general relationships of findings.

Respiratory Gas Exchange

By means of indwelling vascular catheters inserted daily blood sampling could be accomplished at preplanned phases of the study (Fig. 2). Measurements of arterial blood gas tensions were made at pressures to 400 feet of sea water, and venous blood was sampled from the heated hand at 700, 900 and 1200 feet of sea water. Blood sampling failed to be accomplished only for neon breathing at the 1200-foot depth equivalent. From these measurements, performed in the chambers at the elevated ambient pressure, it was learned that no detectable interference with oxygen or carbon dioxide exchange occurred at rest or in exercise with nitrogen to 400 feet, helium to 1200 feet, and neon to 900 feet. The postulated gross diffusion limitation at extreme gas density or pressure (9, 10) therefore was not confirmed.

Pulmonary Mechanical Function

With the imposed progressive increase in density of respired gas, whether helium, crude neon or nitrogen with oxygen, an expected pattern of qualitative change in pulmonary function occurred (29, 31), as encountered by others (33, 34). The pulmonary work required to move respired gas increased as resistance to air flow became greater. The lungs themselves appeared unaffected, as indicated by unchanged pulmonary compliance. Capacity to rapidly move gas decreased, as measured by velocity of a single, Forced Exhalation or Forced Inhalation, as well as in terms of Maximum Ventilatory Capacity (21).

An unexpected and important finding was that as gas density became extreme, the degree of pulmonary ventilation limitation tended to become proportionately smaller, with the result that capacity for useful degree of ventilation was sustained to very high respiratory gas density. It was this previously unrecognized advantage that permitted the attainment of extremely high physical work levels without concurrently developing incapacitating pressures of alveolar carbon dioxide, which inevitably must occur when capacity for metabolic production of carbon dioxide exceeds that for alveolar ventilation.

Respiratory Control

The degree of ventilatory response to rebreathing autogenously produced carbon dioxide was progressively reduced as the density of gas breathed increased. This prominent reduction in total and alveolar ventilation was not found to be correlated with narcotic properties of the inert vehicle gases (He, Ne, N_2) but rather with the same factors of density, pulmonary resistance and respiratory work that were found to limit pulmonary mechanical function. Therefore the diminished ventilatory response to carbon dioxide is not to be interpreted as an index of narcotic depression of the respiratory neurons. These neurons most probably were highly reactive, but with their reactivity masked by resistance to gas flow in the lungs.

Exercise Capacity

The completion of the increasingly severe pattern of exercise on the bicycle ergometer represented performance at approximately 80 percent of the subject's work capacity under normal conditions at 1 atmosphere. Failure to complete this work occurred in only two conditions of the overall study and then it occurred for two different reasons. One form of failure, experienced by each of two subjects during nitrogen–oxygen breathing at the 400-foot depth equivalent, occurred within the last 2 minutes of the most severe work level. This failure was very evidently due to mental confusion and muscular incoordination induced by nitrogen narcosis. It was not due to either dyspnea of respiratory–pulmonary limitation or to muscular fatigue in the exercising limbs.

A second form of failure, again encountered by each of two subjects, occurred when breathing the "crude neon" gas, again during the last 2-minute period of the maximum workload. This failure to complete the planned sequence was due to high density of the respired gas. It occurred only under a condition equivalent to performing the heaviest work at a respired gas density equivalent to that to be expected with helium at a depth of 5000 feet, or with hydrogen at 10,000 feet of sea water (Fig. 5). The degree of elevation of alveolar carbon dioxide pressure encountered in this situation was prominent (about 60 mmHg) but was not in itself limiting.

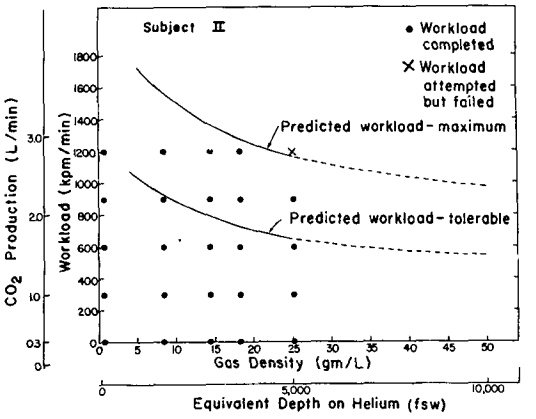

FIG. 5. Prediction of workload tolerable in exposure to increased respiratory gas density or undersea depth. Grid of black dots represents actual conditions of present study, with five levels of gas density at each of five levels of rest and work. The symbol X represents the condition in which failure occurred due to excessive respiratory resistance and work (maximum density together with final and maximum work period). Curve labelled "predicted workload-maximum" is actually not different from that which would induce ventilation equivalent to maximum voluntary ventilation at the indicated respiratory gas densities. The curve indicating "predicted workload-tolerable" represents composite circumstances of workload and gas density which do not result in alveolar P_{CO_2} elevation above 50 mmHg.

Respiratory minute ventilation was found to be diminished with increasing respiratory gas density at each level of work. The degree of this ventilatory interference became severe at 1200 kpm/min, where the degree of exercise ventilation very closely approximated maximum ventilatory capacity. At the more moderate work levels of 300 and 600 kpm/min ventilatory function was symptomatically not distressful and maintained alveolar (end-tidal) levels of P_{CO_2} below 50 mmHg.

On the basis of such findings it is predicted that the respiratory and pulmonary function required at rest and in support of moderate physical work, intelligent activity and manual dexterity should be possible at depths well beyond those studied in any laboratory thus far. With such indications it now remains to be learned how and where factors other than gas density (hydrostatic force, narcotic or other pharmacological effects of inert gases, diffusion limitation) introduce truly detrimental effects upon physiological systems and purposeful activity.

NEUROPHYSIOLOGICAL CHANGES

Probably because of the slow, stepwise compression over several days to the maximum 1200-foot pressure-depth equivalent, no clear indication of a "high pressure nervous syndrome" (4, 7, 8, 15) was observed throughout this entire study. Tremor measurement and spectral analysis of it showed only normal activity or minor deviations (32). Measurement of electroencephalographic activity and frequency analysis of the recordings showed no abnormalities at rest and no evident abnormalities even in the severely narcotized subject exercising while breathing nitrogen at 400 feet of sea water. Visual, auditory and somatosensory-evoked electrocephalographic potentials indicated no important abnormalities, except in the most definite narcosis (nitrogen-oxygen breathing at 400-foot depth

equivalent). Visual function was not detectably altered by any condition of the study. Taste sense was not modified by gas or pressure. Hearing was normal following the composite exposure, but vestibular function was unilaterally inactivated or subnormal in three subjects who developed the "isobaric gas counterdiffusion syndrome" (21-23).

Mental Function

Cognitive tests previously employed to define the dose-effect depression of mental functions by nitrous oxide (12) showed no detectable mental decrement with either helium or crude neon breathing at 37 ata. Nitrogen did produce a progressive central nervous system depression which, as expected (1, 2, 13), became prominent at pressures equivalent to 300 and 400 feet of sea water. Because mental and other neurological functions were essentially normal with helium at the highest pressure (37 ata), it was not possible to detect any antagonism of pressure effect by the actual use of narcotic nitrogen for this purpose (20).

Psychomotor Function

Determination of change in manual dexterity, coordination, and reaction time indicated a definite decrement only during nitrogen breathing at pressures equivalent to 300 feet of sea water or greater. Thus, neither neon nor helium significantly modified any of the psychomotor performance measures employed (19).

Temperature Stress

During the multi-day, stable phases of exposure to helium at increased ambient pressures the subjects collectively selected temperature for comfort. The mean selected temperature became higher as helium density rose, with a narrowing of the comfort range (Table IV).

Weight loss averaged 2 kg over the 11 days of exposure to helium at 37 ata, in spite of a caloric intake averaging approximately 3500 calories/day. Since basal oxygen consumption was not consistently elevated, the degree of any thermal stress was not sufficient to expand metabolic activity.

Blood Chemical, Cellular and Endocrine Characteristics

The combined influences of increased hydrostatic pressure, prolonged exposure to helium at increased partial pressure, and intermittent exposure to respiratory gases of increased

TABLE IV

Thermal Comfort Ranges Selected

Depth (fsw)	Low Limit (°C)	High Limit (°C)
400	28.5	31.5
700	29.0	31.5
900	30.0	32.0
1200	32.5	33.5

density produced no physiologically detrimental changes in blood electrolyte, blood cellular composition, catecholamine or adrenal cortical hormone excretion as compared with pre-exposure controls or with prolonged exposure to increased nitrogen pressures (3, 27, 28).

Unexpected Findings

Isobaric Inert Gas Counterdiffusion Syndrome

During the performance of the integrated investigations described above, an unexpected abnormality was encountered as urticaria, gas-filled skin lesions and vestibular dysfunction developed. These changes, which are considered pathological, are described in detail elsewhere (17, 22, 23). They occurred when nitrogen-oxygen, nitrogen-helium-oxygen or neon-helium-oxygen was breathed while the subjects were surrounded by helium with natural oxygen pressure. Each form of lesion was incapacitating, although the urticaria and the dermal gas lesions were entirely preventable on most of the skin surface by use of a suit ventilated by the gas breathed (23). Occurrence of cutaneous itching and lesions had been observed previously by other investigators (6) who did not associate the phenomenon with development of gas bubbles in the dermal tissues. The unexpected finding, designated "isobaric gas counterdiffusion" (18, 23), has been identified as a gaseous supersaturation generated by unequal rates of inert gas counterdiffusion, at a stable ambient pressure (isobaric state), with continuous evolution of gas bubbles in skin and continuous venous embolization of gas from subcutaneous capillaries to the heart and systemic circulation (18, 23).

It has been deduced from subsequent studies that the most probable basis for vestibular derangement itself was produced not by embolization but by gas bubble generation in the inner ear fluids, as a result of local gas counterdiffusion across the round window, from middle ear to the cochlear fluids (24). These drastic, unexpected findings are further considered to represent a potentially lethal condition. They appear to be fully preventable by avoiding circumstance involving counterdiffusion either at local sites or over the entire body.

Predictions from Composite Studies

The results of all studies performed indicate that no detectable chemical or physiological handicaps should accompany prolonged exposure to a helium–oxygen atmosphere at pressures up to 1200 feet of sea water (37 ata). The subjects should be expected now to be capable of carrying out mental or physical functions essentially as well at this high ambient pressure as at their normal 1-atmosphere environment. Furthermore, helium or neon used as a respiratory inert gas should imposé no detectable narcotic influences upon higher mental functions to pressures well beyond 37 atmospheres of the study described.

When respiratory gas density was grossly increased by use of crude neon or nitrogen as the respiratory vehicle for oxygen, respiratory and pulmonary competence was preserved at rest. Respiration was accomplished even in exercise almost to the subject's demonstrated maximum exercise tolerance, and to a density of respiratory gas equivalent to helium–oxygen breathing at 5000 feet of sea water, or hydrogen–oxygen to 10,000 feet of sea water. These findings indicate that there should be no decrement in pulmonary ventilatory function which would handicap further extension of man into the deep sea. Attention can, therefore, be turned to the still necessary study of shallow exposures and to the influences of hydrostatic pressure itself in rapid, deep diving.

ACKNOWLEDGMENTS

These collaborative investigations were supported by research contracts N00014-67-A-0216-0026 and N00014-67-A-0216-0024 from the Office of Naval Research and U.S. Navy Bureau of Medicine and Surgery, Grant HL 08899-08 from the Heart and Lung Institute of the National Institutes of Health, contract NASA-NGL-39-010-097 from the National Aeronautics and Space Administration, and a special supplementary contract N00014-67-A-0216-0026 from the Office of Naval Research and the U.S. Navy Bureau of Medicine and Surgery. The neon used in the studies was provided as a grant from Ocean Systems, Inc., which also took direct part by providing laboratory staff investigative and technical personnel.

Participation in maintenance, investigation, operations and medical support came from many sources. Planning, preparation and primary investigative participation by Dr. B. Wright, R. Gelfand, Dr. R. Strauss, Dr. R. Peterson, Dr. J. Dickson and Dr. R. W. Hamilton, Jr. provided the cohesive influence upon which the study depended. Special acknowledgment is made of the contributions by Drs. Caroline Leach and C. Alexander of the Biochemistry-Hematology Division of NASA Manned Spacecraft Center, by Dr. Arthur Bachrach and colleagues of the U.S. Naval Medical Research Institute, by Drs. JoAnne Kinney and Robert Sawyer of the U.S. Naval Submarine Medical Center, and medical support by Dr. Charles Knight of the Canadian Institute for Environmental Medicine and Dr. Edward Beckman of Texas A and M University.

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PART I. COMPREHENSIVE STUDIES OF PRESSURE AND DECOMPRESSION*

DISCUSSION

P. Bennett, Chairman

Mr. Hitzig: Dr. Fructus, I was part of the team that investigated the EEG changes in the divers of the University of Pennsylvania Predictive Studies III. We monitored the complete head, as set forth by Silverman, and also eye movements and muscle artefact, looking for artefact in the EEGs. We concluded, using an in-line mean frequency analyzer, that there were no changes we could find attributable to any abnormal or pathological states. Eight channels of EEG were recorded in 10-minute sequences, at the different pressure exposures, with the different gas mixtures. We found that there was only a diminution in amplitude of alpha rhythms, no changes in alpha frequency, and no predictable changes with increasing pressure or different gas mixture in percentage of alpha.

This is different from your findings of prominent changes and I was just wondering if perhaps you could comment on this?

Dr. Bennett: I will offer that the problem here is primarily one of compression rate, that in our particular experiment we were compressing at 16 minutes per hundred feet, a fast rate of compression in comparison to the ones done in Philadelphia. You went up very gently with a lot of little stages on the way, and I think adaptation here was very important. We in a sense were evoking the high pressure syndrome to study it, whereas I think one of the purposes of the Philadelphia dive to some extent was to eradicate it. That is probably the difference, but I think it is primarily rate of compression and also depth. You had stopped at 1200 feet, and the deeper you go (as I think Dr. Agarate's calculations will perhaps confirm) the worse the condition is in terms of reduction of alpha and increase in theta and delta activity.

But if you can compress very, very slowly, you will certainly hold back a lot of these changes and they will come on at a greater and greater depth. The problem in actual diving is probably an economic one—as to how slow a compression you are prepared to accept in order to put your divers down.

Dr. Schaefer: Dr. Fructus, you mentioned that the hematocrit and red cell concentration which normally go down with prolonged oxygen breathing actually went up during the stay at 1500 feet. Would you explain this perhaps on the basis of dehydration, which suddenly might increase this, but if you had a stress symptom you could have release of red cells from reservoirs.

Dr. Agarate: Dr. Fructus thinks this red cell drop could be due either to an osmotic disturbance which creates a water shift, or due to the stress which would provoke a spleen contraction.

Dr. Farmer: Dr. Lambertsen, have we established whether the vestibular phenomena you observed were end-organ events or central events?

Dr. Lambertsen: We have not, except that intensive studies regarding the initial possibility that they were virus-induced have been carried out, with tracing of the immune characteristics of the sera of the individual over the months prior to, during and after the exposure. These studies indicate that no important virus infection had occurred. It is not yet clear whether end-organ or central derangement was induced by the isobaric counter-diffusion process.

Dr. Farmer: I was very intrigued with your suggested possible mechanism, which consisted of counterdiffusion between middle ear and inner ear. Another possibility is transport of gases across the three basic compartments of the cochlea itself. I think that should also be considered. There is some evidence to suggest that with the initiation of breathing of a new inert gas, the gas composition of the middle ear itself does not change rapidly.

**Panelists*: C. Agarate, X. R. Fructus, J. B. Morrison, C. J. Lambertsen.

Dr. Banister: Considering exercise tolerance, it is well-known that exercise limitations are caused not only by decrements in ventilation, but there is some kind of interaction between the anaerobic and aerobic phases of exercise. It is not correct to characterize the limitations of physical function simply by decrements of ventilation. Rather it would be more appropriate to characterize them both by that kind of dysfunction and also the increasing amounts of lactate and pyruvate in the blood.

I noticed, Dr. Lambertsen, that measurements of lactate and pyruvate were carried out in the Pennsylvania study. Would you comment about this kind of limitation in the exercise?

Dr. Lambertsen: You are entirely right in offering at least dual bases for exercise limitation. By choice the effort in summarizing the overall study for you was limited to explaining its score, with a summary of some results. The detailed results do include measurements of changes in blood lactate and pyruvate, under all of the circumstances of exercise, at each of the major levels studied and at all of the pressures on up through 900 feet of sea water, but not to 1200 feet. We failed to get blood samples at 1200 feet of sea water.

The overall pattern of the acid-base changes that occurred showed no indication of a metabolic modification during the exposures, as compared with what would have been expected in exercise at sea level. Therefore, even with N_2 at 400 feet, even with Ne at 1200 feet there were no important changes in the acid-base relationships.

Dr. Banister: I would expect there would be differences apparent between fit and unfit people. It is well-known that athletes can carry on work of long duration at 80–90% of their V_{O_2max}. V_{O_2max} in this case is equivalent to work of up to 2500 kilogram-meters a minute, which is roughly twice the amount that your people did. I would assume that your subjects were relatively untrained technicians, and if we are going to make studies of the actual limitations placed upon people at great depths we should select very fit people—if the optimum conditions of work at those kinds of depths are going to be realized.

It might be interesting to repeat those experiments with very fit people, instead of relatively untrained people, as I would characterize your subjects.

Dr. Lambertsen: I think you are entirely right again. It just happens that one of our unfit subjects made the World Cup Soccer Team last week. Another is a professional basketball player.

Dr. Banister: But these are not trained people. The respiratory function and athletic capabilities of soccer players and basketball players, interspersed by large rest periods, are not those demands made upon divers at work.

Dr. Lundgren: Dr. Morrison's presentation raised the question concerning continuing decompression when you have a subject in pain. Dr. Barnard, I think you mentioned that you had someone come up to the surface with some pain still left.

Dr. Barnard: That is a very easy question to answer in this particular instance because you can only know that the man is in pain if he tells you. In fact, he is here and I think he will admit that for two or three days we did not know it, although he personally knew that he had pain. At a later stage, however, he had pain which we all recognized and it became extremely difficult to do anything about this because both compression and decompression made it worse; and he really wanted us to stop monkeying about and to leave him alone.

So this is what we did and why we did not get him clear of pain during the last part of the decompression, and why he came out of the chamber with pain, but we do not quite see how to avoid it even now.

Mr. Kenyon: Dr. Bennett and Dr. Fructus, when compressing at your so-called high rate of 10 feet/minute, what pressurization gas is used? Is it a pure gas mixture or is it a mixed gas?

Dr. Fructus: In the 1968 experiments it was a mixture. The three studies reported here were effected in pure helium.

Mr. Kenyon: And you still found the HPNS with the mixed gas pressurization?

Dr. Fructus: We mainly found it in the experiments with helium. With mixed gas, it is a very fast compression.

Dr. Weachey: I would like to recall the many anatomical differences and abnormalities in the vestibular system. These should mean an individual sensitivity to impairment in compression and decompression exposures in general.

Dr. Lambertsen: It is important to restate that there have been two kinds of vestibular functional derangements encountered in positive pressure work. One of these kinds has related to decompression after exposure for a long period of time to high inert gas pressure. The other kind, the type that I described to you here, is a new phenomenon which has occurred while subjects were held at the same pressure continuously for many days without decompression at all and merely had the gas that they breathed changed. This was an "isobaric" state. We must distinguish between these two. They are not necessarily in any way related to each other in terms of the initiating mechanism.

Dr. E. B. Smith: I can offer information on compressibility of body tissue, which I have discussed with the

subject Mr. Bevan. The human body will be roughly as compressible as a normal liquid, be it water or any other liquid, which would be about 5×10^{-5} atmospheres^{-1} compressibility coefficient.

If one assumes a 17-L body, one comes out with about 100 cc diminution in total volume at 30 atmospheres pressure. So I think there are real changes of this order of magnitude. The consequence of this, of course, and how far volume itself is an important parameter, I think is not possible to say.

Dr. Halsey: Surely it is the relative volume changes between different phases and tissues rather than the absolute volume change which would matter in terms of the effects.

Concerning the controls in all of these high pressure studies, it must be very difficult to separate the psychological effects of diving at high pressures from the physiological effects. In your studies you must have considered this problem and naively I would have said: Did the subjects know the diving program? Did they know what depths they were at?

Dr. Morrison: We can say that, if you take the changes in the EEG, there have in the past been record dives achieved to the order of 600 feet, and they would be under the same sort of stress—but there were no changes seen in the EEG. These changes crop up with different experiments, and with different depths you see different sorts of changes.

As far as the controls are concerned, we did perform experiments in the chamber at surface. The subjects knew they were at surface. At 1500 feet the subjects knew they were at 1500 feet.

Dr. Bennett: A further check is that in terms of the performance test. For example, the subjects were highly trained so that their performance was at optimum, there was a decrement and they returned to normal at the end.

Dr. Fructus: I think the psychological effects are very minimal because the subjects are very well aware of the program. Furthermore, all of them are professional divers who carry out, roughly, a hundred dives a year in deep water and they are used to it.

Dr. Behnke: Dr. Lambertsen, it is not necessary, of course, to link new discoveries with the ignorance of the past, but for many years divers have shifted from breathing He after a dive to breathing air. The shift usually has been a gradual one in which the N_2 was breathed, let us say, at 150 feet after coming up from a deeper depth (the individual was also surrounded by N_2, so that the skin was surrounded by N_2); the skin phenomena you mentioned have not been observed or have been missed under these conditions. It is apparently necessary, therefore, to have He around the skin.

Regarding the remarkable finding of vertigo in the isobaric condition, is this an isolated instance or was this regularly produced?

Dr. Lambertsen: I think these are instances of a phenomenon which has to be called isobaric vestibular dysfunction. It has not, as far as we know, been encountered before in the laboratory, because the circumstances have not been appropriate before for this kind of phenomenon. It occurred, however, in three of the four subjects in the pressure chamber during the experiments that I described. So it was not a single instance. Very likely it has occurred at sea in diving without being identified.

When an individual in a suit or a compartment is exposed to change of the gas around him, he normally breathes the same gas. Vestibular derangement from counterdiffusion should not be encountered during that kind of exposure. If, however, you generate circumstances artificially with a mask in which one gas is breathed in conjunction with a helmet or a hood in which another gas, He, is contained, the diver could get into the circumstances where he would develop the problems we have described.

We must therefore not think that this is something that can only happen in experimental purposes where divers are in a saturation chamber breathing with a face mask. I think the circumstances can be encountered practically.

Dr. Saltzman: I think the question remains open whether these isobaric phenomena are dependent upon an external He interface, or whether the interface within the tissue is the more vital determinant. The latter has much more ominous implications for decompression.

Dr. Purdy: On vestibular apparatus: one of the things we are apt to forget is that the vestibular apparatus is an electromechanical system. Each hair in the sensory system is essentially an electric generator. We also know that different gases in a fluid against an electrical system will produce electrical gradients. It may be that the mechanism is a process of actually generating some bias in the system.

Dr. Farmer: I would like to echo what Dr. Saltzman said, that this could easily be a tissue interface phenomenon within the cochlea itself. There are different compartments there which have entirely different biochemical composition.

Dr. Behnke, we have not in our current collection of auditory and vestibular incidences associated with

diving collected any of the type Dr. Lambertsen described—these isobaric phenomena. All of our collection so far have been associated with decompression.

Out of 20 cases one of these consists of a diver who was decompressing from a 400-foot He dive and during the stop at 150 feet was transferred into an air chamber. At that time he immediately became vertiginous and, when I was able to see him a year after the event, studies showed that he had a dead labyrinth on one side. So this could be an example of an isobaric phenomenon, but I think it is an incidence of decompression.

One other thing I think we should keep in mind. The oval window and round window open directly into the cochlear part of the inner ear, and with a phenomenon occurring in this area most likely you would not only have vertiginous events but auditory events. I do not believe these have been described. This would tend to indicate—to me at least—that we are dealing more with a phenomenon in the superior part of the inner ear or in the vestibule.

Dr. Lambertsen: It is quite important to comment that with careful search for hearing defects none was found in any of the subjects in spite of the fact that we had done detailed appraisals of their hearing before the exposure. There was clearly a separation between vestibular and auditory influence.

Dr. Farmer: This would again tend to mean to me that it is likely you are dealing with something away from the cochlear part of the labyrinth and up in the semicircular canal (assuming it is an end-organ phenomenon) and that it is probably not a phenomenon of differential gas mixture in the middle ear versus the inner ear, but differential gas mixture at the tissue interface, as Dr. Saltzman suggested.

Part II. VENTILATION AND GAS EXCHANGE

MAXIMAL WORK PERFORMANCE IN HYPERBARIC AIR

D. Linnarsson and L. Fagraeus

The maximal aerobic power, i.e. the maximal oxygen uptake represents a highly reproducible measure of the integrated function of the oxygen transport system. During maximal exercise with large muscle groups the capacity of the circulatory transport of oxygen is usually considered to set the limits for the oxygen uptake when breathing air. This might not be the case under hyperbaric conditions when other limitations induced by pressure-related factors may be present.

Methods

To study the problem, maximal work performance was measured in two sets of experiments: first with seven subjects breathing air at five different ambient pressures ranging from 1.0 to 3.0 ata, and subsequently with another eight subjects at 1.0, 3.0 and 6.0 ata breathing air and at 3.0 ata breathing 21% oxygen in helium. The experiments took place in a dry compression chamber. The subjects exercised on a bicycle ergometer at a standardized supramaximal workload, known to lead to exhaustion within 3–5 minutes at sea level breathing air. Maximal oxygen uptake was measured by means of the Douglas bag method using a low resistance breathing circuit. The CO_2 tension inside the mouthpiece was analyzed continuously with a fast-responding CO_2 meter.

Results

Maximal Oxygen Uptake

Maximal oxygen uptake ($\dot{V}_{O_2}max$) averaged 3.55 $L \cdot min^{-1}$ STPD at 1 ata (range 2.16–5.03 $L \cdot min^{-1}$ STPD) (Fig. 1). At an ambient air pressure of 1.40 ata in the first set of experiments, $\dot{V}_{O_2}max$ was significantly increased by 8%. The time to reach exhaustion was prolonged in proportion to the raised maximal oxygen uptake, and the subjects reported that the same standardized workload was easier to endure. These effects were thought to result from the moderate hyperoxia accompanying the slight rise in ambient air pressure. However, a further rise in ambient pressure to 2.0 and 3.0 ata did not result in any further improvement in maximal work performance or maximal oxygen uptake. This led to the assumption that the increased pressure—and in particular the increased gas density—counteracted the beneficial effect of hyperoxia. Furthermore, at ambient air pressures higher than 3.0 ata,

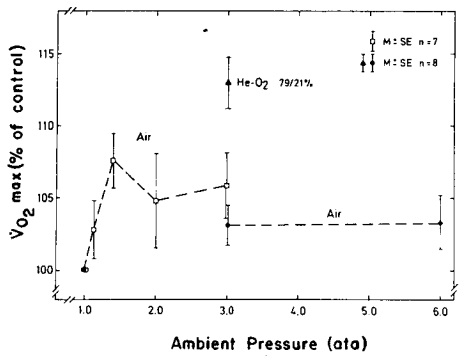

FIG. 1. Maximal oxygen uptake ($\dot{V}_{O_2}$max) as a function of ambient pressure. Mean values ± SE are expressed in percent of control. The open symbols refer to a first set of experiments performed with air at 1.0 ata (control), 1.13 ata, 1.40 ata, 2.0 ata and 3.0 ata. The solid symbols refer to a second set of experiments with eight subjects examined at 1.0 ata (control), 3.0 ata and 6.0 ata breathing air and at 3.0 ata breathing 21% O_2 in He. With little or no increase in gas density, a hyperoxic medium was found to augment $\dot{V}_{O_2}$max significantly. At 6 ata, however, the much more pronounced hyperoxia fails to exert any significant influence on $\dot{V}_{O_2}$max.

maximal work performance and maximal oxygen uptake might not be significantly improved despite a considerable degree of hyperoxia. This reasoning was confirmed in the second set of experiments on eight subjects, who performed maximal work breathing air at 1.0, 3.0 and 6.0 ata, and a helium-oxygen mixture at 3.0 ata giving the same gas density as at 1.0 ata air and the same inspired oxygen partial pressure as air at 3.0 ata. With a helium-oxygen mixture at 3.0 ata, maximal oxygen uptake was increased significantly by 13% ($P < 0.001$) while only a slight and insignificant increase over sea level control was observed at 3.0 and 6.0 ata air.

VENTILATION

Ventilation during maximal work (Fig. 2) usually attained its highest value during the last 30 seconds of the work period. In both sets of experiments a marked decrease in ventilation

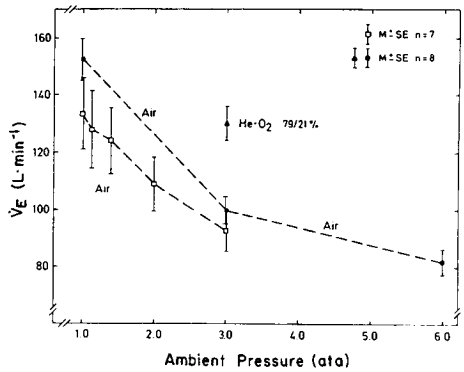

FIG. 2. Ventilation during the last 30 seconds of maximal exercise as a function of ambient pressure. A marked decrease in ventilation was observed with increasing ambient air pressure. Note the reduction in $\dot{V}_E$ also with He-O_2 at 3.0 ata. (For explanation of symbols see legend, Fig. 1.)

was observed with exposures to raised ambient air pressure. The course of the ventilatory decrease with ambient pressure was similar to that described for maximal voluntary ventilation (for review see [5]). The decrease in maximal exercise ventilation did not seem to be solely dependent on the increased gas density. When breathing a helium–oxygen mixture at 3.0 ata, ventilation was 15% lower than control at 1 ata ($P < 0.001$) although gas densities were the same. It was believed this was primarily an effect of hyperoxia. Assuming similar influences of the hyperoxia when breathing air as with a helium–oxygen mixture at 3.0 ata, a considerable part of the ventilatory decrement observed with air breathing at 3.0 ata would be due to hyperoxia, although the major part of the decrement was density-dependent. This was probably also the case at 6.0 ata.

End-Expired P_{CO_2}

End-expired P_{CO_2} during the last 30 seconds of maximal work (Fig. 3) was about 35 mm Hg in the control experiments at 1.0 ata indicating some respiratory compensation for the lactic acidemia of maximal exercise, which in these experiments averaged 15 mEq/L with no significant variations between the various ambient conditions of the study. The progressive ventilatory decrease occurring with raised ambient pressure was accompanied by CO_2 retention leading to raised arterial carbon dioxide tension, as reflected by the end-expired values given in Fig. 3. A mean value exceeding 55 mm Hg was observed at 6.0 ata, indicating that, in this condition, a considerable respiratory acidosis was superimposed on the metabolic acidemia.

Figure 4 shows typical time courses of end-expired P_{CO_2} during maximal work at 1.0, 3.0 and 6.0 ata air and at a 3.0 ata helium–oxygen mixture. During the preceding submaximal "warmup" period, no gross differences occurred between the various experimental conditions. In the two experiments with normal gas density—air at 1.0 ata and $He–O_2$ at 3.0 ata—a typical fall in the end-expired carbon dioxide tension was observed toward the end of the work period, indicating relative hyperventilation. By contrast, breathing air at 6.0 ata led to a progressive rise in end-expired carbon dioxide tension. At 3.0 ata air, an intermediate pattern was observed.

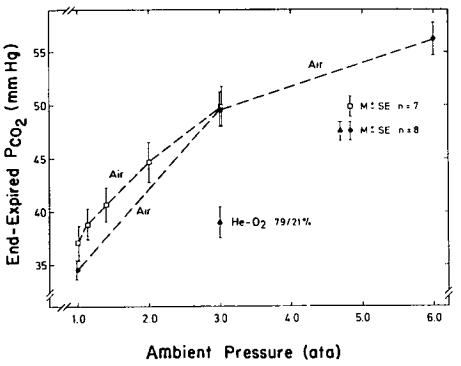

FIG. 3. Mean values of end-expired P_{CO_2} during the last 30 seconds of maximal work vs. ambient pressure. (For explanation of symbols see legend, Fig. 1.) See also Fig. 4.

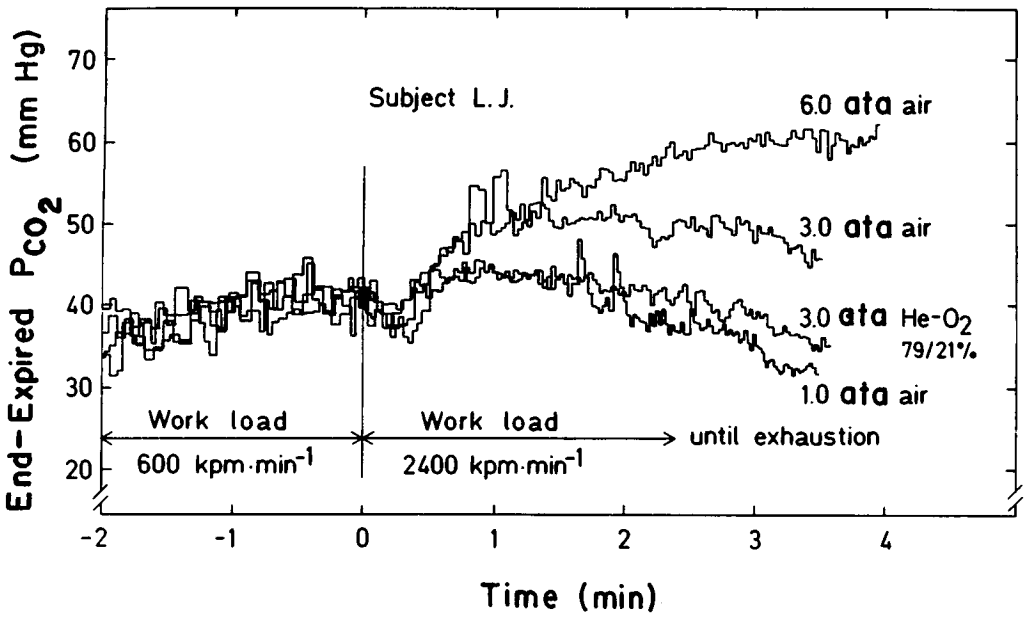

FIG. 4. Typical time courses of end-expired P_{CO_2} shown breath-by-breath during maximal work at 1.0, 3.0 and 6.0 ata, breathing air and at 3.0 ata breathing 21% O_2 in He. The peak value of expired P_{CO_2} for each consecutive breath was detected and displayed as a plateau on the recording. Curves from the four different conditions have been replotted on a common time scale.

HEART RATE (Fig. 5)

It is known that heart rate during submaximal exercise is decreased with exposure to hyperbaric air. In the present study heart rate was found to decrease also during maximal work with air at 3.0 and 6.0 ata. This decrease cannot be readily explained by the influence of any single environmental factor. It is believed, however, that hyperoxia is partially responsible, as shown earlier during submaximal exercise (2).

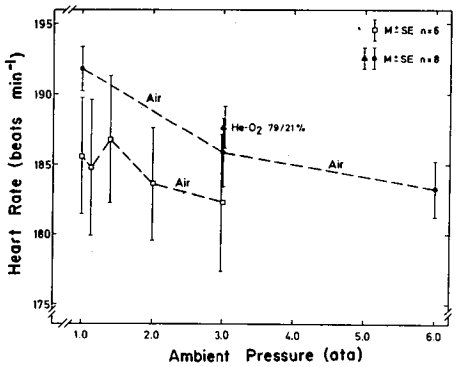

FIG. 5. Maximal heart rate as a function of ambient pressure. A significant reduction was observed at 3.0 ata ($P < 0.01$) and 6.0 ata ($P < 0.001$) compared to sea level control. Maximal heart rate did not differ significantly between the experiments with air and He-O_2 at 3 ata. (For explanation of symbols see legend, Fig. 1.)

Subjective Experience

Of the eight subjects investigated at 6.0 ata, all reported progressive severe dyspnea. In addition three subjects reported dizziness, vertigo and visual disturbances immediately before they interrupted the work task after having exercised 2.5–3 minutes.

Discussion and Conclusions

The present results show that maximal aerobic power is increased when hyperoxia is accompanied by little or no increase in gas density. This observation is in accordance with several other investigations (1, 3, 7–9) and leads to the conclusion that the working muscles have the potential ability to increase their aerobic metabolism above normal when extra oxygen is supplied by the arterial blood. This was the situation at 3.0 ata breathing a helium–oxygen mixture, and it is remarkable that breathing air at the same pressure caused a 10% lower $\dot{V}_{O_2}$max, although almost the same degree of alveolar hyperoxia was prevailing. Deficient oxygenation of the blood does not seem likely with air at 3.0 ata since arterial blood gas data from exhaustive exercise at 3.0 ata breathing O_2 (4) have not shown any abnormal differences between inspired and arterial O_2 tension. A more probable explanation for the deteriorated O_2 uptake with air at 3.0 ata may be found in the combined respiratory and metabolic acidosis. This is in accordance with the observation of Luft et al. (6) who found a significant decrease in $\dot{V}_{O_2}$max even with small amounts of CO_2 in the inspired air. The site of action of the hypercapnia, or the accompanying acidosis, is not evident. An effect on cardiac function is possible as is an influence on the uptake and utilization of oxygen by the working muscles. Also at 6.0 ata, where alveolar hyperoxia and hypercapnia are more pronounced than at 3.0 ata, the hyperoxia fails to improve $\dot{V}_{O_2}$max. Present findings indicate that cardiopulmonary function becomes inefficient during severe exercise and air-breathing at 3.0 ata and even more so at 6.0 ata. This and the experience of severe dyspnea in all subjects and the mental and sensory deterioration in some subjects suggest that 6.0 ata is close to the upper pressure limit for severe physical work with air-breathing, even with very low external breathing resistance.

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THE INFLUENCE OF INERT GASES ON INTRAPULMONARY GAS EXCHANGE

G.v. Nieding, H. Krekeler, K. Muysers, U. Smidt and H. Worth

Intrapulmonary gas exchange involves not only oxygen and carbon dioxide, but any respired gas having a gradient in partial pressure between the blood and the alveolar space. Moreover, the presence of an inert gas may theoretically favor or hinder the transport of O_2 and/or CO_2 in the airways or across the alveolar membrane. Investigations of effects of helium on the arterial P_{O_2} and the $AaDO_2$ under hyperbaric conditions have been described by Saltzman et al. (5) and Krekeler et al. (1).

In further experiments influences of three different inert gases with 21% O_2 upon the transfer of carbon monoxide were measured at 1 ata in 18 healthy male subjects by a modified (4) single-breath technique (2). The gases used included nitrogen–oxygen (ambient air), helium–oxygen, and argon–oxygen. Subjects were surrounded by the gas they breathed.

To ensure comparable conditions the subjects were "equilibrated" before the single-breath maneuver (Fi_{CO} = 0.003) for 10 minutes with the helium–oxygen and argon–oxygen mixtures, respectively. In the last minute of this "equilibration period" there was no further decrease of the difference between the inspiratory and the expiratory partial pressure of the test gas.

The results obtained are demonstrated in Fig. 1. The mean value for D_{LCO} in helium–oxygen was 28.9 ml/min·torr; in nitrogen–oxygen it was 25.1; and in argon–oxygen 20.0 ml/min·torr.

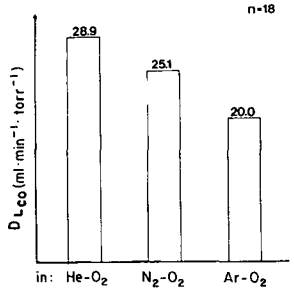

FIG. 1. Mean values of D_{LCO} during breathing of He-O_2, room-air and Ar-O_2.

The probability for significance of the difference was better than 99% in the Wilcoxon test (6).

The correlations of these results to different physical properties of the three gases—i.e., the viscosity (η), the coefficient of solubility in blood (α), the molecular weight (MG) and the coefficient of diffusion for CO in the different mixtures—were then examined. The closest correlation found was to the molecular weight (Fig. 2), which was even better than the correlation with the reciprocal of the square root of molecular weight, which is proportional to the coefficient of diffusion ($D \approx 1/\sqrt{MG}$).

This result leads to the conclusion that the mass of the gas in the lungs has the major influence on the processes which are involved in the gas transport from the bronchi to the blood. This very general conclusion is drawn because the determination of D_{LCO} alone does not allow distinguishing between influences of ventilatory distribution and diffusion across the alveolar membrane and beyond.

However, there is a certain influence on the determination of the alveolar volume, which was calculated as the mean from the simultaneou dilution of 2% Ne and/or He during the single-breath maneuver. It was 2160 ml (BTPS) in the He-O_2; 2200 ml in the N_2-O_2; and 2090 ml in the Ar-O_2 mixture (Fig. 3). These differences between He and N_2 are not significant, but are significant between Ar and N_2. However, they only partially explain the differences in D_{LCO} for they are smaller than the latter.

In addition to Ne and He for the determination of the alveolar volume, Ar and SF_6 were also used. Ar shows the highest dilution, which may be attributed to its relatively high

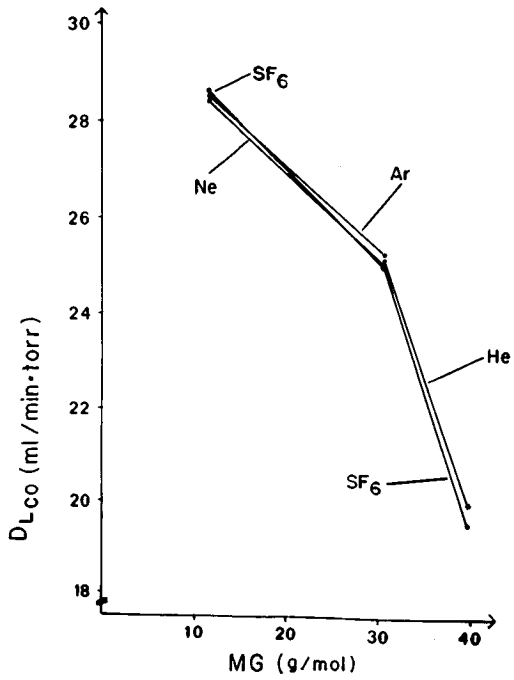

FIG. 2. Correlation between D_{LCO} and molar weight of the test gas mixtures (estimated alveolar concentrations at 37 °C and 1 ata).

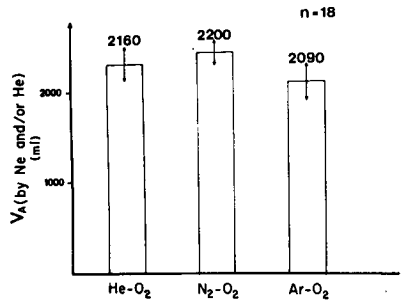

FIG. 3. Mean values and standard deviations of the means of the alveolar volumes (V_A) for the indicators of the test gas mixtures.

coefficient of solubility in blood in comparison to the other test gases. The differences in the results calculated from He and SF_6 with quite similar coefficients of solubility suggest that the distribution of SF_6 is worse than that of He, but it cannot be concluded from this whether the convection in the airways is mainly responsible for the differences or is an effect of stratification.

For the calculation of the effect of stratification, a simultaneous measurement of pulmonary washin of 2% each of He, Ar and SF_6 was performed as well as a simultaneous wash-out in the same subjects.

For the washin up to 37% of the initial concentration difference, the mean expired volume was 2.19 L for He, 2.28 L for Ar and 2.32 L for SF_6 with a mean volume of 0.64 L for every breath (Fig. 4). With helium if a dead space volume for the helium washin of 0.14 L per breath is assumed, the rest of 0.5 L may temporarily be called "alveolar ventilation." From this ratio an "alveolar ventilation" of 1.71 L in the 2.19 L expired volume needed for the helium washin can be calculated.

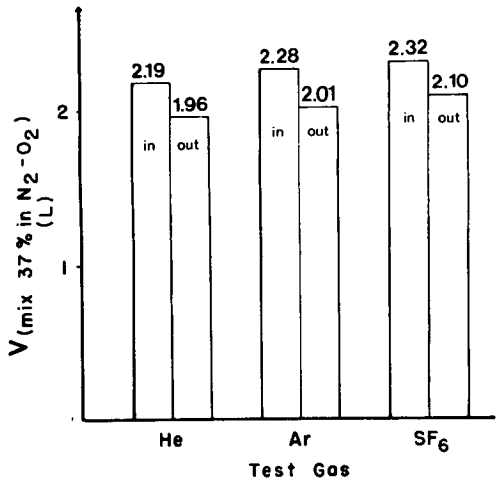

FIG. 4. Mean expired cumulative volume for the washin up to 63% and the washout down to 37% of the initial concentration difference, respectively, for He, Ar and SF_6 in room-air.

A further assumption is that the actual alveolar ventilation for the washin should be the same for all three inert gases, no matter whether it is performed only by convection or partly by gaseous diffusion as well. Then it can be stated that the 1.71 L are contained in 2.28 L for the argon uptake and in 2.32 L for the SF_6 uptake. Since the administration was simultaneous, the volume of the single breath is the same for He, Ar and SF_6; but then the part of the dead space must be higher, for the alveolar ventilation of 1.71 L covers more breaths for the argon wash-out and still more for the wash-out of SF_6.

Assuming that the dead space is expired during the first third of an expiration, the total alveolar ventilation of 1.71 L is subtracted from the total expired volume. The remainder will be four times the dead space volume, since 3.42 breaths were needed for He, 3.56 for Ar and 3.62 for SF_6. Thus an alveolar ventilation per breath of 0.476 L for Ar and 0.466 for SF_6 can be calculated. These values were plotted versus $1/\sqrt{MG}$, which is proportional to the coefficient of diffusion in other gases. When this line is extrapolated to $1/\sqrt{MG} = 0$ (Fig. 5), where the mass is indefinite and where there is therefore no diffusion, alveolar ventilation of less than 0.46 L is reached. So one can hypothesize that at least the additional 0.04 L for helium is due to diffusional gas transport in the alveolar space. This is about 10% for He, 6% for Ar and 3.4% for SF_6. (Instead of 0.14 L, 0.1 or 0.2 L for the dead space may be assumed. But this changes the calculated percentages for the space of stratification by $\pm 1\%$ only.) These figures are calculated under 1 ata conditions. They may change considerably under hyperbaric and hypobaric conditions.

Another observation in the same experiments was that the expired volume for the subsequent washout for all gases was smaller than for the washin particularly in N_2 and Ar (Fig. 6). Actually the washin never came to an end. A difference of about 1–2% of the initial amplitude persisted from the 5th or 6th minute onward as long as the curves were recorded, i.e., up to 20–30 minutes without any further change.

The "indefinite" length of the uptake period may be explained by two factors: 1) The normal N_2 pressure gradient across the skin between ambient air of about 40% humidity and the blood, and furthermore to the alveolar gas with 100% humidity amounts to 12 torr because of this difference. It increases to 54 torr, when the breathing gas contains 2% He, Ar, Ne and SF_6 each. So there is a continuous nitrogen flow from the skin to the lungs, which at least partly causes the nitrogen elimination of the lungs (3) and dilutes the other components of the alveolar gas mixture. 2) A part of the gas to be washed in is—according to its α— dissolved in the blood and leaves the body via the skin, just as for nitrogen, but in

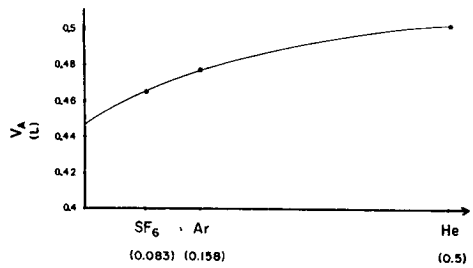

FIG. 5. Correlation between the diffusion coefficient of the test gases and alveolar ventilation per breath.

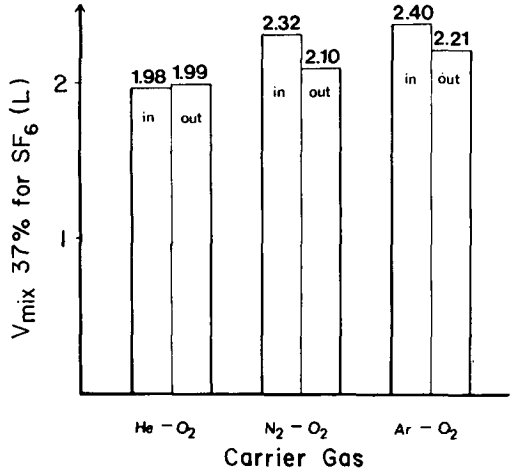

FIG. 6. Cumulative expired volume for the washin up to 63% and the washout down to 37% of SF_6 in $He-O_2$, N_2-O_2 and $Ar-O_2$.

the opposite direction. The higher the coefficient of solubility in blood, the higher the loss of the gas and the persisting difference between inspiratory and expiratory concentration. This is a sort of analog to the steady-state method for D_{LCO}. After achievement of a sufficient equilibrium one can calculate the "diffusing capacity" for Ar or He or any other inert gas from the lungs to the skin, provided the total inspired and expired volume has been measured. The more the inert alveolar gas is dissolved in the blood or in the lung tissue, the more the partial pressures of the other gases will increase. This favors the oxygen transport and hinders the CO_2 exchange and may lead to various compensating reactions. This has to be further investigated.

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PULMONARY MECHANICAL FUNCTIONS IN MAN BREATHING DENSE GAS MIXTURES AT HIGH AMBIENT PRESSURES—PREDICTIVE STUDIES III

*R. E. Peterson and W. B. Wright**

As naval and commercial interest in the sea, particularly concerned with rescue and oil resources, extends to greater and greater depths, the extension of the known limits of man's ability to dive becomes of practical as well as theoretical interest. A factor frequently proposed as limiting man's functional diving depth is pulmonary performance in dense atmospheres. Past studies have shown that the greater gas density associated with descent results in an increase in resistance to pulmonary gas flow and work of breathing, and a depression in maximum respiratory gas flow rates. It has been suggested that the useful work of a diver might therefore be limited by inadequate ventilation resulting either from weakness or fatigue of respiratory muscles or from an effort-independent limitation in expiratory flow.

The purpose of this study was to determine the environmental conditions which would restrict ventilation and gas exchange sufficiently to render useful work impossible and to investigate the source of that restriction. The breathing gas densities required for this could be achieved only by employing inert gases more dense than helium. Correlation of effects upon pulmonary mechanical function of high density breathing gas under moderate pressure conditions with low density breathing gas under extreme pressure conditions was desired to validate predictions of ventilatory limitations of helium breathing at pressures greater than can be achieved in pressure chamber simulation of deep diving.

Methods

These experiments concerning pulmonary function were conducted as part of the comprehensive study of the effects of oxygenated helium, crude neon (76.8% neon and 23.2% helium) and nitrogen at depths ranging from sea level to 1200 fsw (6, 8, 9).

The description of overall program, the chamber systems used, the subjects (II, X), and the general exposure profile are described elsewhere (8). Details pertinent only to pulmonary function will be described here.

Direct comparisons of the three diluent gases (nitrogen, crude neon and helium) were planned at densities of 1.2, 4.7 and 6.2 gm/L in order to validate extrapolation between equal density situations at different ambient pressures. The densities were calculated for a body temperature of 37 °C, an absolute pressure of 760 mm Hg plus the gauge pressure, and a constant 0.21 ata partial pressure of oxygen. Use of the high density inert gases neon and

*The authors, in presenting for this Symposium part of the results of a collaborative study, represent their direct co-investigators in the project.

nitrogen was planned at relatively high pressures to provide the basis for extrapolating results to helium breathing at pressures greater than those studied or even attainable in existing "man-rated" pressure chambers.

Table I shows the density values of the gas mixtures actually employed during each of the experimental conditions. The deviations from the planned exposures were due to: 1) contamination of the helium-oxygen chamber atmosphere with traces of other experimental gases; and 2) deviations from an oxygen partial pressure of 0.21 ata.

At each experimental condition, measurements were made during the following three pulmonary function maneuvers: 15 second maximum voluntary ventilations (MVV), forced expiratory vital capacities (FVC), and tidal breathing (TB). Transpulmonary pressure (esophageal balloon) and instantaneous gas flow (specially-designed pneumotachograph system [5]) were simultaneously recorded on a strip chart recorder and sampled and stored on magnetic tape by a digital computer (PDP-12). The esophageal balloon system was calibrated with a water manometer, while the pneumotachograph system was calibrated with a high-flow Brooks rotameter accurate to ±1% of the instantaneous flow. Calibrations were made both before and after each measurement condition.

When the experimental gas differed from the chamber gas, it was breathed by mask for 5 minutes before each experimental session started and during the time between measurement maneuvers. Before each maneuver the breathing system—including the pneumotachograph, low resistance valves, hoses and dump bag—was thoroughly flushed with the experimental breathing gas.

Data analysis of the three maneuvers cited was subsequently performed on a PDP-6

TABLE I

DESCRIPTION OF ACTUAL EXPERIMENTAL CONDITIONS INCLUDING VISCOSITY (μPOISE) AND DENSITY*

Nitrogen Viscosity = 182		Crude Neon† Viscosity = 307		Helium Viscosity = 201	
Depth (fsw)	Density (gm/L)	Depth (fsw)	Density (gm/L)	Depth (fsw)	Density (gm/L)
				0	0.39
		0	0.78		
0	1.15	18	1.07	167	1.20
				400	3.08
100	4.48	200	4.73		
141	5.86			900	5.65
		272	6.18		
200	7.82			1200	7.15
		400	8.42		
300	11.15				
400	14.49	700	14.37		
		900	18.34		
		1200	25.21		

*Values at body temperature for the breathing mixtures used at each depth.
†Crude neon used was 76.8% neon, 23.2% helium.

digital computer (20). The gas flow values from the MVV maneuver were integrated numerically over time to give the maximum voluntary ventilation rates. The maximum expiratory flows were ascertained from the FVC maneuvers, and the flows from these maneuvers were also integrated over time to determine the lung volumes at which each of the flows occurred. The tidal breathing maneuvers were used to determine flow resistance at each condition. This was done by subtracting the calculated dynamic compliance and calculated inertance (derived by the method of Mead [14]) from the transpulmonary pressure and dividing that value by the measured gas flow (16). Reported values of resistance are actually averages of resistances obtained for flows ± 0.125 L/sec about the given flow. Thus, for example, the resistances specified for a flow of 1 L/sec really include resistances for flows from 0.875 through 1.125 L/sec. Inspiration and expiration are treated separately.

Results

As the density of the breathing gas increased, airway resistance—particularly during expiration—increased, while the maximum voluntary ventilation and maximum expiratory flow decreased. The magnitude of these effects appeared to be influenced by the density and not by the particular diluent gas-pressure combination which provided that density. In spite of these changes in pulmonary function, even the greatest gas density did not prevent substantial ventilation for short periods of time. The specific influences observed in each of the experimental maneuvers are described below.

Forced Expiratory Vital Capacity

As the density of the gas breathed increased from sea level values to about 5 gm/L, peak expiratory flows of both subjects fell rapidly. At greater densities, the rate of reduction in peak flows was much less. Thus about 70% of the total flow reduction caused by increasing density from 1 gm/L to 25 gm/L had occurred by a density of 5 gm/l. Figure 1 shows the values of peak expiratory flows as a function of gas density.

The function which best relates peak flows to density is a power function:

$$\dot{V}_{E\,max} = A\varrho^{B} \tag{1}$$

Values for the coefficients (A) and exponents (B) calculated by the least-squares method for this function are 8.116 and -0.257 for subject II and 10.488 and -0.328 for subject X. Peak expiratory flow occurred at about 92% of the vital capacity for each subject, except at the very lowest densities (those attained by administering helium or neon under normal sea level conditions) where the peak flow was reached at about 82% of the vital capacity. The decrease in peak flow is apparently a function of density alone, as the values for each gas appear to be randomly distributed about the regression lines rather than stratified according to species (Fig. 1).

Maximum Voluntary Ventilation

The relationships of the maximum breathing capacity of each subject to density are shown in Fig. 2. As with peak expiratory flow, the striking feature of these results is that, at high density, there was almost no further reduction in short-term ventilatory ability as gas density

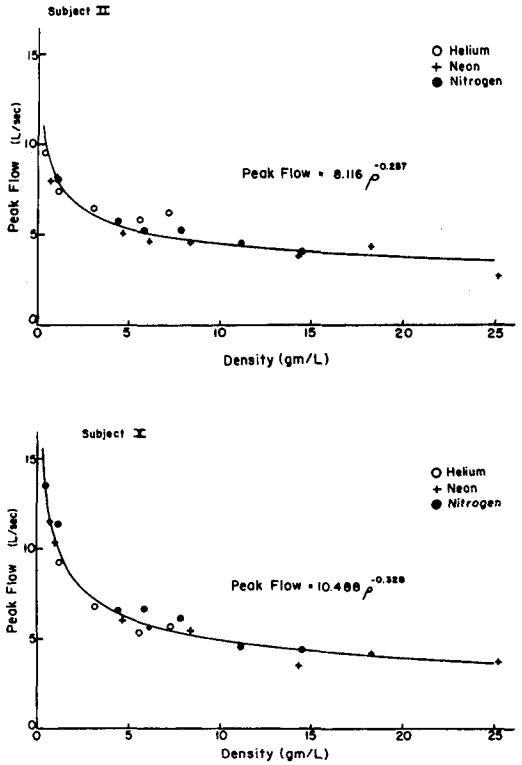

FIG. 1. Peak expiratory flow as a function of density.

increased. At an extreme density of 25 gm/L the maximum breathing capacity of the subjects was still about 50 L/min.

The function which best relates the results of the MVV measurements to the different experimental conditions was also found to be a power function:

$$\text{MVV} = A\varrho^{B} \tag{2}$$

Values for the coefficients (A) and the exponents (B) calculated by the least-squares method for this function are 117.9 and -0.270 for Subject II and 139.0 and -0.298 for Subject X, respectively.

TIDAL BREATHING

The results from the tidal breathing maneuver include values for non-elastic or gas flow resistance and inertance. Linear regression lines relating inspiratory and expiratory non-elastic resistances to density for the flow ranges with median values of 1 and 2 L/sec are shown in Fig. 3.

At a flow rate of 1 L/sec, the inspiratory and expiratory resistances were nearly the same in the low density conditions. However, expiratory resistance increased to a much greater extent than did inspiratory resistance as the density of the breathing mixture was increased.

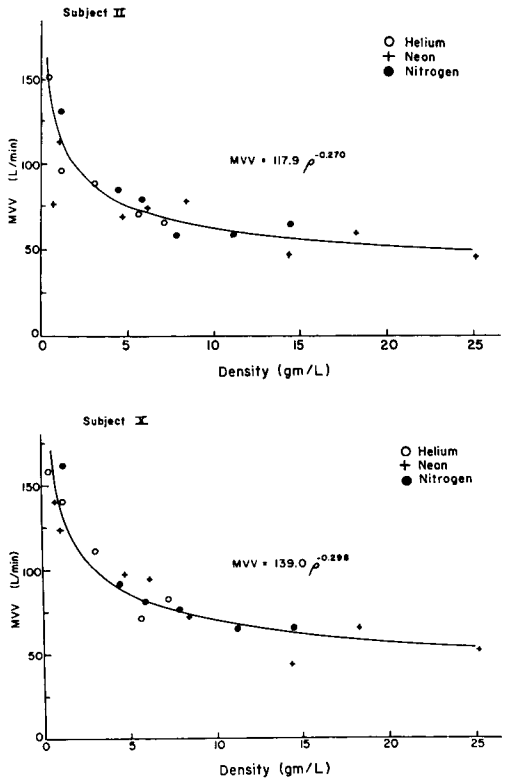

FIG. 2. Maximum voluntary ventilation as a function of density.

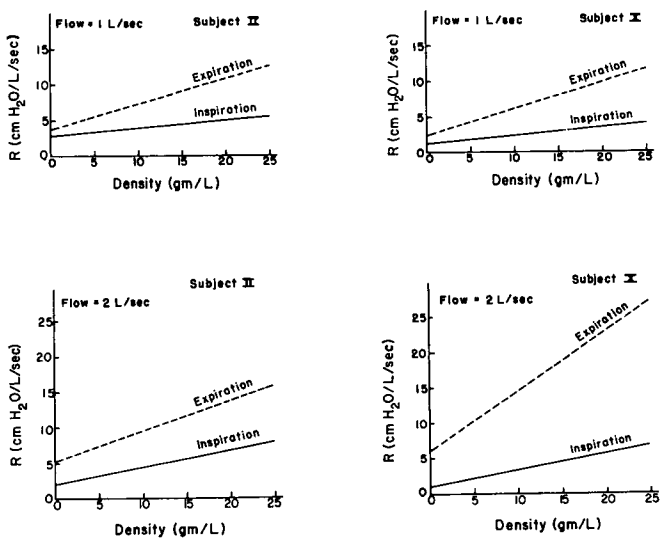

FIG. 3. Non-elastic resistances during inspiration and expiration at flows of 1 and 2 L/sec as a function of density.

At a flow rate of 2 L/sec, the same general pattern was followed. However, expiratory resistance was several times as large as inspiratory resistance for the lower density conditions, and the rate of resistance increase with density was twice the increase at the lower flow rate.

As with the MVV and maximum expiratory flows, there are no obvious differences in the non-elastic resistance-density relationships for the different inert gases. However, the data have considerably more scatter than the FVC and MVV data have.

The inertial pressure drops necessary for the calculation of non-elastic resistance were based upon the instantaneous acceleration and the value of inertance for each specific experimental condition. The inertance values used were derived from a least-squares linear regression analysis relating inertance to density. The parameters describing the relationship are shown in Fig. 4 with the inertance values for the individual experimental conditions.

Discussion

The flow resistance, maximum voluntary ventilation and maximum expiratory flow measurements made in this study appreciably extend the breathing gas density span over which such measurements have been made. Qualitatively, these results match previous work. Flow

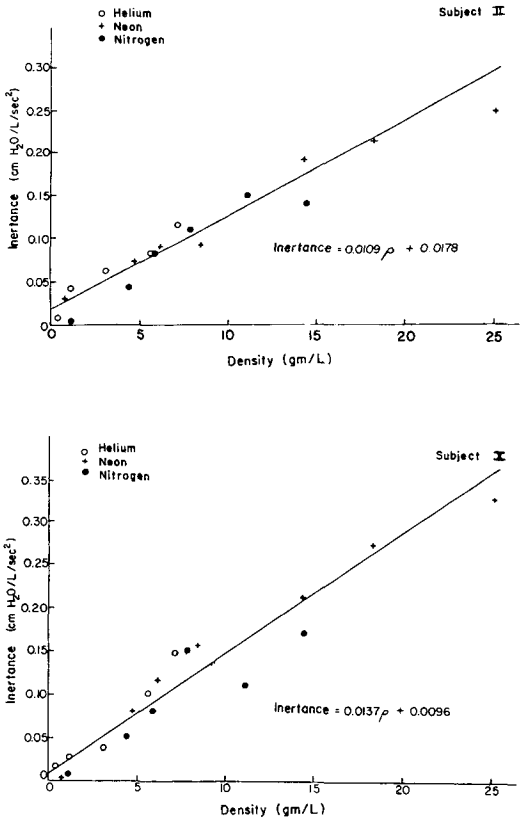

FIG. 4. Average inertance values plotted against density of the breathing gas.

resistance increased with density in a linear manner over the entire density range studied. Maximum voluntary ventilation and maximum expiratory flow decreased sharply as the density was increased from 1 gm/L to 5 gm/L, but the rate of decrement in performance declined appreciably as the density was increased above 5 gm/L. However, the magnitude of the changes measured in pulmonary mechanical function from a density of 1 gm/L to one of 25 gm/L, and the impact these changes had on exercise capacity were not as great as had been expected during planning for the study.

Tidal Breathing–Flow Resistance

The non-elastic resistance of the subjects was similar to previous measurements in the lower density range (3, 12, 13, 23). Although non-elastic resistance increased steadily with density, there was only a threefold increase in resistance during quiet breathing over the entire range of densities. Such an increase in resistance is smaller than that required to produce symptoms of dyspnea in patients with obstructive pulmonary disease or asthma. This level of increase in resistance in acute exposures produced no measurable increase in resting O_2 consumption, no subjective difficulty in breathing, and no change in respiratory pattern toward slower, deeper breathing which is often seen with artificially imposed external breathing resistance.

During periods of hyperpnea, the effect of gas density became more prominent. The greater the increase in ventilation, and hence the greater the gas flow level, the greater was the influence of gas density on pulmonary mechanical function. Thus at a flow rate of 2 L/sec the rate of change of non-elastic resistance with changes in density was twice that found at a flow of 1 L/sec.

Maximum Voluntary Ventilation

Maximum voluntary ventilation is normally performed by a subject breathing in a volume range in which the maximum expiratory flows attainable are limited by airway compression. The gas flows obtained in this maneuver correspond closely to those obtained at the same lung volumes during a forced vital capacity maneuver (15). Consequently, any factor influencing maximum gas flow should have a similar effect on maximum voluntary ventilation. This was found to be the case; a more detailed consideration of the MVV results follows.

Forced Vital Capacity–Maximum Expiratory Flow Rates

Airway compression during expiration (1, 19, 25) may be responsible for reductions of maximum expiratory flow and maximum voluntary ventilation caused by increased gas density (1, 2, 4, 7, 10, 12, 13, 17, 18, 19, 21, 22, 24, 25, 27). Wood and Bryan (26) hypothesized that increased gas density reduced maximum flow in accordance with its effects on the factors (convective acceleration, turbulent flow and laminar flow) determining the resistance of the upstream airways. Of these, convective acceleration is the most important factor in determining the maximum flow at sea level conditions and for lung volumes above 40% of vital capacity; turbulent flow is of lesser significance in the same circumstances (11). Thus, at high lung volumes the maximum flow was expected to be proportional to density raised to a power between -0.5 (convective acceleration) and -0.428 (turbulent flow). At low lung volumes, the density exponent was expected to approach zero (laminar flow) and this was indeed found by Wood and Bryan (26) and Anthonisen et al. (1). Also, in many

cases, the density exponents calculated from maximum expiratory flow, and MVV data in the literature correspond well with the concepts for the determination of the maximum flow outlined above. However, the density exponent values for the maximum flows and maximum voluntary ventilations for subjects II and X are closer to zero than many of the other values cited or calculated from the literature. A less negative density exponent signifies less reduction in maximum voluntary ventilation or maximum expiratory flow with density increases than in cases where the density exponent has a larger absolute value. The density exponents for subjects II and X are also closer to zero than -0.428 and thus ventilatory capacity was greater than what would be expected for a turbulent flow-limiting circumstance.

Influence of Duration of Exposure on the Effects of Increased Gas Density

The disagreement between these results and prior theoretical analysis (26) can be partially explained by comparing measurements of peak expiratory flows and maximum voluntary ventilations made during conditions of prolonged exposure to increased gas density (saturation conditions) with the same measurements made during short duration exposures. Schaefer et al. (21), Hamilton et al. (7) and Dougherty and Schaefer (4) have all shown that there is recovery of ventilatory ability and maximum expiratory flows during a prolonged period at one depth. Under some circumstances a very substantial part (44%) of the ventilatory impairment from increased gas density due to compression has been recovered without reduction of the density which induced the loss (21). The measurements in this study were made ½ to 2½ days after any change in pressure and all pressure changes were made slowly. Thus, they can be classified with the other measurements made after a prolonged period at one ambient pressure.

From the discussion above, it seems likely that the relationships of maximum flow and maximum voluntary ventilation to density are dependent to some extent on the circumstances of the pressure exposure. The density exponents of these relationships should be smaller (more negative) for short exposures (or for short exposures with elevated oxygen tensions) than for longer exposures. In fact, the values of the density exponents for the maximum flow- and MVV-density relationships measured during prolonged pressure exposure are nearer to zero than would have been predicted from the theoretical analysis of density effects on flow limitation (26). The importance of this finding is that maximum expiratory gas-flow and thus maximum ventilatory rates in prolonged pressure exposures are affected less by density increases than had previously been predicted.

Conclusions

Validity of Extrapolation

Based on the findings of this study, it is reasonable to extrapolate pulmonary mechanical function from high density-low pressure to low density-high pressure conditions. For peak expiratory flows, maximum voluntary ventilation and flow resistance there is a good correlation with density and there is no indication of separation according to the inert gas species being breathed. The significance of this finding is that experiments done at obtainable and safe pressures can be used to predict pulmonary function in circumstances unobtainable because of chamber limitations.

Cause of Ventilatory Restriction

For the acute circumstances investigated in this pulmonary function study, ventilatory restriction followed effort-independent expiratory flow-limitation quite closely. Even though flow resistance increased linearly with density, the resistances did not become great enough to prevent high ventilatory rates from being achieved for brief periods of time. Based on the integration of the pulmonary function results with the results of the associated exercise study on the same subjects (9), we would expect extremely heavy exercise to be prevented by expiratory flow limitation at densities greater than about 5 gm/L. Brief periods of medium to heavy work, however, may never be restricted by expiratory flow limitation, even at densities far in excess of 25 gm/L. On the other hand, increased density of breathing gas and the associated increase in flow resistance may limit the duration of useful work by causing excessive fatigue of the respiratory muscles. The data do not permit quantitative description of this effect.

Conditions Producing Failure

The pulmonary mechanical limitation imposed by the environmental conditions of this study was sufficient to restrict the work capacity of the subjects at extreme levels of exertion (9). This limitation, however, changed very little with breathing densities greater than 10 gm/L and the ventilatory capacity at the most severe condition (crude neon at 1200 fsw) was sufficient to support a work level of 900 kg·m/min for the planned short period of time. As the density of this latter condition (25 gm/L) is equivalent to the density of normoxic helium at 5000 fsw, and extrapolation based on equal density conditions appears valid, it is believed that the pulmonary mechanical function of an unencumbered man will be sufficient at depths up to 5000 fsw to support useful activity. Because of the relationships between maximum voluntary ventilation and density and between maximum expiratory flow and density, it also appears that adequate ventilation can be achieved at densities greater than 25 gm/L and therefore at depths even greater than 5000 fsw. Thus, for practical purposes, if man is given an adequate, low resistance breathing device, his pulmonary mechanical function should not limit the depth to which he can dive.

Acknowledgments

The specific work reported here was performed at the Institute for Environmental Medicine, University of Pennsylvania, as a component of its Predictive Studies III Program, with the direct collaboration of Dr. C. J. Lambertsen and R. Gelfand. The presentors are indebted to Mr. J. Forster and Mr. J. Rosowski for their expert assistance during the experiments in data acquisition and recording and subsequently in data processing. This study was supported by contract HL 08899-08 from the National Institutes of Health, and contract N00014-67-A-0216-0026 with the Office of Naval Research.

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RESPIRATORY FUNCTION DURING A SIMULATED SATURATION DIVE TO 51 ATA (500 METERS) WITH A HELIUM-OXYGEN MIXTURE

B. Broussolle, J. Chouteau, R. Hyacinthe, J. Le Pechon, H. Burnet, A. Battesti, D. Cresson and G. Imbert

A series of experiments of simulated saturation dives with helium-oxygen (P_{IO_2} = 300 mm Hg) was performed with a two-diver team, to 26 and 41 ata respectively. Carbon dioxide retention at a workload of 110 watts at 26 ata and at a workload of 55 watts at 41 ata was not observed.

This study presents the results of a third experimental dive to 51 ata for 18 hours at that depth, followed by a stop at 41 ata for 27 hours. The essential theme of the simulated dive was the study of respiratory function by gas exchange measurements on the one hand, and by measurements of ventilatory mechanics on the other. The object was to elucidate eventual mechanical limitations to pulmonary ventilation which could reduce the muscular work capacity at such pressures. Monitoring of electrocardiogram and EEG of the two divers was performed throughout, and quantitative measurements of these were made before, during and after the dive. However, only the results of the respiratory physiological measurements are reported in this paper.

Conditions of the Dive

The experiment took place in the simulated diving complex of the Advanced Naval Studies Centre (CEMA, Marseille). The respiratory physiological measurements were performed by the Naval Bio-physiology Research Centre (CERB, Toulon). The actual pressure-time profile of the dive is shown in Fig. 1.

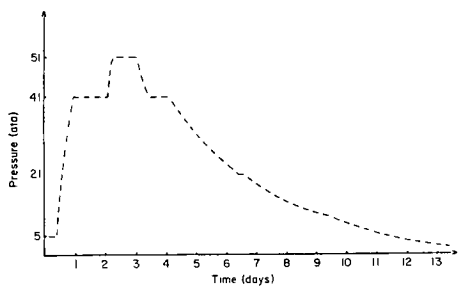

FIG. 1. Dive profile to 41 and 51 ata. Saturation III—CEMA-CERB.

During the dive, the P_{IO_2} was maintained at 300 mm Hg ± 3 (400 millibars), relative humidity at about 50%, temperature at 30°C, and chamber P_{ICO_2} was always less than 0.5 mm Hg. The physiological measurements were performed 12 hours after arriving at 41 and 51 ata. For comparison, similar measurements were performed in previous weeks with air at sea level and with a helium-oxygen mixture at 3.5 ata, the relative density of the mixture being equivalent to that of air at sea level, but with the P_{IO_2} maintained at 300 mm Hg.

Experimental Subjects

Both subjects were experienced divers of the CEMA staff: R. G., 30 years old who had participated in the 250-meter saturation diving experiment in 1970, and Y. O., who had participated as a diver and cameraman in Cousteau's pre-continent experiment. Their basic biometric data are summarized in Table I.

TABLE I

BIOMETRIC DATA

Subject	Height (m)	Weight (kg)	Age (yrs)	V.C. (L_{BTPS})	F.E.V.$_1$ (L/sec, BTPS)	R.V. (L_{BTPS})	$\frac{\text{F.E.V.}_1}{\text{V.C.}}$	M.V.V. (L/min, BTPS)
R.G.	1.66	72	30	5.34	4.27	1.69	0.80	122.9
Y.O.	1.72	60	30	5.59	4.07	1.89	0.73	111.3

Gas Exchange Measurements

The following parameters were measured or calculated at rest or at work:

$\dot{V}_E$ = Expiratory flow in liters/minute BTPS

f = Respiratory rate/minute

$\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ = CO_2 production and O_2 uptake in liters/minute STPD

P_{ACO_2} and P_{AO_2} = Alveolar partial pressures of CO_2 and of O_2 in mm Hg, respectively

P_{ICO_2} and P_{IO_2} = Inspired partial pressures of CO_2 and of O_2, respectively

P_{ECO_2} and P_{EO_2} = Partial pressures of CO_2 and O_2 in expired gases.

PROCEDURE OF MEASUREMENT

The same procedure was employed at each pressure at which measurements were made, i.e., 1 ata air and 3.5, 41, and 51 ata helium-oxygen. A first set of measurements was taken at rest followed by a second set at a muscular workload level of 110 watts, using an ergometer of our own design. The subject sits on a seat operating pedals with his legs which stretch rubber bands. The workload required to stretch the rubber bands had been previously

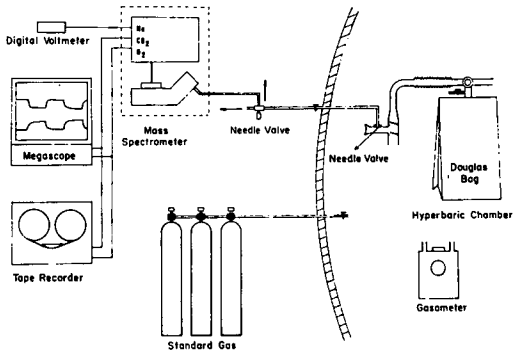

FIG. 2. Experimental system for respiratory measurements.

established. The pedalling rhythm was provided by a metronome. The bands were chosen so that the work done by their lengthening and the frequency of pedalling gave a total workload equivalent to 110 watts. This is roughly equivalent to the power produced by an underwater swimmer using fins and underwater-breathing apparatus at a speed of 1200 meters/hour.

For each group of measurements performed at rest or at work the subject inspired the gas mixture in the chamber through a low resistance double valve. Expiratory gases passed through a three-way cock to a Douglas bag via large bore ring tubing. The volume of gas in the bag collected during a given period was measured by dry gas meter at the end of the experiment.

A small needle valve inserted into the mouthpiece allowed part of the expired gas to be collected, passed out through the chamber wall through a fine copper tube connected to a small needle valve located outside the chamber. By means of appropriate connections continuous gas analysis was facilitated by the use of a Thomson CSF SM 100 R rapid response mass spectrometer (response time, 60 milliseconds). The mass spectrometer employs four fixed collectors for oxygen, CO_2, nitrogen and helium. The helium signal is displayed on a digital voltmeter the output of which controls the stability of sampling, while oxygen and CO_2 signals are displayed on a cathode ray oscillograph for constant monitoring, and also on magnetic tape for subsequent analysis. The values for end-tidal P_{CO_2} and P_{O_2} have been taken to represent alveolar P_{CO_2} and P_{O_2} (P_{ACO_2} and P_{AO_2}). A 1-liter sample of mixed expired gas was collected from the Douglas bag in a balloon, passed through the collecting system and analyzed, thus obtaining P_{ECO_2} and P_{EO_2}. The chamber gas was also analyzed to obtain P_{ICO_2} and P_{IO_2}. Calibration of the mass spectrometer was performed using three standard gas mixtures.

Having measured the expired volume for the collection period ($\dot{V}_E$), CO_2 production was calculated as follows (Eq. 1):

$$\dot{V}_{CO_2} \text{ liters/minute STPD} = (\dot{V}_E \text{ liters ATPS}) \text{ (coefficient STPD) } (F_{ECO_2} - F_{ICO_2}) \quad (1)$$

The respiratory quotient or gas exchange ratio (R) was calculated:

$$R = \frac{P_{ECO_2} - (P_{ECO_2} \times F_{IO_2})}{P_{IO_2} - P_{ECO_2} - (P_{ECO_2} \times F_{IO_2})} \quad (2)$$

From the calculations of $\dot{V}_{CO_2}$ and R, $\dot{V}_{O_2}$ was estimated:

$$\dot{V}_{O_2} \text{ liters/minute STPD} = \dot{V}_{CO_2}/R \quad (3)$$

Alveolar ventilation was calculated, using an estimation of total (instrument and anatomical) dead space, as being 200 ml:

$$\dot{V}_A = \dot{V}_E - (200 \times f/1000) \quad (4)$$

Expired gas collections during rest and exercise were made for the final 2 minutes of an 8-minute period. The EKG was recorded throughout the whole period. A trial period of exercise at 110 watts was carried out for 10 minutes under EKG control at 51 ata, since it was the first time such work had been performed at such depth. The actual respiratory measurements were made during the subsequent test and recovery period, again performed under EKG control. For both exercise runs at 41 and 51 ata, only subject R. G. performed the 110-watt load. Subject Y. O. had developed articular pains with joint crepitations, classically related to helium influx but favored by an old joint injury. His exercise was restricted to 55 watts only.

Results. In Figs. 3, 4 and 5 the values for CO_2 production ($\dot{V}_{CO_2}$), ($\dot{V}_{O_2}$) oxygen consumption, and $P_{A_{CO_2}}$ are presented. The CO_2 output and oxygen uptakes were slightly less at 3.5

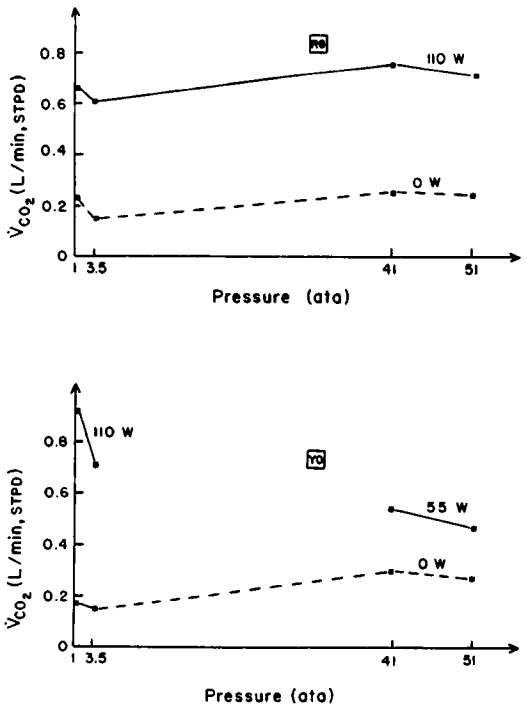

FIG. 3. CO_2 production at increased ambient pressure. Dashed line shows resting state; solid line shows diver at work.

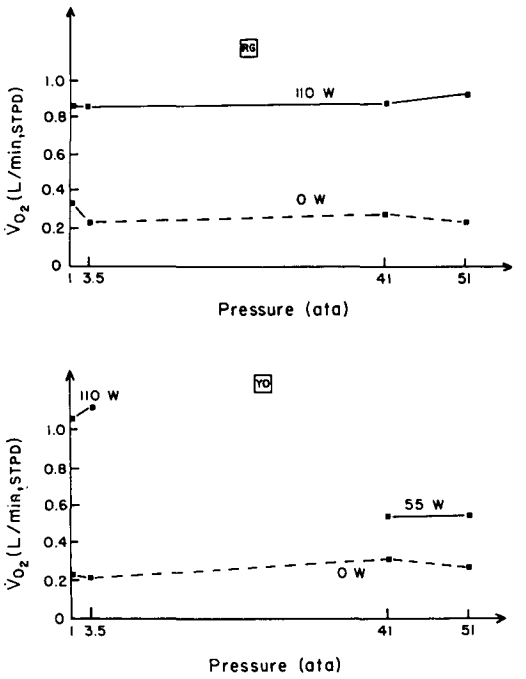

FIG. 4. Oxygen consumption at pressure. Dashed line indicates resting state; solid line shows diver at work.

ata with helium-oxygen than at 1 ata with air. This can be explained by the difference in thermal conditions: namely, 20°C and reduced humidity at 1 ata with air, 33°C and 50% humidity at 3.5 ata with oxyhelium. In addition, the $P_{I_{O_2}}$ at 3.5 ata was twice that at 1 ata in air. The $\dot{V}_{CO_2}$ and $\dot{V}_{O_2}$ increased slightly when the pressures increased from 3.5 ata to 41 and subsequently to 51 ata, increasing in proportion to the increase in dynamic respiratory work due to the progressive increase in gas mixture density. The $P_{A_{CO_2}}$ levels recorded at rest and at work were slightly higher at 3.5 ata in oxyhelium than in air at 1 ata. It can be seen that subject Y. O.'s $P_{A_{CO_2}}$ during work increased while that of subject R. G. decreased. At 41 ata, when compared with 3.5 ata, Y. O.'s $P_{A_{CO_2}}$ rose by 4 mm Hg at rest and by only 1 mm Hg during work. However, Y. O. performed only 55 watts of work at 41 ata. In subject R. G. the $P_{A_{CO_2}}$ was higher at work than at rest, increasing by 8 mm Hg. At 51 ata, $P_{A_{CO_2}}$ levels differed little from those recorded at 41 ata.

In summary, despite a slight tendency to rise, the $P_{A_{CO_2}}$ never exceeded normal physiological values, demonstrating that carbon dioxide retention is not a problem at 51 ata during work of 110 watts in power. However, at still higher workloads, hypercapnia would be expected due to the limitation imposed on the increase in ventilation by expiratory mechanical factors.

Measurement of Ventilatory Mechanics

Measurements on both subjects were always performed under the same conditions—in the afternoon, at 1 ata in air, and 3.5, 41 and 51 ata in helium-oxygen. Similar measurements

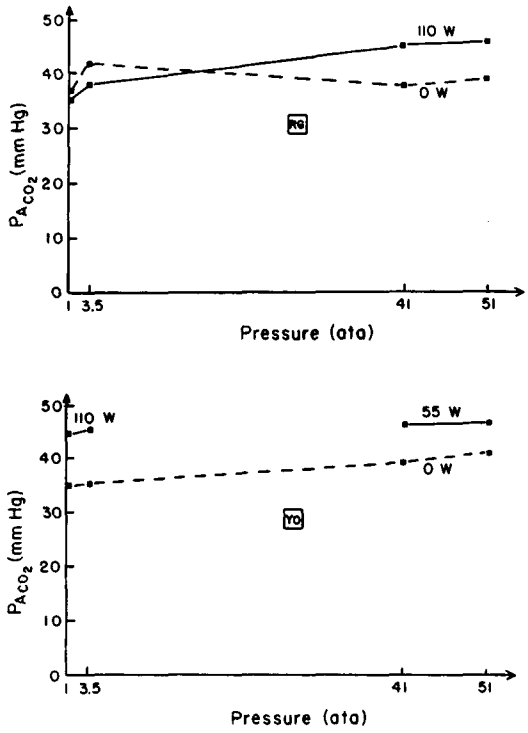

FIG. 5. CO_2 alveolar partial pressure at pressure. Dashed line indicates resting state; solid line shows diver at work.

were performed on subject R. G. during decompression at 21 and 9.7 ata in helium–oxygen. These measurements were not performed on subject Y. O. who developed labyrinthine problems during decompression at 41 ata.

The measurements were made as follows: during normal ventilation at rest; during forced expirations at rest; and during maximum minute ventilation maneuvers (maximum voluntary ventilation).

MEASUREMENTS PERFORMED DURING VENTILATION AT REST

Dynamic lung compliance, dynamic respiratory resistance, and vital capacity were measured. Esophageal pressure measurements were made by esophageal balloon coupled to a pressure transducer. Flow was obtained by a Fleisch no. 1 pneumotachograph coupled to a differential pressure transducer. Outputs of the transducers measuring esophageal pressure, flow and volume, by means of integration of the flow signal, were recorded on magnetic tape and printed out on an ultraviolet photographic recorder. Dynamic compliance was obtained graphically by the classical method, relating tidal volume to the difference in esophageal pressure at the instant of zero flow, at the beginning and end of each breath. Respiratory resistance was obtained by plotting the integral of the difference between esophageal pressure and elastic recoil pressure of the lung with time against the integral of instantaneous flow recorded during each breath.

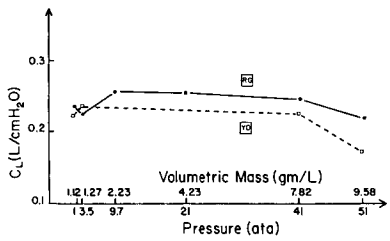

FIG. 6. Lung compliance at increasing pressure or volumetric mass.

Results. Dynamic compliance did not vary significantly between 1, 3.5 and 41 ata. However, at 51 ata there was a slight diminution in dynamic compliance in both subjects, which is significant (Fig. 6).

Dynamic respiratory resistance did not change between 3.5 ata on oxyhelium and 1 ata on air where the volumetric mass is approximately the same (Fig. 7). Subject R. G.'s resistance was somewhat higher than subject Y. O.'s. In both subjects, dynamic respiratory resistance rose regularly and in parallel at each depth at which measurements were performed to 51 ata, where the volumetric mass of the gas breathed was 9.58 g/liter, and density relative to air: 7.54. In R. G., the expiratory resistance increased by a factor of 1.87 in changing from 1 ata air to 51 ata in oxyhelium, and in Y. O. by a factor of 1.64.

The vital capacities of both subjects did not change significantly between sea level and 51 ata regardless of the gas mixture breathed.

MEASUREMENTS PERFORMED DURING FORCED EXPIRATIONS

The techniques employed were the same but a Fleisch no. 4 pneumotachograph was required to cope with the higher flows generated.

Results. The Tiffeneau coefficients (FEV_1/VC) decreased from 0.83 at 1 ata breathing air to 0.57 at 51 ata breathing helium-oxygen in subject R. G., and from 0.81 to 0.66 in subject Y. O. The differences between the two subjects are explicable, since R. G.'s expiratory resistance was the higher of the two.

Instantaneous flow maxima were measured at three levels of the vital capacity (75, 50 and 25%), and are shown as a function of atmospheric pressure and volumetric mass for each subject in Figs. 8 and 9. Values recorded for subject R. G. are shown in Fig. 8 and for Y. O. in Fig. 9. The limitation in instantaneous flow rates is particularly well-demonstrated

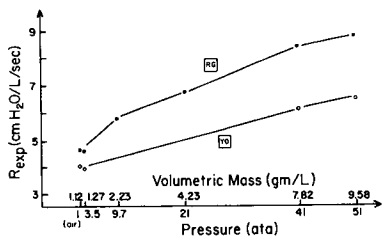

FIG. 7. Expiratory resistances at increasing pressure or volumetric mass.

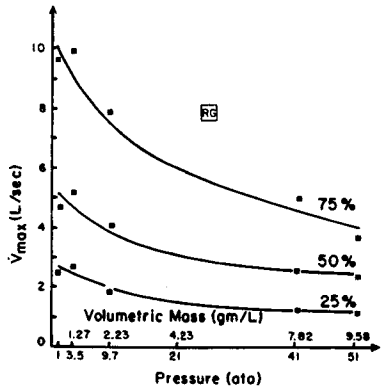

FIG. 8. Maximum expiratory flow-volume curves for three values of vital capacity (75%, 50%, 25%) at different ambient pressures. Subject: R.G.

at high lung volumes: at 75% vital capacity, maximum flow ($\dot{V}_{max}$) decreases from about 10 liters/second at sea level to about 4 liters/second at 51 ata.

Different instantaneous flow values obtained during a series of expiratory maneuvers with decreasing force provide the data for isovolume pressure-flow curves. Figure 10 shows the curves obtained from subject R. G. upon whom more measurements were performed at different pressures. The curves obtained at 75% of the vital capacity are presented, and a progressive decrease in maximum flow can be seen. At ambient pressures of 41 ata and above, expiratory flow reaches a plateau when the esophageal pressure increases above a value called P_{max}. The employment of a higher esophageal pressure than P_{max} allows no greater efficiency since it is not accompanied by a higher flow.

Isovolume pressure-flow curves have been described by numerous authors at atmospheric pressure (3, 5, 6, 9). A maximum flow plateau has never been found at 75% vital capacity in normal subjects at sea level, but it is found in patients with obstructive airway disease. It occurs by a flattening of the bronchiolar lumen when the peribronchic pressure becomes

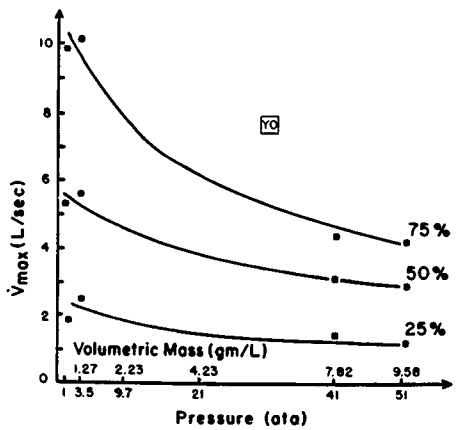

FIG. 9. Maximum expiratory flow-volume curves for three values of vital capacity (75%, 50%, 25%) at different ambient pressures. Subject: Y.O.

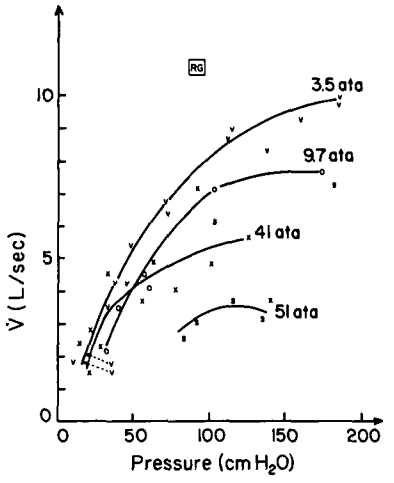

FIG. 10. Isovolume (75% vital capacity) pressure-flow curves at different ambient pressures. Subject: R.G.

higher than the intrabronchic pressure, which progressively diminishes along the airways by the loss of energy. The loss of energy is much greater when the density of the gas mixture is increased.

The study of such isovolume pressure-flow curves on the divers in this study has pointed out once again the important limitation to expiratory flow seen at 41 and 51 ata.

MEASUREMENT OF MAXIMUM VOLUNTARY VENTILATION (MVV)

Measurement of MVV was made at each ambient pressure level by electronic integration of the flows generated during each breath over a 15-second period. The value so obtained is then multiplied by four to obtain the MVV. The decrease in ventilation in the subjects, shown as a function of depth, was practically linear (Fig. 11). At 51 ata, MVV was decreased by 58% for subject R. G. and by 54% for Y. O. This reduction of more than half of the MVV was due to the increase in respiratory resistance and to the decrease in the instantaneous expiratory flow generated by each subject at the increased depth.

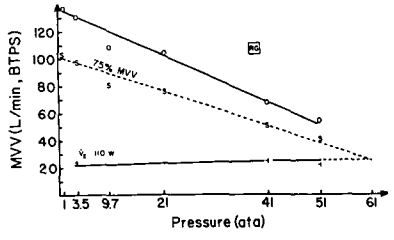

FIG. 11. Maximum voluntary ventilation (MVV) and ventilation $\dot{V}_E$ at workload of 110 watts. Variations with increasing ambient pressure. Subject: R.G.

Discussion

The results obtained during this simulated dive to 51 ata may be compared with those obtained by us and by other investigators at roughly the same relative gas density, i.e., air at 7.5 ata, or with the same helium–oxygen mixture at different absolute pressures. First the gas exchange measurements made will be discussed and then the ventilatory mechanics.

Gas Exchange at Rest and at Work

A moderate increase in $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ with depth at rest and at work has already been described at 7 ata in air (2), and in helium–oxygen mixtures at 600 feet (182 meters) (4) and at 1000 feet (304 meters) (11). The nature of this increase has already been explained.

Alveolar P_{CO_2} levels (P_{ACO_2}) increased slightly in these experiments but not as much as was seen in experiments at 7 ata in air. However, in the 7 ata experiments the P_{IO_2} was about 1500 millibars as opposed to the 400 millibars P_{IO_2} employed in these helium–oxygen experiments. There was no evidence of CO_2 retention at 51 ata during the work level performed, indicating that there was no mechanical limitation to the ventilation (2, 10).

Ventilatory Mechanics

Measurements of dynamic respiratory resistance under hyperbaric conditions have been performed in air to 7 ata. In comparing the measurements performed by Varène (12) in helium–oxygen at 47 ata, the increase in dynamic respiratory resistance over that measured at sea level is less than might be expected (a factor of 1.42). These studies confirm the findings at 51 ata (a factor of 1.76) and at 41 ata (a factor of 1.45). It is generally assumed, that respiratory resistance varies as the square root of the density relative to air (8). At 51 ata where the relative density was 7.54 the factor should have been 2.74 instead of 1.76. Therefore measured respiratory resistances are less at 51 ata in helium–oxygen than would be predicted by calculation from the generally accepted formula. Impairment in breathing afforded by resistances such as those measured is little for the moderate work performed, since, as Marshall et al. (7) have stated, it is necessary for respiratory resistance to increase by a factor of 3 before a subject will perceive any respiratory embarrassment. However, the increase in resistance has an unfavorable influence upon those ventilatory tests which require high flow rates, for example: MVV and isovolume pressure–flow curves. The MVV is reduced at 51 ata by about 57%, and the reduction is of the same order as that measured by Wood in air at 7 ata, the relative at 51 ata being 7.54.

Maximum expiratory flow rates, expressed as a function of absolute pressure at different values of vital capacity, show that expiratory flow becomes very much reduced, particularly at lung volumes greater than 25% vital capacity. At this volume expiratory flow was 1.51 liter/second at 51 ata. As Varène has noted (12), maximum instantaneous expiratory flow required to maintain pulmonary ventilation at a workload of 100 watts is about 1.5 liter/second. It can be seen, therefore, that the diver is obliged to ventilate his lungs at a lung volume range which does not fall below 25% of the vital capacity.

Conclusion

Despite important reductions in ventilatory capacity at 51 ata, no problems in gas exchange

for the work level performed by these divers were observed. There was no mechanical limitation to the ventilation because the expiratory flows generated during this work did not reach the values obtained during maximum voluntary ventilations. However, by extrapolating the curves obtained from subject R. G. it can be demonstrated that the straight line represented by 75% of the MVV (i.e., maximum ventilation available for a steady-state effort) and the straight line showing the $\dot{V}_E$ at a workload of 110 watts intersect at about 61 ata. At 600 meters, therefore, mechanical limitation of ventilation will no doubt be reached very soon at such workloads.

It is necessary to emphasize that care be taken to select divers on a basis of respiratory function. It should be worthwhile to choose divers whose pulmonary ventilation during heavy work represents as low a fraction of the maximum ventilation as possible.

ACKNOWLEDGMENTS

We wish to thank Dr. J. N. Miller of the Royal Naval Physiological Laboratory for his assistance in preparing this manuscript in English, and Surgeon Commander E. E. P. Barnard for presenting it in English for us.

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LIQUID BREATHING: EXPIRATORY FLOW AND CO_2 ELIMINATION USING FLUOROCARBON AND AQUEOUS SOLUTIONS

W. H. Schoenfisch, G. D. Blenkarn, B. A. Hills and J. A. Kylstra

The maintenance of a normal P_{aCO_2} in liquid-breathing mammals has been a major problem thus far, and inadequate CO_2 elimination through liquid-filled lungs would seem to preclude the application of liquid breathing in man. Experimental evidence is presented here which indicates that maintenance of a normal P_{aCO_2} at moderate levels of exercise should be feasible in liquid-breathing divers if a suitable breathing fluid is used.

The elimination of CO_2 from liquid-ventilated lungs is dependent upon the solubility of CO_2 in the liquid (α_{CO_2}) and the effective alveolar ventilation ($\dot{V}_A^e$). Under steady-state conditions when there is no CO_2 in the inspired liquid:

$$\dot{V}_{CO_2} = P_{aCO_2} \cdot \alpha_{CO_2} \cdot \dot{V}_A^e \quad (1)$$

where the units of $\dot{V}_{CO_2}$, P_{aCO_2}, α_{CO_2} and $\dot{V}_A^e$ are ml (STPD)/min; mmHg; ml (STPD) ml^{-1} $mmHg^{-1}$; and ml/min, respectively, and $\dot{V}_A^e$ = the virtual volume of exhaled liquid in which P_{ECO_2} is uniformly equal to P_{aCO_2}.

If CO_2 elimination in liquid-filled lungs is inadequate, as evidenced by a greater than normal P_{aCO_2}, either $\dot{V}_A^e$ or α_{CO_2} or both must be deficient. $\dot{V}_A^e$ is determined by alveolar ventilation ($\dot{V}_A$), the balance of ventilation and blood flow, and the time allotted for diffusive mixing within the liquid-filled alveoli (5).

Clearly, in order to obtain an adequate CO_2 elimination during liquid breathing, an effort must be made to increase α_{CO_2}, $\dot{V}_A^e$, or both. This paper reports on experiments undertaken to 1) estimate the maximum feasible minute ventilation ($\dot{V}_{E_{max}}$) of liquid in man; 2) estimate $\dot{V}_{CO_2}$ at $\dot{V}_{E_{max}}$ and a normal P_{aCO_2}, using aqueous solutions or a synthetic perfluoronated hydrocarbon; 3) evaluate the merits of an organic buffer (THAM) as a means of increasing α_{CO_2} and 4) assess CO_2 diffusion in the liquid-filled human lung.

Methods and Materials

Pressure-Volume and Volume-Flow Relationships of Liquid-Filled Lungs

Pressure-volume and volume-flow relationships of air- and liquid-filled lungs were deter-

mined by volume displacement plethysmography in excised dogs lungs. The sequence of experimental procedures and measurements was the following: 1) quasi-static pressure-volume relationship of the air-filled lung; 2) relationship between lung volume and maximal expiratory flow of air; 3) quasi-static pressure-volume relationship of the same lung filled with liquid; and 4) relationship between lung volume and maximal expiratory flow of liquid. The plethysmograph and the technique used for these measurements have been described in detail elsewhere (8, 12).

CO_2 Content of THAM Solutions Equilibrated with CO_2

Solutions (0.1 *M* and 0.3 *M*) of THAM (Tris(Hydroxymethyl)Aminomethane*), titrated with 6 *N* HCl to a pH = 7.4 at 37°C, were equilibrated with CO_2 at partial pressures ranging from 7 to 64 mmHg, and the CO_2 content of samples of the equilibrated solution was then measured by the Van Slyke manometric technique.

Use of 0.1 *M* THAM Solution as Breathing Fluid in Dogs

Anesthetized dogs were intubated in such a way that their right and left lungs were functionally separated. They were ventilated with 100% oxygen for approximately 1 hour and then rendered apneic by intravenously injecting a muscle relaxing agent.

The experiments were done in a hyperbaric chamber. During pressurization of the chamber to 58.5 p.s.i. (abs), the gas in the right lung was replaced by 500 ml of an isotonic 0.1 *M* THAM solution (pH 7.4) at a rate of 50 ml/min. When priming of the right lung was complete, ventilation of the left lung with O_2 was discontinued. The oxygen-filled left lung was maintained at a pressure of 20 cm H_2O greater than the pressure in the chamber for the remainder of the experiment. Tidal volumes of 500 ml of the prewarmed THAM solution were alternately infused into and drained from the right lung by gravity. The duration of the "respiratory" cycle (i.e., the time from the start of the infusion to the end of draining of a tidal volume) was measured with a stopwatch. Oxygen and carbon dioxide content and partial pressures as well as pH of mixed venous and arterial blood were measured with electrodes and the Van Slyke manometric method. Mixed "expired" CO_2 content was measured with the Van Slyke technique.

CO_2 Diffusion and Expiratory Flow in Saline-Filled Human Lungs

These measurements were made in patients suffering from alveolar proteinosis who were treated with lung lavage. The technique of volume-controlled lung lavage has been described in detail elsewhere (7). Briefly, the patients were anesthetized and intubated with a Carlens double-lumen bronchospirometry catheter. The functional residual capacity of gas in one of the patient's lungs was gradually replaced by a volume of prewarmed normal saline. Once the lung had been thus "primed," tidal volumes of 500 ml prewarmed normal saline were then alternately infused into and drained from the lung by gravity, while the other lung was ventilated with a mixture of oxygen and anesthetic gas (Fig. 1).

Samples of expired liquid were withdrawn at various distances along the outflow tube and partial pressures of carbon dioxide in arterial blood and expired liquid samples were

*Fisher Scientific Co., Raleigh, N.C.

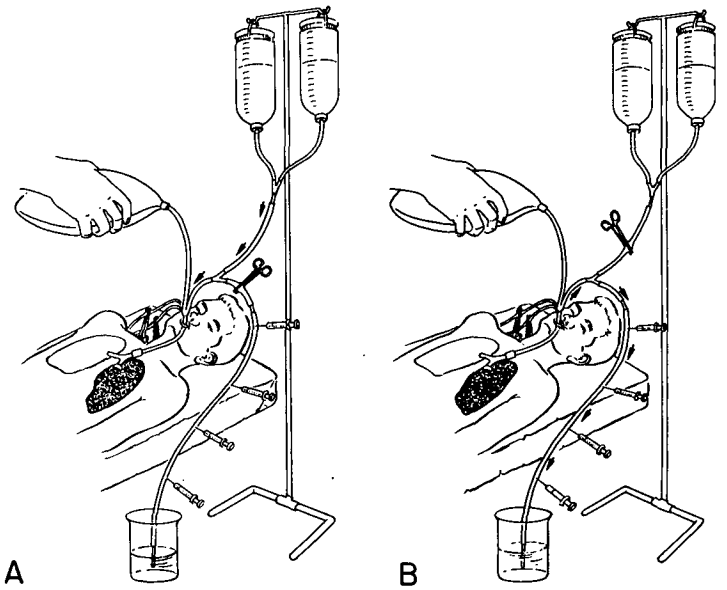

FIG. 1. Experimental setup for diffusion studies in human subjects. **A**, Tidal volume of saline enters lung. **B**, Tidal volume of saline drains from lung.

measured with a Severinghaus electrode. The time required for "inspiration" and "expiration" of a tidal volume was measured with a stopwatch.

Results

AIR AND LIQUID PRESSURE-VOLUME AND VOLUME-FLOW MEASUREMENTS

Figure 2 shows the quasi-static pressure-volume curves for the excised lungs of two dogs, one filled with air and then saline, the other filled with air and then fluorocarbon. Figure 3 is a maximum flow-volume record for a lung first filled with air and then saline. It was constructed by taking simultaneous flow and volume points from the air and saline expiration recordings at a transpulmonary pressure of 50 cm H_2O.

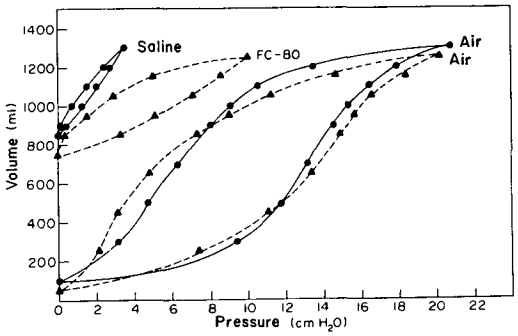

FIG. 2. Quasi-static pressure volume curves of saline, FC-80 fluorocarbon, and air-filled lungs of two dogs. (Adapted from [8].)

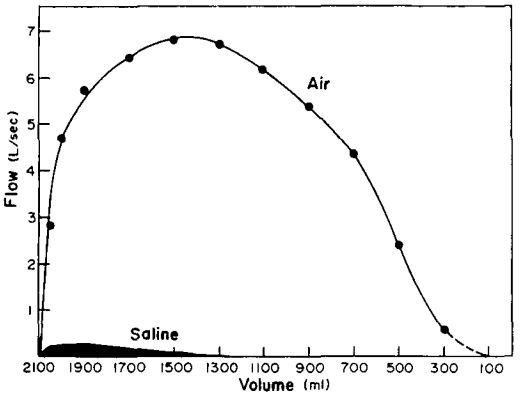

FIG. 3. Flow-volume curves comparing maximal expiratory flow of air and saline in the same lung.

Table I presents the total volume of liquid drained from the lungs at a transpulmonary pressure of 50 cm H_2O, and the time interval between the beginning and cessation of flow. The "end-inspiratory" lung volume is the volume at which the quasi-static recoil pressure (P_{stat}) of the same lung filled with air was 20 cm H_2O. This volume ranged from 800 to 2050 ml for the seven dogs reported here. The total volumes of liquid "exhaled," which ranged from 310 to 850 ml, were not directly related to the end-inspiratory lung volumes. The time required for the first 50% of the total volume of liquid to be exhaled was read directly from the oscilloscope tracing.

Table II presents typical results obtained with a saline-filled lung. The exhaled volume and time required for expiration of that volume (t_{exp}) were taken from the volume-time recording. Assuming the time required for inspiration to be equal to the time required for

TABLE I

VOLUME AND FLOW DATA FOR LIQUID-FILLED, EXCISED DOGS' LUNGS

Dog No.	Weight (kg)	V[a] (ml)	P_{stat}[b] (cm H_2O)	V_{exp}[c] (ml)	t_{exp}[d] (sec)	$t_{exp0.5}$[e] (sec)
1-S[f]	16	2050	—	850	14.7	2.7
2-S	15	1200	—	650	13.6	2.8
3-S	15	1240	3.5	310	10.4	1.9
4-S	15	850	5.0	650	12.7	2.5
5-F[g]	15	1250	10.0	340	13.7	3.3
6-F	15	800	7.7	450	10.0	1.7
7-F	17	1080	12.0	800	13.3	3.1

P_{pl} = 50 cm H_2O.

[a] V = end-inspiratory volume.
[b] P_{stat} = static recoil pressure of liquid-filled lung at V
[c] V_{exp} = total volume of liquid exhaled
[d] t_{exp} = time required for exhalation of V_{exp}
[e] $t_{exp0.5}$ = time required for exhalation of 50% of V_{exp}
[f] S = saline-filled lung
[g] F = fluorocarbon-filled lung

TABLE II

CALCULATED $\dot{V}_{A_{max}}$ FOR AN EXCISED, SALINE-FILLED DOG'S LUNG

Vol (ml)	t_{exp} (sec)	f (breaths/min)	$\dot{V}_{E_{max}}$ (L/min)	$\dot{V}_{D_{anat}}$ (L/min)	$\dot{V}_{A_{max}}$ (L/min)
675	15	2.00	1.350	0.120	1.230
640	12	2.50	1.600	0.150	1.450
609	10	3.00	1.827	0.180	1.647
578	8	3.75	2.167	0.225	1.942
516	6	5.00	2.580	0.300	2.280
469	5	6.00	2.814	0.360	2.454
406	4	7.50	3.045	0.450	2.595
344	3	10.00	3.440	0.600	2.840
250	2	15.00	3.750	0.900	2.850
195	1.5	20.00	3.900	1.200	2.700
156	1	30.00	4.680	1.800	2.880

expiration, and assuming an anatomical dead space ($V_{D_{anat}}$) of 60 ml (2), $\dot{V}_{D_{anat}} = f \cdot 60$ and $\dot{V}_A = \dot{V}_E - \dot{V}_{D_{anat}}$. At a frequency of 5 breaths/minute, the mean calculated $\dot{V}_{A_{max}}$ for the four excised saline-filled lungs is 2.18 L/minute, and 2.16 L/minute for the three excised fluorocarbon-filled lungs (c.f. Table II).

Figure 4 illustrates the results of tonometry of 0.1 *M* and 0.3 *M* THAM solutions, where the CO_2 content (C_{CO_2}) in ml/L is plotted on the ordinate and the P_{CO_2} of the test gas in mm Hg is plotted on the abscissa. Each point represents one sample. Also shown in Fig. 4 are the computed CO_2 contents of saline, air and FC-80 fluorocarbon liquid (13) at 37°C. At a P_{CO_2} of 40 mm Hg, the C_{CO_2} of the 0.1 *M* and 0.3 *M* THAM solutions is 250 and 390 ml/L, respectively.

The results of an experiment where isotonic 0.1 *M* THAM solution was used to ventilate a dog's right lung whose O_2-filled left lung was not ventilated are illustrated in Fig. 5. P_{aCO_2}

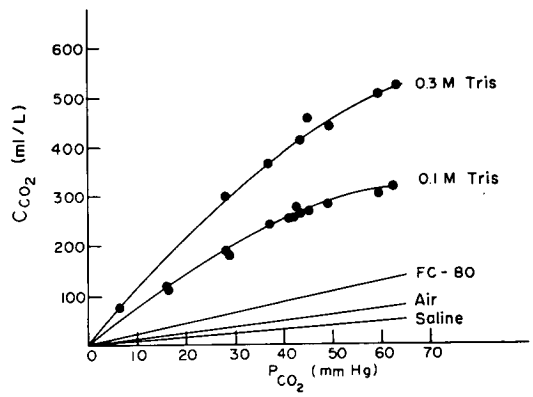

FIG. 4. CO_2 content (C_{CO_2}) vs. P_{CO_2} of 0.1 *M* and 0.3 *M* isotonic THAM solutions, FC-80 fluorocarbon, air, and saline. Each point represents one sample.

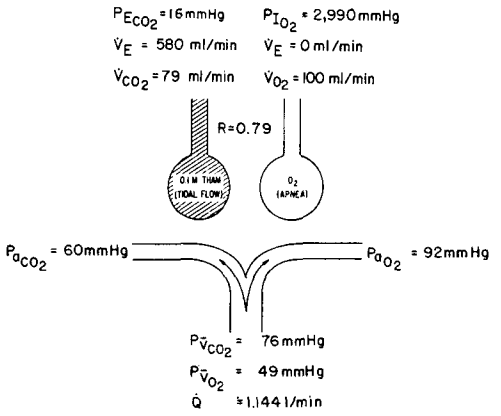

FIG. 5. CO_2 elimination through one lung of an apneic dog by lavage with an isotonic 0.1 *M* THAM-NaCl solution.

climbed sharply during the priming procedure but stabilized around 60 mm Hg shortly after tidal ventilation with liquid was begun. The mean $\dot{V}_{CO_2}$ was 79 ml/minute at a mean respiratory gas exchange ratio (R) = 0.79 (c.f. Fig. 5). The arterial, venous and expired P_{CO_2} and P_{O_2} values are the means of four samples taken periodically during the 25-minute lavage. $\dot{V}_{CO_2}$ is calculated as follows:

$$\dot{V}_{CO_2} = \dot{V}_E \cdot C_{SCO_2} \tag{2}$$

where C_{SCO_2} = CO_2 content (vol %) of the mixed expired THAM solution. Cardiac output ($\dot{Q}$) can then be calculated by application of the Fick principle:

$$\dot{V}_{CO_2} = \dot{Q}\,(C_{\bar{v}CO_2} - C_{aCO_2}) \tag{3}$$

where $C_{\bar{v}O_2}$ and C_{aO_2} are mixed venous and arterial CO_2 contents, respectively. Substitution for $\dot{Q}$, C_{aO_2}, and $C_{\bar{v}O_2}$ permits the calculation of $\dot{V}_{O_2}$:

$$\dot{V}_{O_2} = \dot{Q}\,(C_{aO_2} - C_{\bar{v}O_2}) \tag{4}$$

The respiratory gas exchange ratio (R) is $\dot{V}_{CO_2}/\dot{V}_{O_2}$.

The ratios of CO_2 partial pressures in expired liquid and arterial blood during lung lavage in patients with alveolar proteinosis are given in Table III. As can be seen, the partial pres-

TABLE III

RATIOS BETWEEN PARTIAL PRESSURES OF CO_2 IN END TIDAL LIQUID FRACTIONS ($P_{et_{CO_2}}$) AND ARTERIAL BLOOD

	Patient							
	V.W. (Left Lung)			V.W. (Right Lung)		R.W. (Right Lung)		
Time (sec)[a]	18	19	33	43	47	70	80	80
$P_{et_{CO_2}}/P_{a_{CO_2}}$	1.03	0.96	0.90	0.90	0.88	0.87	0.99	1.03

[a] Time = interval between beginning of infusion and end of drainage.

sure of CO_2 in "end tidal" samples, i.e. a sample of expired liquid following removal of 400 ml, was approximately equal to P_{aCO_2} regardless of the differences in the duration of the lavage cycles.

In the left lung of one patient, mean expiratory flow rates were measured over the 500 ml tidal volume from end-inspiratory volumes of 2500 ml and 3000 ml. The flow rates were 62.5 ml/second ($\dot{V}_E$ = 3.75 L/minute) and 71.5 ml/second ($\dot{V}_E$ = 4.29 L/minute), respectively.

Discussion

The difference in shape and position of the pressure-volume curves of lungs filled with air, saline and fluorocarbon liquid (Fig. 3), precludes a meaningful isovolume-pressure-flow comparison. Therefore, other criteria are needed to compare the maximum expiratory flow of saline and fluorocarbon, for instance, by comparing the time required to exhalation of the first 50% of the total volume of liquid expired over approximately equal lung volume ranges.

The means of the average maximum expiratory flows of the first 50% of the total volume of saline and fluorocarbon exhaled were 109 and 94 ml/second, respectively (Table I). This indicates that $\dot{V}_{Emax}$ of fluorocarbon and saline is about equal over approximately equal lung volume ranges, despite the fact that fluorocarbon has a density almost twice that of saline and a viscosity half again that of saline at 37 °C. The static pressure-volume characteristics of saline- and fluorocarbon-filled lungs, illustrated in Fig. 2, may serve to explain this phenomenon. At comparable lung volumes, the static recoil pressures of the two air-filled lungs were almost identical, while the static recoil pressure of the fluorocarbon-filled lung was, on the average, twice as great as the static recoil pressure of the lung filled with saline, at comparable lung volumes. Based on the EPP concept of Macklem and Mead (11), the greater static recoil pressure of the fluorocarbon-filled lung would account for the approximately equal maximal expiratory flow rates achieved with saline and the denser, more viscous fluorocarbon liquid (12).

For the estimation of $\dot{V}_{CO_2}$, an alternative method of data presentation is given in Table II. Kylstra, Paganelli and Lanphier (5), ventilating the lungs of anesthetized dogs with hyperbarically oxygenated saline, found that CO_2 elimination through the liquid-filled lungs was inadequate and attributed this largely to the presence of a diffusion dead space (V_{Ddiff}). Using Crank's (1) solution of the diffusion equation and assuming spherical gas exchange units, the diffusion dead space can be computed by the expression derived by Kylstra, Paganelli and Rahn (6). The reported average computed radius of the dog's hypothetical spherical gas exchange units was 419 μ (5). Using this value and the appropriate diffusion coefficients*, the computed V_{Ddiff}/V_A = 0.11 for a dog breathing saline and 0.21 for a dog breathing FC-80 fluorocarbon at a rate of 5 breaths/minute.

At a frequency of 5 breaths/minute, the mean calculated $\dot{V}_{Amax}$ for the four excised saline-filled lungs is 2.18 L/minute (c.f. Table II) and 2.16 L/minute for the three excised fluorocarbon-filled lungs. In the absence of a distribution dead space, the effective alveolar ventilation ($\dot{V}_{A^e max}$), would be 1.94 L/minute for the four saline-filled lungs and 1.70 L/minute

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* D_{CO_2} in saline at 37 °C is 2.55 × 10^{-5} cm²/sec (2). D_{CO_2} in FC-80 fluorocarbon at 37 °C has been measured, using the dilatometer technique of Hills (3, 4), and found to be 1.57 × 10^{-5} cm²/sec. Using the same technique, D_{CO_2} in saline at 37 °C was 2.88 × 10^{-5} cm²/sec.

for the three fluorocarbon-filled lungs ($\dot{V}_A^e = \dot{V}_A - \dot{V}_{Ddiff}$). $\dot{V}_{CO_2max}$ can now be calculated according to Eq. (1). Assuming a normal P_{aCO_2} of 40 mm Hg, the calculated $\dot{V}_{CO_2max}$ for the saline- and fluorocarbon-filled lungs are 56 and 143 ml/minute, respectively.

If it is assumed that D_{CO_2} for the THAM solution is the same as for saline, then $\dot{V}_{CO_2}$ can be estimated from Eq. (1), using the $\dot{V}_A^e$ of our saline-filled lungs. For 0.1 *M* and 0.3 *M* THAM solutions, titrated to pH 7.4, our estimates of $\dot{V}_{CO_2max}$ at P_{aCO_2} = 40 mm Hg are 545 and 850 ml/minute, respectively. Clearly, the addition of THAM to a saline-breathing solution would permit the maintenance of a normal P_{aCO_2} at moderate levels of exercise in dogs.

The above calculations have been made assuming an inspiration time (t_{insp}) equal to the time required for expiration (t_{exp}). Kylstra and Tissing (10) observed in an anesthetized, spontaneously saline-breathing dog, that the animal apportioned the time required for inspiration and expiration in an approximate 1:2 ratio. Decreasing t_{insp} will result in an increase in respiratory frequency. $\dot{V}_{Danat}$ and $\dot{V}_{Ddiff}$ will increase also, but not enough to offset a gain in $\dot{V}_A$. The net effect of decreasing t_{insp}/t_{exp} is a progressively decreasing increase in $\dot{V}_{CO_2}$ (12).

During volume-controlled lung lavage in two patients with alveolar proteinosis, the partial pressure of CO_2 in end-tidal samples was approximately equal to P_{aCO_2} at a time interval of 20 seconds between the beginning of infusion of 500 ml of saline and the end of drainage of that tidal volume (9). Thus, diffusive equilibrium of CO_2 between alveolar capillary blood and alveolar saline appears to be complete or very nearly so within 20 seconds in saline-filled human lungs. This indicates that, at a respiratory frequency of 3 breaths/minute, diffusion dead space would be negligible in saline-breathing man. In one of the patients, saline could be drained from one lung at a rate of 71.5 ml/second, indicating that he should be able to breathe saline at a rate greater than 4 L/minute. Thus, a diver breathing an isotonic THAM solution at a tidal volume of 1330 ml and a frequency of 3 breaths/minute should be able to maintain a P_{aCO_2} of 40 mm Hg during exercise requiring an oxygen consumption of approximately 1.7 L/minute, assuming a negligible distribution dead space and an anatomical dead space of 150 ml.

Acknowledgments

This research was supported by Grant HE-07896 from National Institutes of Health and Contract NR-101-758 with the Office of Naval Research.

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EFFECTS OF EXTERNAL RESISTANCE ON MAXIMUM EXPIRATORY FLOW AT INCREASED GAS DENSITY

J. Vorosmarti, Jr. and E. H. Lanphier

Maximum expiratory flow below approximately 80% of vital capacity has been shown by several investigators (3–5, 8) to be limited by intrathoracic factors. Investigations at increased ambient pressure have indicated that the basic relationships of lung volume, pleural pressure and flow remain the same with increased gas density (9). The effect of density on this relationship is to decrease the flow obtainable at a given driving pressure and, therefore, to limit ventilation. In the practical diving situation, the fact that external resistance in the form of breathing apparatus is also present must be considered. Although Mead et al. (5) have shown that the addition of some external resistance at 1 ata does not decrease maximum effort-independent flow, this situation has not been investigated at depth. This study was undertaken to investigate the influence of external resistance on maximum expiratory flow with increased gas density.

Methods and Materials

Five subjects (Table I) participated in the study, which was conducted in a standard double-lock compression chamber, using air at 1, 4, and 7 ata. At each pressure the subjects, who were seated comfortably in the erect position, performed at least three forced

TABLE I

PHYSICAL CHARACTERISTICS OF THE SUBJECTS

Subject	Sex	Age (yr)	Height (cm)	Weight (kg)	Predicted Vital Capacity (cc)	Measured Vital Capacity (cc, BTPS)
1	M	30	183	78.3	5300	4900
2	M	29	179	86.5	5100	6406
3	M	36	188	91	5450	6400
4	F	25	180	66	4100	4512
5	F	30	157	50	3050	3032

expiratory vital capacity maneuvers through a 27-mm-diameter mouthpiece and through each of a series of resistance orifices which could be placed in the mouthpiece. The orifices were 12-mm-thick plastic discs with holes of the following diameters located in their centers: 15, 10, 7.5, 5 and 3.5 mm. The mouthpiece was connected to a low resistance wedge spirometer* with large bore tubing. Volume and flow signals from the spirometer were recorded simultaneously on a multichannel oscillograph† and on magnetic tape using a Honeywell Model 7600 recorder. Pressures were measured with Statham P23AC pressure transducers modified for use in high pressure environments. One transducer was also modified to measure esophageal-mouthpiece differential pressures. The space between the diaphragm and cap of these transducers was filled with distilled water to obviate any errors that might be introduced by compression of this volume of gas. Mouthpiece pressure was obtained through a tap about 1 inch proximal to the orifice. Esophageal pressure was obtained through an 80-cm length of 1.7-mm I.D. polyethylene tubing, the distal end of which was pierced and covered with a 12 × 1-cm latex balloon containing 1 cc of air. The balloon was positioned in the lower esophagus in a region free of cardiac artifacts. Esophageal, mouthpiece and esophageal-mouthpiece pressure signals were recorded in the same manner as flow and volume signals. The spirometer volume signal was calibrated prior to each experiment using both the electronic calibration source and the introduction air with a 2-L calibrated syringe. The flow signal was checked electronically before each experiment after having been previously calibrated with air at an accurately known flow. A water manometer was used to calibrate the pressure transducers prior to each experiment.

Each subject performed several slow vital capacity maneuvers and several forced expiratory vital capacity maneuvers before the actual experiment to insure that he was performing the maneuver correctly. The subjects were also coached during the experiment by a physician who accompanied them on the dive.

Flow-volume, esophageal-mouthpiece differential pressure-volume, and mouthpiece pressure-volume curves were constructed for each experimental situation by reproducing the taped signals through an X-Y recorder‡ at 1/8th the recording speed. Flows and pressures at 80, 50 and 25% vital capacity and pressures at peak flow were taken from these curves for further analysis.

Results

Changes in average pleural pressure (P_{pl}) at 50% vital capacity due to density and orifice size are presented in Fig. 1. With the open mouthpiece and the 15-mm orifice, pleural pressure rose with density. At 1 and 4 ata there was an increase in pleural pressure as orifice size decreased to 10 mm, while at 7 ata the use of the 15-mm orifice caused only a slight increase in pleural pressure. With smaller orifices the pleural pressure reached a plateau at 1 ata, while at 4 and 7 ata pleural pressures were very nearly equal and decreased slightly with decreasing orifice size. At peak flow pleural pressures leveled off at both 1 and 4 ata with smaller orifices while at 7 ata it also decreased from its peak on going to the 10-mm orifice. At 80% vital capacity this pattern was again found. At 25% vital capacity there

*Medscience Electronics, model 170.
†Grass model 7B.
‡Honeywell model 530.

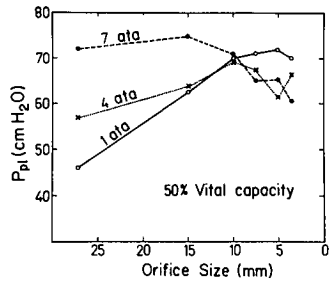

FIG. 1. Changes in pleural pressure (P_{pl}) at 50% vital capacity with decreasing orifice size at 1, 4 and 7 ata.

was little difference (<10 cm H_2O) between pleural pressures at different densities, and a reversal of the above pattern, i.e., pleural pressures at increased density were smaller than at 1 ata. At this lung volume decreasing orifice size had little effect on pleural pressure. Thus, the relationship of pleural pressure to density showed a decreasing effect of density as lung volume decreased. The effect of smaller orifices on pleural pressure was also decreased as lung volume decreased.

Figure 2 shows the changes in average esophageal-mouthpiece pressure differential (transpulmonary pressure, P_{tp}) related to orifice size and gas density at 50% vital capacity. With the open mouthpiece and 15-mm orifice, the transpulmonary pressure increased with density by an amount equal to the increase in pleural pressure with density (Fig. 1). As the orifice size decreased so did the effect of density such that the transpulmonary pressures at orifices smaller than 15 mm were for practical purposes equal. As orifice size decreased, the transpulmonary pressure also decreased, reaching minimum values at the 5- and 3.5-mm orifices. The effects of density and orifice size as lung volume decreased were similar to those found with the pleural pressure; the differences due to density decreased with decreasing lung volume and the effect of increasing external resistance diminished at lower lung volumes.

The relationships of average flow, density, mouthpiece pressure and orifice size at 50% vital capacity are shown in Fig. 3. The most important result shown is that at a given gas density the addition of external resistance does not affect maximum flow until the orifice

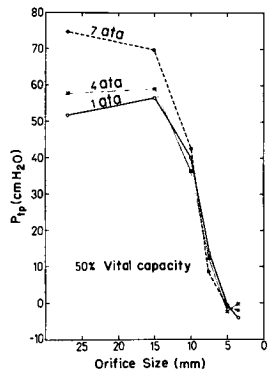

FIG. 2. Changes in transpulmonary pressure (P_{tp}) at 50% vital capacity with decreasing orifice size at 1, 4 and 7 ata.

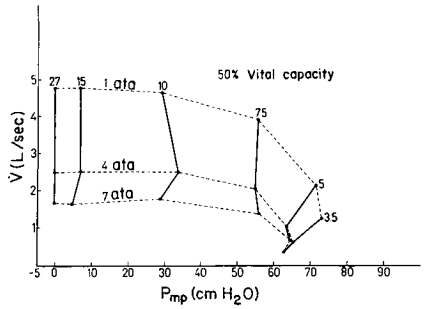

FIG. 3. Relationship of flow at 50% vital capacity and mouthpiece pressure (P_{mp}) at 1, 4 and 7 ata with variously sized orifices. Orifice size in mm is shown at points along the 1 ata flow curve.

size is smaller than 10 mm. The "limiting orifice" has been defined as the largest orifice that causes a decrement in maximum flow. In this particular case, the limiting orifice is 7.5 mm. However, it can be discerned from Fig. 3 that the actual limiting orifice would lie somewhere between 10 and 7.5 mm, presumably close to 10 mm. It should also be noted that the limiting orifice did not change with increasing gas density, nor did the mouthpiece pressure change appreciably with density, as long as the flow was stable. The results obtained at peak flow, and 80% and 25% vital capacity showed similar changes except for differences in the limiting orifice. At peak flow the 15-mm orifice was limiting. At 80% and 25% vital capacity the limiting orifices were 10 and 5 mm, respectively.

The relationships of pleural pressure, transpulmonary pressure and mouthpiece pressure at 50% vital capacity and 4 ata with the different orifices are shown in Fig. 4. As the pressure drop due to external resistance increases, the transpulmonary pressure decreases by the same amount. The apparent exception with the 15-mm orifice is due to the increase in pleural pressure. The point at which the transpulmonary and mouthpiece pressures are equal is also the point at which flow began decreasing (i.e., between the 10-mm and 7.5-mm orifices, but extremely close to the 10-mm orifice, Fig. 1). At 1 and 7 ata the mouthpiece and transpulmonary pressures were slightly different but the point at which they become equal remained the same. When the results obtained at the other lung volumes studied are graphed in this manner, the same relationships are found between mouthpiece and transpulmonary pressure and the orifice size at which flow is initially decreased.

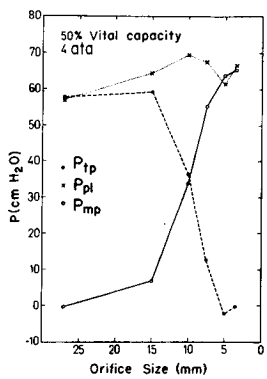

FIG. 4. Changes in P_{tp}, P_{pl}, and P_{mp} at 50% vital capacity and 4 ata with decreasing orifice size.

Discussion

The increase in pleural pressure with increased gas density and external resistance (Fig. 1) can be attributed to the associated decrease in the rate of change of lung volume. As rate of shortening of the expiratory muscles is decreased, more force can be generated (1), and higher pleural pressures should result. This effect is less evident at smaller lung volumes where the decrease in lung volume is already very slow. The drop in pleural pressure with smaller orifices at 4 and 7 ata is contrary to the expectation that maximum dynamic pressure would be reached and maintained as is the case at 1 ata. This difference in the behavior of pleural pressure has not yet been explained. It may be related to fatigue and to the order in which the maneuvers were conducted.

The effects of external resistance upon expiratory flow (Fig. 3) and associated changes of transpulmonary and mouthpiece pressures (Figs. 2 and 4) are consistent with a familiar analysis of the determinants of maximum expiratory flow (5). The basic concept focuses attention upon a point in the intrathoracic airways where pressure within the airway is equal to the surrounding pleural pressure (equal pressure point, EPP). Maximum expiratory flow at a given lung volume is determined by the static recoil pressure and the airway resistance between the alveoli and the EPP. The P_{pl} provides the driving force from the EPP to outside. A certain P_{pl} is required to reach maximum flow at a given lung volume. This pressure is defined as P_{max}, and increases in P_{pl} above P_{max} are dissipated by increasing compression of intrathoracic airways downstream from the EPP. When there is no resistance external to the mouth transpulmonary pressure, P_{tp} is by definition equal to P_{pl}.

The addition of a small external resistance may cause either a rise in P_{pl} or more generally a drop in P_{tp}. In any case the difference between P_{pl} and P_{tp} must by definition be equal to the pressure drop across the external resistance, P_{mp}, and the sum of P_{tp} and P_{mp} must equal P_{pl}. Also by definition, the driving force for flow between the EPP and the mouth is now P_{tp} instead of P_{pl}.

Flow at a given lung volume should remain maximal as long as P_{tp} remains greater than P_{max} and P_{pl} is greater than P_{max} plus P_{mp}. The fact that maximum flow is maintained while P_{tp} continues to decrease must indicate a decrease in airway resistance. This is due to the increase in airway pressure caused by "back pressure" from increased P_{mp}. This increase in pressure within the airways reduces the pressure difference across the walls of the airways and decreases airway compression.

Continuing to add external resistance results in a rapid drop in P_{tp} and rise in P_{mp} until they are equal, and P_{mp} then rapidly exceeds P_{tp} (Fig. 4). At 50% vital capacity the point of most rapid change occurred between the 10- and 7.5-mm orifices. It was also between these same orifices where flow began to decrease (Fig. 3). This combination of changes indicates that airway resistance in the downstream segment can no longer be decreased because airway compression no longer exists. P_{tp} at this point must therefore be very nearly, if not exactly, equal to P_{max}. If orifices between 10 and 7.5 mm had been used, the "limiting" orifice diameter as reflected by decreased flow should have been very close to the point where P_{tp} and P_{mp} are equal.

Varying external resistance to determine P_{max} has been alluded to by Mead et al. (5) in their analysis of P_{mp}-flow curves. This was also the basis of a method used by Olafsson and Hyatt (7). The latter investigators showed that P_{max} determined in this manner was identical

to P_{max} derived by the conventional method of varying expiratory effort. If P_{max} dropped with increasing density, a greater amount of external resistance could be added without decrement in maximum flow with depth. Results here show that this is not the case at least to 7 ata. This is at variance with the results of Wood and Bryan (9) who reported a decrease in P_{max} with increasing density.

As external resistance continues to increase, flow decreases because external resistance and P_{pl} now govern flow. P_{tp} continues to decrease with flow and finally becomes negative, indicating that the driving force between the alveoli and the mouth is the static recoil pressure of the lung. The EPP is now extrathoracic.

The addition of increasing density to this system decreases the maximum flow obtainable and may increase the P_{pl} with external resistance. Nevertheless, the basic relationships of the important variables do not change. The increased density apparently affects the internal and external resistances equally. At lung volumes between 30% and 80% of vital capacity, maximum flows found in this experiment and in others (2, 9) decreased by a factor approximately equal to the inverse of the square root of density. This is also the relationship of flow to density for an orifice smaller than the tube in which it is located. Since static recoil pressure is unaffected by density, the upstream segment at a given lung volume apparently responds to increased density as if it were an orifice. This suggests that the human airway with an external orifice resistor can be viewed as a system containing two orifices in series. With constant driving pressure and increasing density, the upstream orifice will cause flow to decrease in proportion to $1/\sqrt{D}$. With this decrease in flow, the pressure drop across the downstream orifice should remain constant. That such a model is valid is supported by the lack of change in mouthpiece pressure with density while flow is maximum (Fig. 3) and by the lack of any difference in the limiting orifice with increased density.

The basic fact established by this study is that a certain amount of external resistance can be tolerated at depth without any decrement in maximum effort-independent flow. Of more practical importance is the fact that any resistance which does not interfere with maximum effort-independent flow at the surface will not interfere with this flow at depth. If a breathing apparatus could be characterized as being equivalent to a certain sized orifice this would greatly simplify the testing procedure of such apparatus. No testing at depth would be required to determine whether the apparatus would allow the diver to achieve maximum flow. This approach ignores such effects as hydrostatic imbalance and changes in breathing bag compliance, which are important in some apparatus.

The fact that a certain amount of resistance can be tolerated before maximum flow is impaired does not indicate that such resistance can be allowed in a breathing apparatus. Information related to maximum flow applies only in the situation where a diver must do severe exercise for short periods of time and can tolerate the added respiratory work required to produce maximum flow. Other investigators Miller et al. (6) have shown that work requiring oxygen consumptions of 2.8–3 L/min can be done even at a relative gas density of 6 without reaching maximum effort-independent flow. This level of work is seldom reached by a diver, so flows will ordinarily be well below maximum. In this case the respiratory work required is directly related to the external resistance during both expiration and inspiration, requiring external resistance to be minimal. Minimal external resistance will also insure that maximum expiratory flow will be limited by the lungs and will be higher and will require less work than if limited by external resistance.

ACKNOWLEDGMENTS

This study was supported in part by ONR Contract N00014-71-C-0342. The authors gratefully acknowledge the assistance of LCDR Edward T. Flynn, MC, USN, Mr. D. Markey and Mr. J. Cunningham.

Opinions and conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or the endorsement of the Navy Department.

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PART II. VENTILATION AND GAS EXCHANGE*

DISCUSSION

J. A. Kylstra, Chairman

Dr. Sturr: Dr. Wright, have you looked upon the frequency characteristics of the pneumotachographs at high pressure and timed them? We have done that and observed a damping of the system, which made it difficult to measure during high flow rate.

A second question concerns the calibration procedures. I might mention the article of Finucane et al. in *Journal of Applied Physiology*† about the significance of upstream geometry in steady-state flow calibrations. Different upstream geometries cause different flow response from the pneumotachograph.

Also, he studied the effects of periodic flow and found—especially with high gas densities—a phase lag between the pressure and flow, and also an amplitude decrease. This could mean that the results of measurement with the pneumotachograph, when these have not been examined, are questionable.

Dr. Wright: The pneumotachograph is a very difficult instrument to work with and we spent quite a bit of time dealing with the problems that you have outlined as well as others. With regard to the upstream geometry, calibration was carried out through identical upstream geometry. The mouthpiece was separated from the pneumotachograph by a large bore tubing, which was approximately a meter and a half in length. This calibration was carried out through this same tubing.

With regard to the frequency response and phase lag between the pressure signal and the pneumotachograph signal: these were measured and corrections for the difference between the phase lag of the signal in different gases were applied to the computer program in which the calculations were made.

Another problem with the pneumotachograph is that as gas density increases, flow in the pneumotachograph becomes turbulent, at lower and lower flow rates. When flow becomes turbulent, the pneumotachograph is no longer linear. We circumvented this difficulty by building a special pneumotachograph which had a linear flow range from 0 to 60 L/sec at sea level, and verified the linearity of the pneumotachograph with each gas at each depth before and after each measurement series.

Dr. Sturr: This is not completely satisfactory. What I am pointing at in my question about frequency characteristics is the following: that the natural frequency and the damping of the system goes down when gas density increases. This means that while you may, for instance, measure maximum voluntary ventilation in the normal circumstances, the frequency content of periodic breathing at maximum ventilation is no longer covered by the linear frequency range of the pneumotachograph.

Dr. Wright: I do not completely understand the comment.

Dr. Kylstra: Have any of you systematically compared the pneumotachograph and wedge spirometer at depth?

Dr. Wright: We have not done that.

Dr. Morrison: I have in fact had some trouble with high-frequency vibrations in the pneumotachograph when trying to use the pneumotachograph to measure gas velocities from an open-circuit demand valve. We were having frequency vibrations of up to 100 cycles/second at depths down to about 8 ata, using air—which is eight times normal air gas density. We finally had to go down to 20 cycles/second in order to get rid of these vibrations.

So I do not think that frequency limitations are going to be problems in measuring normal ventilatory factors.

Dr. Davidson: Dr. Wright, about the compressible compartment in the intrathoracic airways, have you used or considered using radiology—I was thinking of bronchography with a small focus tube and magnification—to demonstrate these changes?

**Panelists:* N. R. Anthonisen, B. Broussolle, L. Farhi, D. Linnarsson, G. v.Nieding, W. H. Schoenfisch, R. H. Strauss, J. Vorosmarti, Jr., W. B. Wright.

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Dry. Kylstra: This has been demonstrated morphologically. You can easily demonstrate collapse or at least narrowing of intrathoracic airways upon a forced expiration. There are beautiful cinefluoroscopy studies on that.

Participant: I would like some comment about the protocol of exercise studies that Dr. Linnarsson and Dr. Wright have talked about. When you are doing a work task the power output is a combination—especially on the bicycle ergometer—of the frequency of pedaling and the load that is presented. Theoretically, the same power output could be generated by pedaling at twice the speed and half the load in one case—as at half the speed and twice the load in the other.

This represents the same external work power output but yet imposes a very different metabolic load upon the organism. The European schools have regularly used 60 cycles/minute or revolutions/minute as the frequency of pedaling, and this imposes rather high resistances to the legs when you get into larger work outputs.

I wonder if the same kind of ventilatory mechanics and end points of exhaustion are reached if the other method of presenting the work task to the individual is followed, i.e., to ask him to pedal with high frequencies and keep the load small.

Dr. Linnarsson: We used the standard 60 rpm because our subjects are used to it. It is quite a difficult task to make people do maximum work and it is not less difficult at 6 atmospheres. The subjects must perform in a way they are used to. We have not tested higher rates of pedaling, and we do not know what it will result in. I would not say that it would not make much difference for the maximum oxygen uptake; maybe it would make some difference for the performance time and the subjective experience of the work done.

Dr. Kylstra: Dr. Wright, have you compared your studies with the results of Wood and Bryan? I was particularly struck by the discrepancy of the negative exponent in your experiments—if I am not mistaken it was -0.3; whereas Wood and Bryan consistently found an exponent of something like -0.49 or -0.46. Have you made a systematic comparison of your data, and do you have any explanation for this discrepancy?

Dr. Wright: Dr. Peterson of our group went through all the literature in which flow-limited functions were measured as a function of density and either calculated himself or took the density exponents that were given by the authors. About ten or twelve papers were surveyed, and we found that during acute exposures to pressure the exponents fell nearer to what Wood and Bryan reported three years ago—around 0.4 to 0.5. And in the studies in which the subjects remained at pressures for a longer period of time, as suggested by Dr. Schaefer, the exponents were closer to zero—that is, they showed improved function.

We do not know why this is. Dr. Schaefer suggested some years ago that there may be a vagal influence on bronchomotor tone that is decreased with time. This is a possibility. We do not understand it, but we feel that our study, which was a chronic exposure with very slow pressure changes, probably fits in rather well with the other observed chronic studies.

Dr. Kylstra: Maybe atropine is something that could be used to study this.

Dr. Wright: I think Dr. Dubois at the University of Pennsylvania has shown that atropine in the normal individual will reduce respiratory resistance significantly, so that all of us here now are showing the effects of vagal influence on our bronchomotor system. Atropine may be able to distinguish this relationship at depth as well, although we have not tried this.

Participant: Dr. Anthonisen, I wonder if you could postulate an explanation for the alleged improvement in V/Q relationships in allegedly stiff portions of the lung with dense gas.

Dr. Anthonisen: The A-a DO_2 is due to alveolar units with low ventilation-perfusion ratio generally. You can assume that dense gas probably does not change perfusion distribution very much. That means that it changes ventilation distribution. So what you have to do is increase the ventilation to units with a low ventilation-perfusion ratio. What dense gas does—if you have a shift ventilation from units with high resistance to units with low resistance—is increase ventilation to units which are badly ventilated and which have low resistance. If they have low airway resistances they can only be badly ventilated for one reason—that is, they have very, very low compliances.

Dr. Smith: I wonder how far the effects that Dr. Anthonisen has observed are due to the use of SF_6, which has some very curiously anomalous properties when used at high pressure. For instance, in decompression sickness one can produce death with SF_6. In the total absence of visible bubbles within the body what one gets is a white or pink foam which spreads in the lungs and I wonder how far it interacts with the lung in a specific way. I think this could be tested by the use of CF_4 which at, say, 6.5 atmospheres would have a comparable density to SF_6 at 4 atmospheres while not producing foaming to the same extent in decompression, suggesting it does not interact specifically with the lung (if that indeed is the explanation) as SF_6 does.

SF_6 also has an anomalous property in that when one measures its narcosis level produced in mixtures one gets a less than additive potency of SF_6, which again one does not get with CF_4. Dr. Anthonisen, did you consider CF_4?

Dr. Anthonisen: It is very expensive, of course, and I think that terminated our consideration of it. The SF_6 we regarded as just an inert gas species, and it may not be such—which is what you are suggesting. I will say that we had a difficult time doing studies after surfacing on our dogs because it's very difficult to dive a dog on SF_6, anesthetized at least, and have him alive when you get back to the surface. They are very prone to get fatal decompression sickness, pulmonary edema being the main problem we saw.

Dr. Kylstra: Talking about the effects or possible effects of SF_6 on pulmonary function, I wonder whether anyone has ever computed how high you have to raise the pressure of the inspired gas to get physical action of the gas on the gas/aqueous interface. In other words, if you pack the gas molecules close enough together, theoretically you should get an interaction attraction between the gas molecules in the gas phase and the gas molecules in the aqueous layer. At normal pressures this, of course, is negligible, but I wonder whether this could not conceivably at very high pressures affect the interfacial tension at the alveolar interface.

Dr. Karin: Dr. Linnarsson, if I understood you correctly, you have concluded that most or all of the reduction in $\dot{V}_{O_2max}$ at high densities was due in some way to the combined metabolic and respiratory acidosis rather than to some hindrance to delivery of oxygen from the lung to the blood. This could be checked by actually measuring the oxygen content in the arterial blood to see if it was not saturated or by exposing the subjects to a period of hyperventilation and/or infusing bicarbonate before they exercise. Did you try it, or what would you predict?

Dr. Linnarsson: The arterial measurements during exercise still remain to be done and we are very anxious to know the results. There have been some experiments done with pure oxygen at 3 atmospheres, and inspired-arterial oxygen difference is the same in rest and with severe or maximal exercise. So with pure oxygen you do not see those differences, but with more or less high fractional inert gas it remains to be done.

Dr. Sass: Dr. Schoenfisch, we have been using the FC-80 fluorocarbon for about 2 years at the Mayo Clinic to ventilate dogs and have had from the start no trouble maintaining normal CO_2 tensions for periods of 4–8 hours while the dog was breathing the oxygenated liquid fluorocarbon.

However, our dogs are anesthetized and paralyzed and are ventilated with mechanical assistance. We use a water immersion respirator to ventilate the dogs and we are able to control the CO_2 tension at normal or hypocapnic levels only when we ventilate the dogs with mechanical assistance. Other investigators who have used FC-80, other liquid fluorocarbons and silicon oil, have not been able to maintain normal CO_2 tension because the dogs were spontaneously breathing the liquid. I think that should be pointed out in your presentation: control of CO_2 is possible only when mechanically assisted ventilation is used.

Dr. Schoenfisch: Contrary to what we may have discussed before, we have revised our predictions for FC-80 fluorocarbon. With your dogs in particular, though, am I correct in assuming that you do not measure expired CO_2 values?

Dr. Sass: We have not reported the measurements. We have made measurements of right and left bronchial P_{O_2}, CO_2 and mixed expiratory liquid gas tensions, and we find that, because fluorocarbon is approximately twice as heavy as blood, we get a preferential flow of the liquid to the dependent lungs and at the same time a flow of blood to the upper lung; so that what you end up with is a ventilation perfusion inequality that is just 180° out of phase with the normal animal breathing gas.

We have found, for example, that whereas the blood flow is greater in the upper lung, the fluorocarbon flow to the upper lung is less than it is to the lower lung, but the greater ventilation is in the upper lung. The CO_2 tension in the upper lung would be roughly 56 mm Hg, whereas the CO_2 tension in the dependent lung would be on the order of 10 or 12 mm Hg. The mixed fluorocarbon CO_2 would be on the order of 50 mm Hg, while normal arterial gas tensions were maintained.

Dr. Schoenfisch: There is no controversy regarding fluororcarbon for anesthetized dogs, but I hesitate to make any prediction about its use for anything more than an anesthetized dog.

Dr. Sass: I agree with you. Furthermore, I do not think FC-80 would be a good liquid fluorocarbon to use even for anesthetized dogs, although the greater experience has been accumulated with FC-80. I think all published reports, other than Dr. Kylstra's with saline breathing, have been done with FC-80 fluorocarbon or DC-200. The point I am making is that FC-80 has a number of other problems. The biggest one is that the vapor pressure of FC-80 is roughly 70 mm Hg at 37 °C and—as you know from Holiday's studies which we have verified in a number of dogs—FC-80 is absorbed into the circulatory system while the animals are breathing the liquid. Whether FC-80 is injected intravenously in an air-breathing dog or absorbed into the circulatory system of the liquid-breathing dog, the fluorocarbon evaporates.

There is a difference of about 60 mm Hg between the total gas pressure or gas tension and the total liquid pressure in the circulatory system. So, for example, when FC-80 appears in the circulation with a vapor pressure of 70 mm Hg it boils off, literally causes a gas embolism and you get bubbles; a large quantity of gas exists in the circulatory system and the animals die within a few hours.

However, if, for example, either P-12-F fluorocarbon (which is manufactured by the Allied Chemical Company) or PP-3 fluorocarbon (manufactured in London by IFC) were injected—both liquids have a vapor pressure of less than 60 mm Hg and a gas embolism is not produced.

In any future studies done with liquid-breathing I would recommend that either PP-3 or the P-12-F fluorocarbons be used. They have a high enough vapor pressure to facilitate evaporation from the lungs after the animal is switched from liquid to gaseous ventilation, but also have vapor pressure low enough as not to cause gas embolism.

Dr. Schoenfisch: The CO_2 solubility in either of the other fluorocarbons that you mentioned is not much greater than in FC-80 fluorocarbon.

Dr. Sass: True, but I do not think CO_2 retention is the problem. There are other problems with liquid-breathing and one is that the liquids cannot be allowed to appear in the circulatory system unless they have a vapor pressure of less than 70 mm Hg.

Dr. Kylstra: Why do you believe that CO_2 elimination is not a problem? I think what Dr. Schoenfisch presented indicates that indeed in an anesthetized dog with a basal oxygen consumption you might get by. That, of course, does not guarantee any adequate CO_2 elimination during normal awake basal states, let alone some degree of exercise. I think that is indeed dependent to a large extent upon the CO_2 solubility in the liquid used. On what basis do you feel this is not the case?

Dr. Sass: I meant that I did not think on the basis of our studies that CO_2 retention is a problem if the dogs are ventilated with mechanical assistance and are anesthetized and paralyzed—that really there is no problem in controlling arterial CO_2 tension in such a condition.

Dr. Kylstra: But there is one defect, I think, in this conclusion: that you measured arterial CO_2, but never how much CO_2 was in fact exhaled through the lungs. In other words, the mixed expired CO_2 was not measured and multiplied by the solubility coefficient and the minute ventilation. So it is conceivable that these dogs would be at a very minimal metabolic rate, and that could easily explain your impression that the CO_2 removal would be very simple with fluorocarbon because you apparently had to be careful not to overventilate. But I find it difficult to really believe that no matter what fluorocarbon you use that you would succeed in maintaining a normal arterial P_{CO_2} with any degree of significant exercise.

Dr. Sass: There is just no data. I agree with that. In fact, my major point was that this is possible in those mechanically assisted ventilated dogs.

Dr. Kylstra: I would like to expand a little bit on what Dr. Schoenfisch said about Tris buffer.

We have obtained direct evidence concerning CO_2 elimination in human patients who were undergoing treatments for a variety of diseases, such as alveolar proteinosis, and had one of their lungs washed with a saline solution. In these patients we could measure what the flow rates were—i.e., the amounts of saline you could drain from one lung/minute—and from that it is simple to estimate what the feasible alveolar ventilation would be.

We found in these patients that if you sample the liquid that comes out of one lung it has a CO_2 partial pressure which is approximately equal to that in arterial blood. This was a great surprise to us and would only indicate one thing: that at the slow rate of ventilation as it occurred in these patients apparently diffusive equilibrium was complete or almost complete within the time available for diffusion of CO_2 within the air spaces of the lung. On the basis of such observations it was possible to calculate that if we would have ventilated not one lung but two lungs of these patients who had alveolar proteinosis with a 0.3 molar isotonic THAM solution, CO_2 elimination through the lung at an arterial P_{CO_2} of 40 mm Hg would have been 1.4 L/minute, corresponding to an O_2 consumption of about 1.6 or 1.7 at the normal respiratory exchange ratio.

This would indicate it would indeed be feasible for a diver to do relatively heavy work while breathing liquid at the rate of approximately 3 breaths/minute, taking tidal volumes of close to 2 L at the time.

In short, it looks indeed as if the problem of CO_2 elimination, which has so far frightened away even the thought of possible application in diving, can be overcome by using adequately buffered saline solutions.

Dr. Wirtheson: Dr. Kylstra, have you considered that the addition of Tris buffer is inducing a solution, dissolving the buffer, and thereby permitting better wetting of the surface of the alveoli and therefore a better passage of the gas? Is this a possible explanation of the effect of the buffer?

Dr. Kylstra: We have thought of the possibility and actually it has worried us a little bit, because this Tris buffer has been approved by the Federal Food and Drug Administration as a therapeutic agent to be given intravenously or by mouth, but not by lung. It is apparently completely innocuous. It has been used in tissue culture and does no damage to cells. Nevertheless, we are not certain whether it conceivably could damage, or modify or alter the pulmonary epithelial layer. We still do not have the answer to that. If so, that would in fact rule it out as a useful substance because you would end up with denuded lungs and that would be unacceptable.

Dr. Kylstra: I had expected quite a few questions after listening to Dr. Farhi's predictions that if you looked closely enough you should find evidence of an impaired gas exchange caused by diffusion impairments in the gas phase in the gas exchange unit of the lung.

Dr. Farhi: Yes, this is an implication, and from paper and pencil "experiments" it turns out that out of the normal approximately 10 mm alveolar-arterial difference you might well be able to attribute 1 or 2 mm to the gas diffusion problem.

Dr. Kylstra: I have performed a few of these paper experiments myself—as a matter of fact, using the diffusion distance we found in the liquid-breathing dogs—using the same spherical model and substituting the diffusion coefficient of O_2 and CO_2 in air instead of in saline, which makes a difference of about 5 or 6 thousandths. I think if you substitute these values with all other factors remaining equal in these dogs you end up with a diffusive equilibrium in the dog's lung being reached within a few milliseconds.

I fully agree that this is no comparison—the bulk flow profile may be situated at a completely different level. Yet the difference between these predictions is so large that it is hard to reconcile.

Dr. Farhi: Yes, the difference is extremely large and it makes all the difference in the world whether you take an interface that is at 0.5 mm or 2 mm from the point of gas exchange. My own particular experiments were based on going from our Freon-acetylene experiments which are in a gas-breathing animal; assuming that the profile diffusion for O_2 corrected for macroweight is the same you end up with that figure of 1 or 2 mm P_{O_2} difference between the center of the alveolus and the point at which gas comes out.

Dr. Kylstra: I think that by making this calculation you assume that the boundary conditions for diffusion of inert gas are the same as for the diffusion of O_2 or CO_2. I remember when I first presented this diffusion model at the CIBA symposium several years ago, Wyvil jumped on my neck and said, "Look here, you are talking about spheres where you get diffusion from the periphery to the center and you forget that in fact we have all these alveolar ducts and all these alveolar walls and it is not a sphere from which CO_2 diffuses from the outside toward the center through a void and where oxygen is taken up from the center only at the periphery. In fact, it is a very complicated structure, which is filled with alveolar ducts which all have their little capillaries and perfusion there."

He opened my eyes really and made me realize that perhaps the boundary conditions for diffusion of inert gases may be widely different from those of physiological gases O_2 and CO_2, and I wonder if that would not perhaps explain the discrepancy between the undeniable inert gas gradients that are there and the fact that it is so difficult to really demonstrate the presence of any O_2 or CO_2 diffusion impairment in man even under high pressure conditions.

Dr. Farhi: The difference is not between inert gases and O_2 and CO_2. It is between gases for which no sink exists; in other words, insoluble inert gases and gases for which there is a sink and for which the profile changes drastically. In this respect acetylene and Freon, although biologically inert, go the way oxygen goes. They are taken up and you have the same diffusion gradient.

Dr. Kylstra: You mean because they have such a high solubility?

Dr. Farhi: Because they are soluble. That's the point. Unfortunately we have not been able to demonstrate an O_2 gradient due to diffusion. What one usually does is assume equilibrium between alveolar gas phase and arterial blood in terms of CO_2—and the minute you have done that you have canceled the diffusion gradient for CO_2, which is essentially the same as the diffusion gradient for O_2, and you are back where you started.

Dr. Kylstra: Dr. Farhi, would you comment on Dr. Nieding's paper?

Dr. Farhi: In terms of CO you are at an advantage because in a single breath the gradients you are creating are enormous, while for oxygen or CO_2 in the steady state the gradients are much smaller and therefore you can either say more difficult to demonstrate or of less importance—which is exactly the same thing but places you on opposite sides of the argument. In other words, there is a sizeable difference between a steady-state experiment and a single-breath experiment in terms of the magnitude of gradients you are creating in reference to the time available for equilibration. For O_2 and CO_2, where you have a steady-state situation, I think it is much more difficult to demonstrate the existence of those diffusion gradients.

Dr. Nieding: Yes, it was single breath CO, but we found inferences also on steady-state experiments, as I mentioned. When we washed in simultaneously different test gases with different solubility in blood there remained the difference between the inspiration and expiration, not only due to respiratory quotient; in that case the difference should have been the same for all inert gases. But it was different, and so I think all these results have to be interpreted as well according to the solubility coefficient of the gases on the one hand and to their mass on the other hand.

Part III. PATHOPHYSIOLOGY OF BONE

DEVELOPMENT OF BONE NECROSIS LESIONS

M. Bonfiglio

Development of Bone Necrosis Lesions

The development of bone necrosis, secondary to fracture or dislocation, in which disruption of blood supply to susceptible sites such as the head of the femur, the body of the talus, the carpal navicular and lunate bones, is generally understood. The pathogenesis of bone necrosis in the nontraumatic situation remains a subject of considerable controversy. The exact nature of the development of bone lesions in decompression sickness in caisson workers or divers is as uncertain ~~(9,11,14,16,24)~~ as is that in instances of nontraumatic osteonecrosis occurring in patients with a whole host of associated diseases such as alcoholism, gout, hyperlipidemia, platelet disturbances, corticosteroid effects and others ~~(4,5,8,21,22,26,27) (Table I)~~. Anatomic factors which put certain anatomic sites at risk must be taken into consideration. McCallum et al. (23), and Elliott and Harrison (10) describe the sites of involvement in patients with bone necrosis secondary to decompression

TABLE I

CAUSES OF BONE NECROSIS

Fracture femoral head, talus, navicular
Dislocation hip
Decompression disease
Systemic diseases
Hyperlipidemia
Thrombocythemia
Thrombocytopenia
Gout
Marrow hypoplasia (drugs)
Raynaud's phenomenon
Sickle cell disease
Angiokeratoma
Gaucher's disease
Lupus erythematosis
Cortisone effect
Alcoholism

sickness. These sites have similarly been implicated in cases of bone necrosis secondary to nontraumatic causes: namely, the humeral heads, femoral heads, diaphysis of the distal femur and proximal tibia. The only difference is that the exact incidence of involvement in sites other than the femoral head in the nontraumatic region is not known. Jaffe (18), Kahlstrom (21), and Phemister (27,29) have described bone infarcts in relation to arteriosclerosis and idiopathic nontraumatic lesions other than caisson disease. They suggested that these are probably more common then generally appreciated. The variable blood supply to the femoral head has been extensively studied by numerous investigators (17,30,31,34) and could account for the variability in frequency, occurrence and degree of involvement in the development of bone necrosis in both the traumatic and nontraumatic situations.

Current Theories of Etiology

In decompression disease the bubbling of compressed air in the tissues and blood is thought to be the basis for the circulatory embarrassment leading to bone lesions. Since nitrogen is less soluble, it presumably remains in a gaseous state and causes intravascular air embolism or extrinsic compression in lipoid-rich bone marrow. Elliott and Harrison (11,13) state that even without overt manifestations of decompression sickness, any diver runs the risk of damage by "silent" bubble formation in the body, particularly with prolonged decompression.

A number of investigators postulate fat embolism as the mechanism of production of bone necrosis (19,20,35,36). This concept has been disputed by Hartman (14) who postulates, on the basis of the size of the commonly observed necrotic areas, that occlusion takes place more proximal than in the capillary bed—that is, at the level of arterioles. He based his views on the study of 31 patients who underwent lymphangiography using a poppy seed oil preparation in which fat embolization occurred but no instance of femoral head necrosis resulted. Similarly, there are no known reports of bone necrosis following posttraumatic fat embolization. The incidence of fat macroglobulinemia, according to Tedeschi and coworkers (33), is quite high in a wide variety of clinical situations. Their observations deny any diagnostic significance to the presence of fat macroglobulinemia.

Lagier (22) suggests mechanical overloading as an etiologic factor in bone necrosis based on the presence of microfractures inducing changes in the blood supply. Most of the pathologic studies reported indicate that fractures occur in relationship to repair once bone necrosis has occurred. The only exception to this may be in steroid-induced osteoporosis which subjects the femoral head to stress fractures and secondary necrosis.

Based on a study of 50 patients with nontraumatic necrosis of the femoral head, it has been postulated that a constellation of events alters coagulation homeostasis, resulting in sludging thrombosis or hemorrhage in an area of susceptible blood supply (4,12). Thus femoral head necrosis, in effect a skeletal expression of systemic disease, becomes a complex problem of a multifactored nature in patients with a wide variety of systemic problems. Harrison (13) has stated that a large number of factors could be considered relevant to the etiology of aseptic necrosis of bone, yet the total number of positive cases is small. He was studying the incidence in decompression sickness. Does the individual at risk in decompression sickness, in some instances, have other factors such as alcohol intake or a subclinical manifestation of a systemic disease as contributing factors to the development of necrotic bone lesions? The answer to this question must await a well-designed protocol to

study the individuals at risk. The study initiated by Elliott and Harrison (10) in naval divers to identify radiologic changes is particularly pertinent.

Correlative Pathologic Aspects of Necrotic Bone Lesions

Regardless of the etiology, once bone necrosis occurs, knowledge of the process of repair determines its recognition. The clinical, radiographic, and pathologic changes must be considered simultaneously. Excellent articles on the subject by Catto (6,7), Jacqueline and Rutishauser (17), Jaffe (18), Phemister (29), and Lagier (22) are available for study. Since the exact moment of interruption of blood supply by the incident or incidents producing nontraumatic bone necrosis is usually not known, one must use pathologic material from experimental sources and traumatic cases to describe the early repair process and time sequences. In addition, nonsymptomatic femoral head necrosis in patients with bilateral disease has been biopsied at the time of prophylactic bone grafting. These specimens provide additional information to assist the radiologic interpretation of the bone lesions.

Bone Necrosis After Trauma

Interpreting the radiographic signs of bone necrosis requires knowledge that dead bone has the same density as living bone so that at the moment of ischemia there are no visible alterations in the bone. After trauma, either fracture or dislocation, the period of protective inactivity produces atrophy of living bone permitting the dead bone to stand out providing, however, one includes the uninvolved side for comparison. The initial attempt at bone repair includes a cellular and capillary invasion of the necrotic marrow. In the osteoporotic female, following a fracture of the neck of the femur, this proceeds quite rapidly (3). With marrow necrosis of fat, calcification may occur within a matter of months to permit an increase in bone density. In addition, new bone is deposited on old dead bone so that one of the earliest signs is an increased density in the head-neck junction in nontraumatic bone necrosis of the femoral head. In the early stage of segmental collapse, a subchondral radiolucent zone appears. Its presence, as will be shown, invariably indicates a fracture of the subchondral cortex as a result of partial repair reaching the subchondral cortex. Later a zone of increased density surrounds areas of segmental collapse of varying size.

The time between femoral neck fracture with union and the onset of symptoms heralding segmental collapse usually ranges from 18 to 20 months (7,28,29,32), but it may occur as long as 8 years after fracture (personal experience). A 13-month interval elapsed in one patient who developed hip pain from a segmental femoral head necrosis secondary to a drug-induced pancytopenia and a hypocoagulable state (2).

Femoral head necrosis studies in dogs, produced by dislocating a dog's hip and osteotomizing the neck, describe the invading front of repair by fibrovascular tissue in the marrow spaces, with capillaries penetrating the trabeculae (1).

A review of human femoral head specimens showed vascular invasion of the subchondral cortex and new bone on old bone as early as 2 weeks after femoral neck fracture in a 79-year-old osteoporotic woman (3). My findings were similar to those reported by Catto (6).

A 49-year-old man, who sustained a fracture of the neck of the femur 3 months earlier, illustrates several of the early features of necrotic bone repair. The pelvic radiograph shows

FIG. 1. Radiograph of pelvis 3 months after fracture of the left femoral neck in a 49-year-old man illustrates relative density difference with atrophy of the inferior neck and intertrochanteric region and retention of original femoral head density.

a fracture of the neck of the left femur fixed with a Smith-Peterson nail (Fig. 1). Disuse atrophy of the femoral head inferiorly and the intertrochanteric area permits the head to appear more radiopaque. This is an instance of relative density difference. The right femoral head and superior-lateral aspect of the left are of comparable density. This man was treated by removal of the Smith-Peterson nail and insertion of two tibial bone grafts. A core biopsy shows the fibrovascular marrow repair without any evidence of new bone as yet. Another photomicrograph illustrates the fatty marrow necrosis with lipophages and foam cells. These changes are well described by Catto (6,7), Jaffe (18), Phemister (28) and Sherman and Phemister (32) and need not be elaborated upon further other than to note that, once a fracture of the femoral neck unites, the femoral head necrosis undergoes the same evolution to be described for the nontraumatic cases.

Nontraumatic Necrosis of Bone

The juxta-cortical lesion without collapse is illustrated by a 60-year-old male with bilateral femoral head necrosis secondary to gout and alcoholism. The patient had pain in the right hip for 10 months. The left hip was asymptomatic. A tomogram delineates the increased density changes from the inferior neck to the subchondral cortex (Fig. 2-**A**). The patient was treated by drilling and bone grafting. Photographs and radiographs of the biopsy cores show the zone of necrosis. A low power view of the microscopic section indicates the zone of fibrous tissue replacement, delineating the remaining necrotic area (Fig. 2-**B**). Changes of nonsymptomatic necrosis, similar to those described in this patient, have been reported by McCallum and colleagues (23).

Another example of an early neck lesion, a so-called "cyst," is illustrated by a 50-year-old male who sought help for 14 months before diagnosis of bilateral femoral head necrosis (Fig. 3-**A**-**E**). The symptoms were confined to the right hip. The left illustrates slight

FIG. 2. **A**, Radiograph of right hip and tomogram illustrates a juxta-cortical necrotic lesion without collapse in a 60-year-old male with bilateral femoral head necrosis secondary to gout and alcoholism.

B, Microscopic section of a core biopsy shows the zone of fibrous tissue replacement and increased bone formation delineating the necrotic area. (x2)

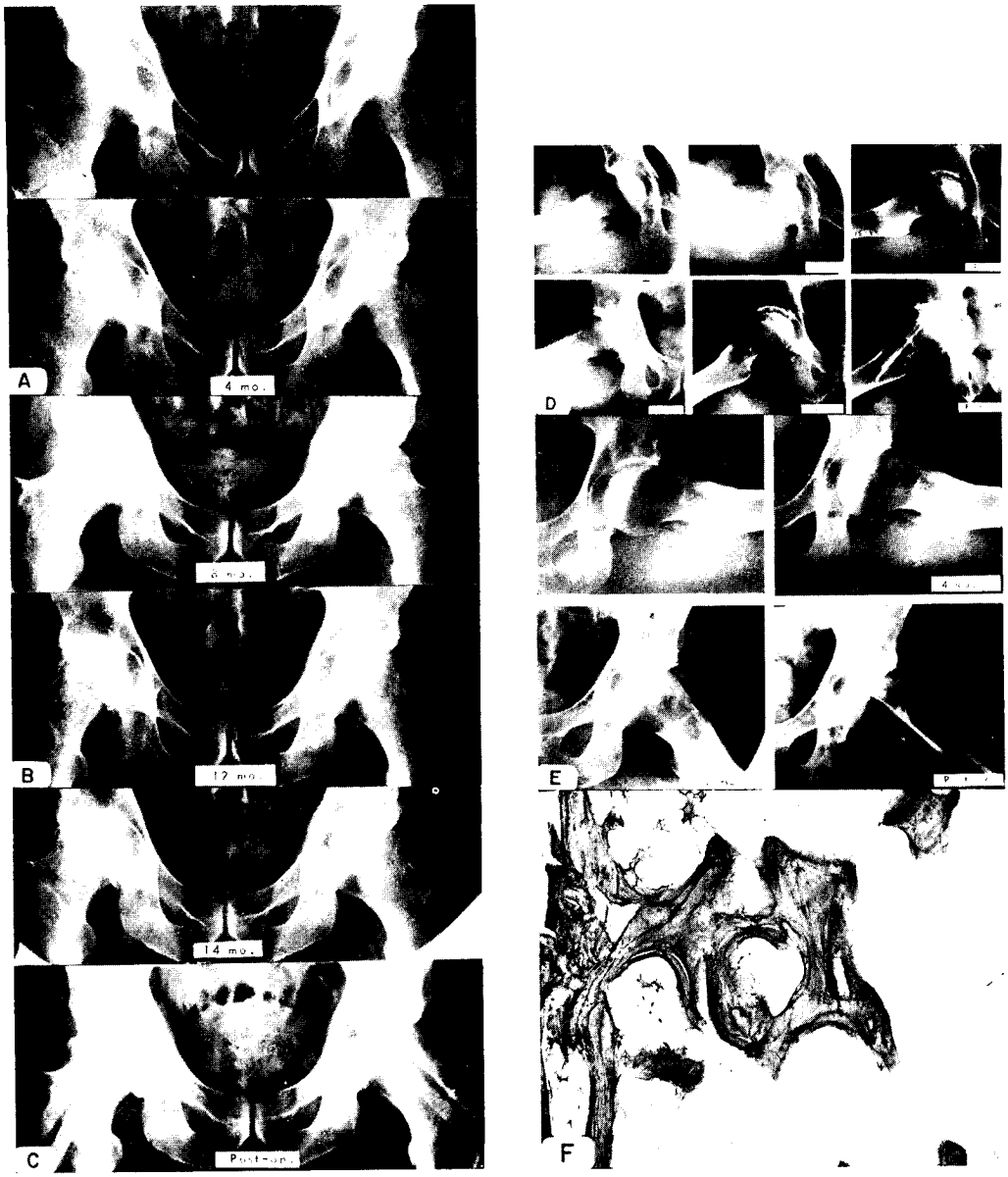


FIG. 3. **A**, A 50-year-old male, who sought help 14 months before a diagnosis of bilateral femoral head necrosis was made, shows an early neck lesion on the left and a juxta-cortical lesion on the right. The evolution of these lesions over time period of 14 months is shown.

Pelvic radiographs anteroposterior views at the onset and at 4 months. Note a juxta-cortical lesion on the right and a spherical opacity on the left with a juxta-cortical lesion.

B, Radiograph of pelvis anteroposterior at 8 and 12 months. Further progression of the right hip lesion is evident, with early segmental collapse.

C, Anteroposterior radiograph of the pelvis at 14 months and after bone grafting. Further collapse is present.

D, Lateral radiographs of the right hip at the onset, 4, 8, 12 and 14 months. Note the subchondral cortical radiolucency in the radiograph at 4 months.

E, Lateral radiographs of the left hip at the onset, 4 and 8 months. The 8-month view illustrates spherical density in the anterior neck.

F, A microscopic section of increased density made from a biopsy of the left hip, neck density corresponding to the radiographic neck density shown in Fig. 3**C** and 3**E**. (x50)

FIG. 4. **A**, Pelvic radiograph 1 year after onset of right hip pain in a 38-year-old male shows segmental collapse of the right femoral head and increased density without collapse of the left femoral head.

B, A lateral radiograph shows the marked increase in density and collapse of the weight-bearing area.

C, Eight years after onset, a radiograph of the pelvis demonstrates a Colonna arthroplasty on the right, and further increase in density of the left femoral head which has remained asymptomatic.

D, A radiograph of the femur illustrating a classical bone infarct at the diaphyseal-metaphyseal junction.

E. A gross photograph of the femoral medullary contents and of a core of the left femoral head shows white necrotic bone in both areas.

F. A microscopic section of the core shows the new bone on old in the subchondral cortical region as well as distally, outlining the extent of the necrosis which corresponds to the radiographs shown in Fig. 4**C** and the gross photo in Fig. 4**E**. (x3)

G. A photomicrograph of the junction between living and dead bone with marked calcified fibrous tissue between necrotic trabeculae (x80).

H. A photomicrograph from the femoral shaft lesion indicates calcified connective tissue and dead bone. (x100)

increase in density in the inferior neck and a zone of increased density comparable to the neck lesion described in Fig. 11 of the paper by McCallum and colleagues (23). The evolution of changes in both hips at 4,8,12,14 months illustrates the juxta-cortical subchondral radiolucent zone in the lateral view on the right at 4 months and increased density and persistence of the neck density on the left hip. A photomicrograph obtained from the left femoral head core biopsy specimen indicates new bone on old dead bone at a site deep in the neck, corresponding to the radiographic neck density (Fig. 3-**F**). The remaining femoral head repair resembled that of the previous patient with a subcortical lesion without collapse.

Another patient illustrates the evolution of a juxta-cortical femoral head necrotic zone over an 8-year period. In addition, he had a segmental collapse of the other hip and a distal femoral shaft infarct. At the age of 38 the patient had a crushing injury about the hips which did not require an interruption of his work. He also had a moderate alcohol intake. He developed gradually increasing pain in his right hip as a result of femoral head collapse as illustrated on a roentgenogram made 1 year after the onset (Fig. 4-**A**). The left hip shows increased density without collapse. A lateral radiograph shows the marked increase in density and the collapse of the weight-bearing area (Fig. 4-**B**). Eight years after the onset, a roentgenogram of the pelvis demonstrated a Colonna-type arthroplasty on the right and an increase in density of the left femoral head (Fig. 4-**C**). A roentgenogram made of the femur at this time shows a calcified zone of increased density at the diaphyseal-metaphyseal junction (Fig. 4-D). The left hip was treated by drilling and bone grafting and the right femoral shaft biopsied. A gross photograph of the specimens shows the white necrotic bone in both areas (Fig. 4-**E**). A microscopic section of the core shows increased density in the subchondral cortical region as well as in the trabeculae distally (Fig. 4-**F**). A high-power view of the junction between living and dead bone shows very marked calcified fibrous tissue adjacent to necrotic trabeculae, which accounts for much of the increased radiographic density (Fig. 4-**G**). A photomicrograph from the femoral shaft lesion indicates calcified connective tissue and dead bone (Fig. 4-**H**). This patient shows all the features usually ascribed to caisson dissease; however, others have reported generalized lesions in non-traumatic situations, particularly arteriosclerosis (18,27).

A 28-year-old male seen 6 months after the onset of right hip pain, with bilateral nontraumatic necrosis of the femoral head, presented with an intermediate stage of minimal femoral head collapse. The right femoral head shows minimal collapse at the weight-bearing area opposite the lateral margin of the acetabulum (Fig. 5-**A**). There is increased density in the foveal region as well as inferiorly in the neck. At the time of drilling and biopsy of the right femoral head it was apparent that this depressed segment represented a fracture. A microscopic section of the articular cartilage and subchondral cortex, at the junction between the repair zone and necrotic bone, demonstrates a fracture zone which has extended into the cartilage (Fig. 5-**B**). The cartilage shows loss of normal staining. Articular cartilage over necrotic bone usually remains well-preserved until there has been repair by vascular invasion of the subchondral cortex. The histologic appearance of the left hip biopsy corresponds to changes described earlier with a zone of new bone on dead surrounding unreplaced necrotic trabeculae.

A 34-year-old male with polycythemia and a history of alcoholism presented with experience of pain in his right hip for several years. His radiographs illustrate minimal collapse of the femoral head (Fig. 6-**A**). A tomogram delineates the extent of increased

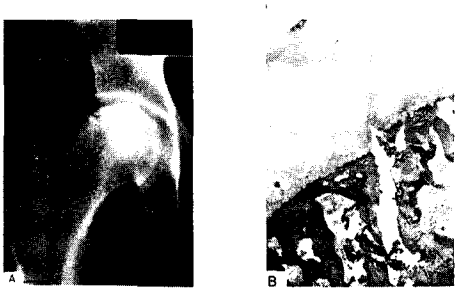


FIG. 5. **A**, Radiograph of the right femoral head in a 28-year-old male with bilateral nontraumatic necrosis of the femoral head demonstrates minimal collapse at the weight-bearing area opposite the lateral margin of the acetabulum. Increased density extends from the foveal region to inferior neck.

B, A microscopic section of the articular cartilage and subchondral cortex at the junction between the repair zone and necrotic bone demonstrates a fracture which has extended into the cartilage (x50). The cartilage shows loss of normal staining but is otherwise well-preserved.

FIG. 6. **A**, Radiographs, including tomogram of the right hip, show minimal collapse of the femoral head but with rather marked increase in density distally to the head-neck junction. A small subchondral cortical zone of radiolucency is also present.

B, Radiograph, gross photo and microscopic section of the illustrate the juxta-cortical fracture line and permit correlation with the increased density of the margin of the lesion in all three components. (x1)

C, A photomicrograph of the necrotic bone between the fibrous tissue and the subcondral fracture shows calcified fatty marrow. (x40)

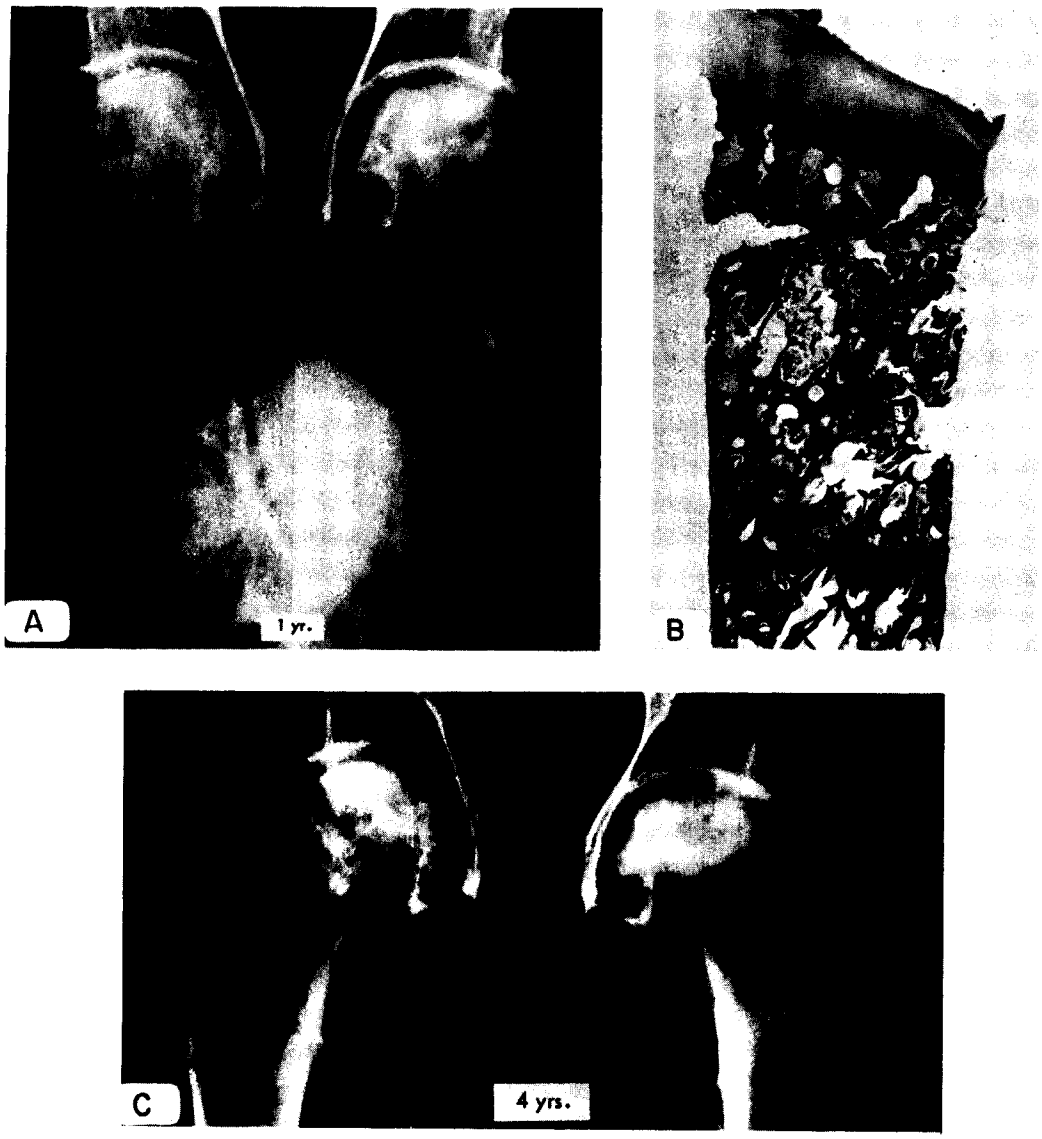
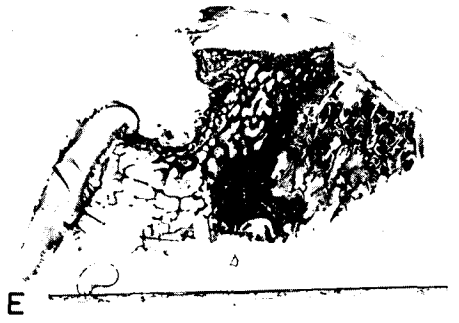
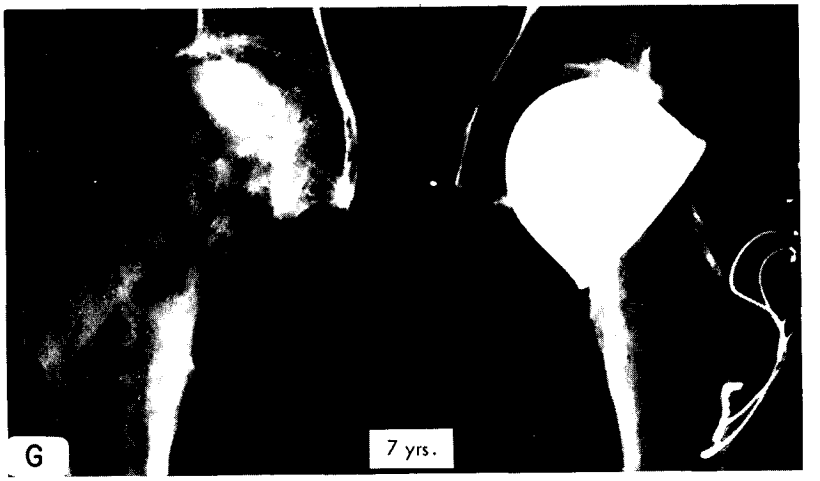


FIG. 7. A. A 49-year-old woman with idiopathic thrombocythemia presented with pain in the left hip for 10 months followed by pain in the right hip within a few months. The radiographs of both hips show segmental collapse, more marked on the left as compared with the right. A moderate degree of degenerative arthritis is present.

B, A histologic section of the right femoral head biopsy shows a subchondral cortical fracture, the increased thickness of trabeculae corresponding to the zone of new bone on old bone. (x3)

C. Radiographs of the hip made 4 years later. The left hip shows marked increase in femoral head density, flattening of the femoral head in the weight-bearing area and an increase in the medial osteophyte formation.





D. A radiograph of the specimen slab, removed at the time of mold arthroplasty for the left hip, shows the zone of increased density surrounding the necrotic segmentally collapsed fragment.

E, A microscopic section of the same specimen shows the sclerotic zone of new bone on old dead bone and the necrotic fragment. The articular cartilage is still well-preserved. (x1)

F, A photomicrograph of the junction between the sclerotic zone of bone and the necrotic fragment. (x32)

G, Radiographs made 7 years after bone grafting and 3 years after mold arthroplasty are shown. The patient maintains comfortable function of both hips.

density at the head-neck junction. In addition, there is a small subchondral cortical zone of radiolucency. A core biopsy illustrates the juxta-cortical fracture line in the radiograph, gross photo and microscopic section of the specimen (Fig. 6-**B**). The correlation with the increased density at the margin of the lesion is easily delineated in all three components of the specimen. A photomicrograph of the necrotic bone between the fibrous zone and the subchondral fracture shows calcified fatty marrow (Fig. 6-**C**). The mechanism of production of calcification in fatty marrow is presumed to be by saponification of necrotic fat to produce amorphous calcium deposits.

Another patient, a 49-year-old woman, depicts a somewhat more advanced bone lesion. She experienced pain in the left hip for 10 months followed within a few months by pain in the right hip. The etiology of her necrosis was due to an idiopathic thrombocythemia. The patient had a platelet count of 3,000,000/mm^3. The disease was controlled by treatment with busulfan (myleran). Her right hip was treated by drilling and bone grafting. Treatment of the left hip was delayed to determine what effect the treatment of her disease might have on it. The radiographs of the right hip show the subchondral cortical radiolucency, a moderate degree of femoral head density and early marginal osteophytes (Fig. 7-**A**). The left hip illustrates a segmental collapse with marked increase in density of the collapsed fragment with a zone of radiolucency surrounding this. The lateral view demonstrates that zone in the anterior portion of the head. Slightly more advanced secondary marginal osteophytes are present. The histologic section of the right femoral head biopsy shows the subchondral cortical fracture, the increased thickness of trabeculae corresponding to the zone of new bone on old bone and, although not shown on this lower power view, extensive marrow fibrosis and calcification (Fig. 7-**B**). Four years later the patient had minimal discomfort in the right hip but increasing pain in her left hip. A mold arthroplasty was performed at this time. The roentgenogram shows a marked increase in femoral head density, flattening of the femoral head in the weight-bearing area and an increase in the medial osteophyte formation (Fig. 7-**C**). The specimen removed at the time of the arthroplasty indicates quite clearly that the necrotic fragment had not been replaced. There is a zone of markedly increased density surrounding the original density of the dead bone. The subchondral cortex has separated from this fragment which appears dead-white (true mortification of bone) on the gross photo and as a zone of radiolucency on the slab radiograph and microscopic section of the specimen (Fig. 7-**D**-**E**). A photomicrograph of the junction between the sclerotic zone of bone and the necrotic fragment indicates a fracture which undoubtedly accounted for the continuation of the patient's pain (Fig. 7-**F**). Continual compression of the femoral head on segmentally collapsed fragments prevents advancement of repair through the pseudarthrosis intervening between the living and dead bone. Radiographs made 7 years after bone grafting and 3 years after mold arthroplasty are shown (Fig. 7-**G**). The patient maintains comfortable function of both hips.

A 57-year-old male who sustained a fracture dislocation 3½ years earlier illustrates a more advanced state of the necrotic bone lesion. A slab radiograph of the femoral head specimen removed at the time of replacement arthroplasty shows a segmental collapse of a central fragment with marked increased density surrounding it (Fig. 8-**A**). The photomicrograph of the entire specimen illustrates the marginal degenerative joint changes, loss of articular cartilage, particularly in the sclerotic zones adjacent to the necrotic fragment, but also at this time over the necrotic fragment (Fig. 8-**B**). A photomicrograph of the junction between the very dense and the necrotic fragments shows considerable fibrocartilaginous connective tissue comparable to what one would see in a pseudarthrosis (Fig. 8-**C**). There

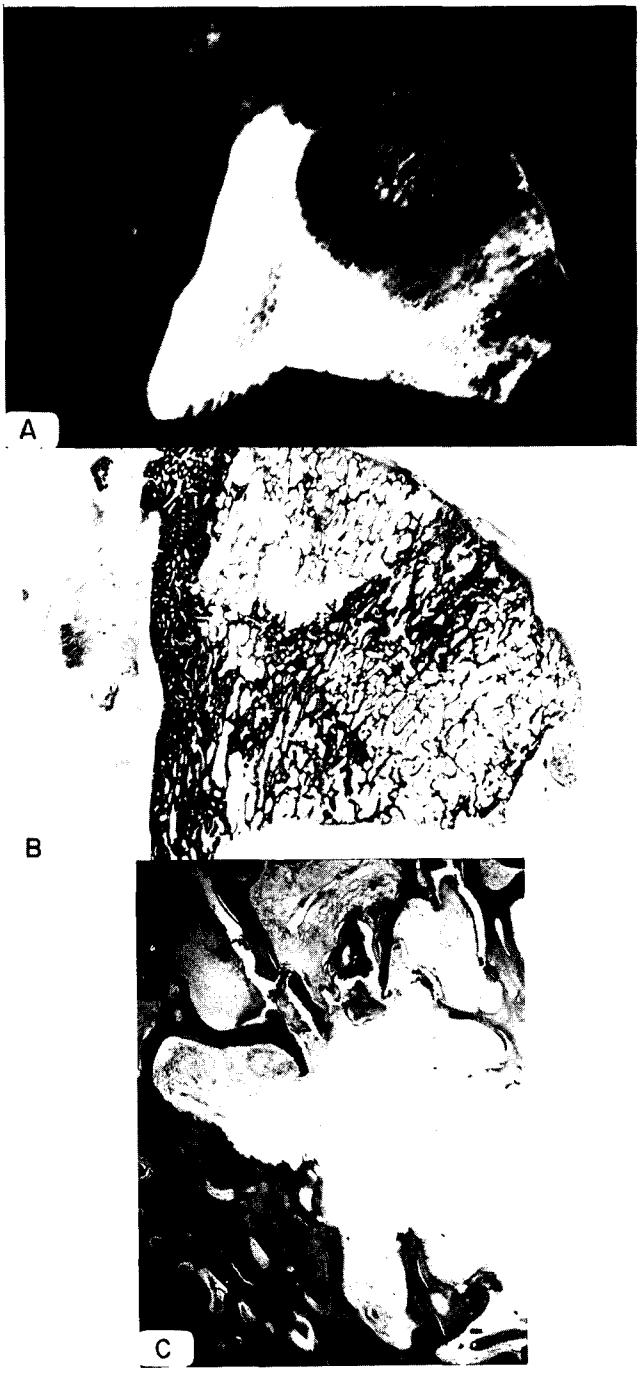


FIG. 8. **A**, Slab radiograph of a femoral head specimen removed 3½ years after hip dislocation in a 57-year-old male. Segmental collapse of the central fragment with marked increased density surrounding it.

B, Photomicrograph of the specimen shows marginal degenertive joint changes, loss of articular cartilage, particularly in the sclerotic zone adjacent to the necrotic fragment. (x1)

C, Photomicrograph at the junction between the dead bone and the necrotic fragment illustrates pseudarthrosis tissue. (x30)

has been some resorption of the necrotic trabeculae and the ever-present hallmark of repair of dead bone, the thickened trabeculae with new bone on old necrotic cores. In addition, marrow calcification persists in the necrotic segment of the femoral head.

Although most authors assume some kind of disturbance to the peripheral blood supply of the femoral head, rarely are thrombosed vessels observed. Studies of the ligamentum teres by Catto and Chandler (6,8) revealed some obliterated vessels. The AP and lateral radiographs of the left hip of a 50-year-old male alcoholic with bilateral femoral head necrosis shows segmental collapse (Fig. 9-A). A photomicrograph made from a core biopsy of the femoral head illustrates obliterated and thick-walled arterioles in the femoral head in the repair zone of thickened trabeculae (Fig. 9-B). Whether they represent a manifestation of the generalized disease or a local phenomenon cannot be stated.

The progression of bone lesions beyond those demonstrated is in many instances one of progressive degenerative joint disease with increasing marginal osteophytes, loss of articular cartilage, development of degenerative cysts and further fragmentation of the necrotic bone.

As etiology becomes defined, efforts should be directed toward the early identification of the lesion and early treatment. The views of the Iowa group have been recorded on the subject of treatment (2,4).

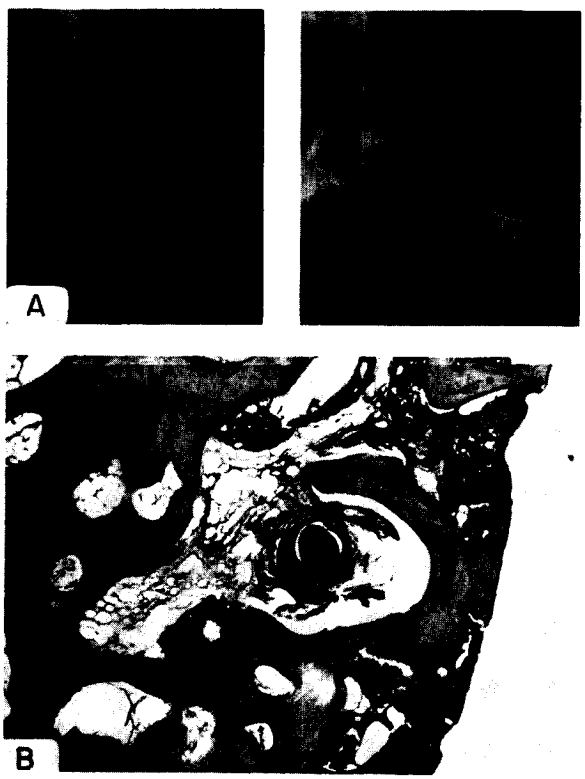


FIG. 9. A. Radiograph of femoral head with extensive necrosis and segmental collapse in a 50-year-old alcoholic.

B. Photomicrograph of thrombosed, thick-walled arteriole from space between thick trabeculae at repair zone (x80).

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THE EARLIEST RADIOGRAPHIC EVIDENCE OF DYSBARIC OSTEONECROSIS

J. K. Davidson

Dysbaric osteonecrosis—aseptic necrosis of bone or caisson disease—is a hazard to divers, and compressed air workers or sand-hogs. If these young men, often in their 20s or 30s, develop a severe painful arthritis of their shoulder and hip joints, secondary to the bone lesions, they will be severely disabled.

Clearly it is important to reduce the incidence of the condition and, since radiology provides the method whereby dysbaric osteonecrosis can be recognised before symptoms develop, any serious attempt to control this condition must involve regular skeletal radiographic surveys of those at risk. If the condition is to be recognised early, it is most important that radiographs of high quality be obtained.

The classical changes of osteonecrosis are well demonstrated in the head of the femur following a fracture of the neck (Fig. 1 A-C). When a segment of bone loses its blood supply it dies; initially necrotic, this bone is radiographically indistinguishable from the living bone (Fig. 1A). Revascularisation of the dead area takes place with gradual laying down of new bone on the dead trabeculae causing an increase in bone bulk and so an increase in radiographic density (Fig. 1B). It takes about 4 months for this to occur. Later there may be a structural failure of the joint surface (Fig. 1C) and symptoms will develop.

The earliest radiographic evidence of bone necrosis is therefore an increased density of the affected area. This normally takes between 8 months and a year after the causal exposure—the shortest interval observed being 4 months.

Similar radiographic changes develop in osteonecrosis from other causes such as, for example, that which follows the use of steroids, especially when given in large doses, and that of dislocation of the hip. Osteonecrosis also occurs in association with the haemoglobinopathies—especially with Hb SC variant; pancreatitis; and disorders of fat metabolism.

The later changes of dysbaric osteonecrosis are well known. Figure 2 shows osteonecrosis of the head of the right humerus with sequestration of a large part of the articular surface and increased density of part of the humeral head. This 33-year-old commercial diver first noticed pain in his right shoulder after lifting some heavy timber. Presumably it was at this time that the weakened joint surface collapsed into the underlying necrotic bone. He had been diving to 180 feet for over 4 years. Because of persistent pain the joint had to be fused.

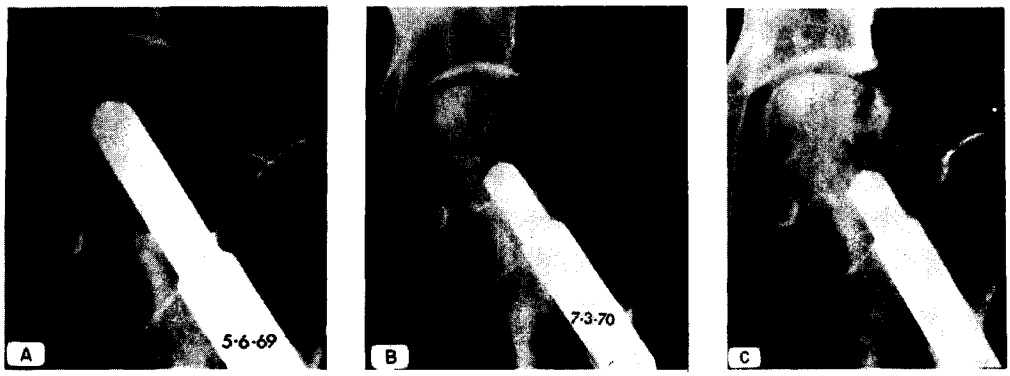

FIG 1. A, Fracture of femoral neck. Radiograph on completion of pinning and within 6 days of fracture. Bone structure of femoral head is indistinguishable from surrounding bone but it is, in fact, necrotic (see B).

B, Same patient 9 months after fracture. Increased density is now present in femoral head resulting from revascularisation with laying down of new bone on dead trabeculae causing an increase in bone bulk.

C, Same patient 15 months after fracture showing structural failure of the superior part of the articular surface with collapse into the underlying bone.

It is, however, the earliest radiographic changes which are of interest because the presence and absence of bone lesions in radiographic skeletal surveys have been used as criteria of success when assessing the effective uses of new decompression schedules. Although the radiographs from a number of skeletal surveys on divers have been examined, there has been much greater experience in studying surveys of compressed air workers. The radiographic features of osteonecrosis appear to be identical in these two groups.

Examples of the earliest radiographic features are shown—in the head of the humerus (Fig. 3A), in the femoral head (Fig. 4A), and in the medulla of the femur (Fig. 6A). Evidence that these are, indeed, early osteonecrosis stems from the fact that each has been followed, for periods of up to 6½ years by regular radiographic examination, and each has developed into a classical lesion.

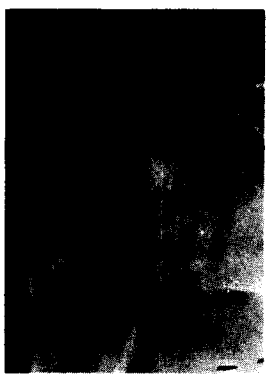

FIG. 2. Thirty-three-year-old commercial diver showing typical dysbaric osteonecrosis of the head of the right humerus. Structural failure with sequestration and increased density of a large part of the humeral head.

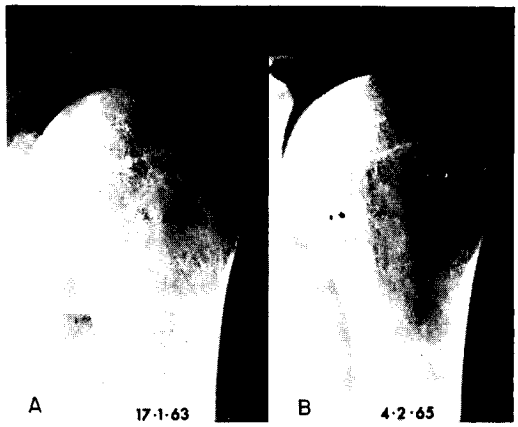

FIG. 3. **A**, Ill-defined dense areas in the head of the left humerus representing early radiographic evidence of osteonecrosis. Patient had worked in compressed air for 17 months at pressures up to 37 p.s.i.g. and never had type I or type II bends (see **B**).

B. Same patient, now developed linear opacity, serpiginous in shape. Still has no symptoms and has continued working in compressed air.

The earliest radiographic abnormality may appear as a number of dense areas lying adjacent to an intact articular cortex varying in diameter between 3 and 20 mm. Trabeculae passing through these areas may appear thickened and fused. The margins are indistinct in contradistinction to bone islands which are usually well defined. These dense areas are seen more commonly in the head of the humerus (Fig. 3A). In the femoral head detail is often obscured by the overlying acetabulum and tomography may be required to improve definition. When several areas of increased density become confluent, the appearance is that of a "snow-cap" as described by Poppel and Robinson (3).

These ill-defined dense areas may progress to a "linear opacity" which may be curved or serpiginous (Fig. 3B). Alternatively this may be the initial radiographic abnormality. The opacity extends to the intact cortex on each side enclosing as much as two-thirds of the joint surface. The thickness varies between 2 and 5 mm and the margins may be either ill defined or distinct. The necrotic bone between the "linear opacity" and the articular cortex is usually radiographically indistinguishable from living bone on the opposite side. There may be further areas of increased density at the shaft side of the opacity. The lesion is more commonly seen in the head of the humerus than the head of the femur. In the femur the opacity tends to follow a more S-shaped course (Fig. 4A and B). This type of linear opacity presents a typical appearance and could be described as a hallmark of osteonecrosis.

Not all of the lesions seen progress radiographically. Some, however, go on to a structural failure of the joint surface (Fig. 4C) as weight-bearing or excessive muscular activity cause the weakened articular surface to collapse into the underlying subchondral bone. This disruption of the bone contour may appear as a translucent subcortical band representing a fracture through the necrotic bone, or as depression or collapse of the cortex, or as sequestration of either a large part or several fragments of the joint surface. These features are now well known.

Accurate interpretation of bone changes observed radiologically has been made possible

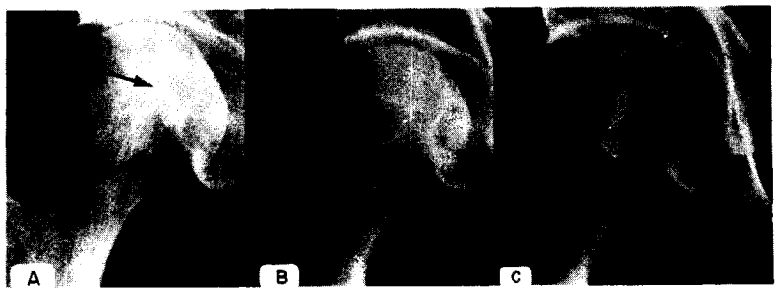

FIG 4. A, Zone of increased density running vertically through the right femoral head in a symptomless compressed air worker (see B).

B, Same patient 2 years and 10 months later. More definite linear opacity, serpiginous in shape. Articular surface still intact, symptoms absent (see C).

C, Same patient. Serpiginous zone of increased density involving a large part of the femoral head. Structural failure with sequestration of the articular surface inferiorly; symptoms present.

only because the concurrent histological changes have been studied. In the Clyde Tunnels Report (1), the histological changes which occurred in the head of the humerus of a man who died after considerable experience of work in compressed air were described. These sections (Fig. 5A and B) show that a large proportion of the head of the humerus had been necrotic but was being revascularised. The area of subarticular necrotic bone was separated from the living bone by a thick layer of new bone laid upon dead trabeculae. Between this dense area and the remaining necrotic part there was a layer of fibrous tissue indicating perhaps that further revascularisation was unlikely. Similar correlation between histological and radiological features has been demonstrated in other cases. In one it was possible to confirm that the small dense areas adjacent to the intact articular cortex in the head of the humerus represent thickened trabeculae resulting from the laying down of new bone on dead trabeculae during revascularisation. In another a band of thickened trabeculae in the head

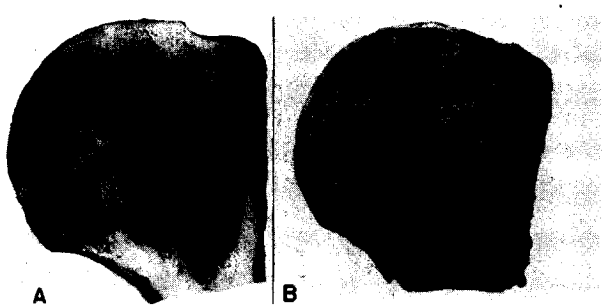

FIG. 5. A, Slab radiograph of the humeral head in a compressed air worker known to have osteonecrosis. Serpiginous zone of increased density involves the large part of the femoral head (see B).

B, Photomicrograph showing an intact articular cortex covered by articular cartilage of normal thickness. Beneath the cortex there is a shallow saucer of dead bone bordered by a greyish line of fibrous tissue representing the furthest advance of revascularisation process. Broad trabeculae (arrow) are found immediately distal to this consisting of new bone laid on the trabeculae of dead bone. Same patient as A.

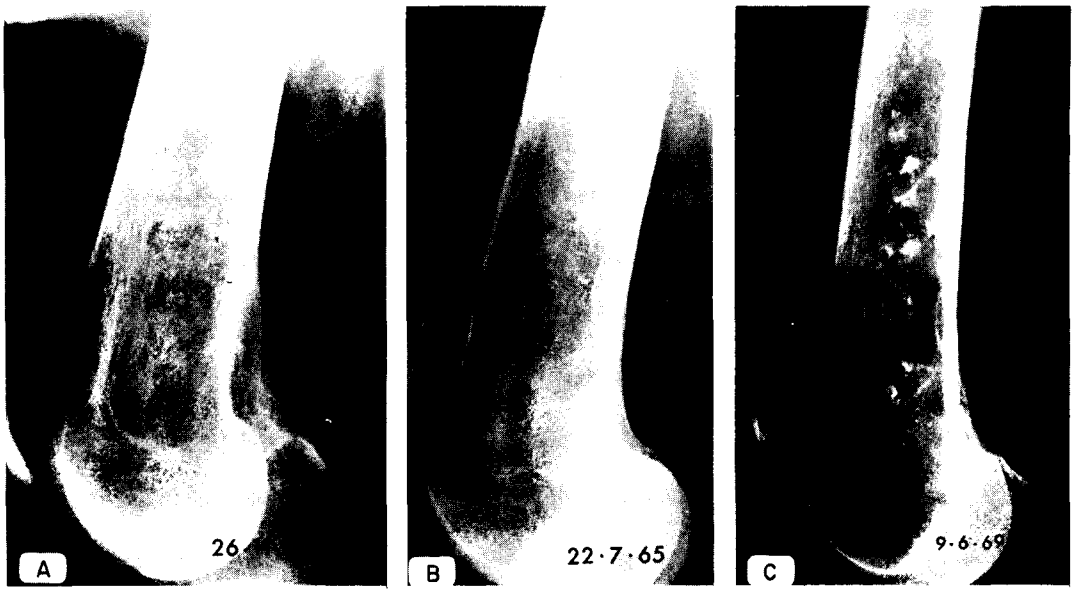

FIG. 6. **A**, Examples showing faint speckles of irregular calcification and increased density within the medulla of the femur (see **B**).

B, Same patient as **A**, 2 years and 8 months later showing more definite calcification (see **C**).

C, Same patient. More extensive calcification involving a large part of the femur.

of the femur was seen as the explanation for the radiological linear opacity running through the femoral head.

Turning now to the early radiographic features of bone lesions in the femoral shaft, these first appear as small speckles of calcification lying within the medulla at some distance from the joint surface (Fig. 6A). They may also appear as small areas of increased density similar to those already described adjacent to a joint surface. Sometimes extensive calcification is seen occupying a large part of the medulla of the femoral shafts (Fig. 6B and C). These areas of increased density and calcification seen in the medulla may be due to new bone laid on dead trabeculae, to calcification laid down in the fibrous capsule surrounding a necrotic area of bone, and even to calcification in the dead marrow itself.

The relative frequencies of the different types of lesions have been analyzed in a review of 334 compressed air workers with 820 positive lesions (Table I). From Table I it can be seen that the most frequent early abnormalities are: 1) head, neck and shaft—irregular calcification; 2) head, neck and shaft—dense areas; and 3) juxta-articular dense areas with intact articular cortex.

In all, the shoulder, hip and knee joints of 1,694 compressed air workers have been examined and the occurrence of lesions found are shown in Table II. Some of this data has already been published (1, 2).

The incidence of definite osteonecrosis is 19.7% with 11% having a lesion next to the joint surface and so liable to cause symptoms. One interesting point is that in 13.5% of the men the diagnosis had to be suspected osteonecrosis: this underlines the difficulty in identifying the earliest radiographic changes, often because the radiographic detail is not

TABLE I

RELATIVE FREQUENCY OF DIFFERENT TYPES OF LESIONS AND PRESENCE OR ABSENCE OF SYMPTOMS[a,b]

Type of Lesion	Relative Frequency (%)	Symptoms
A) Juxta-articular		
A1) Dense area with intact articular cortex	15.5	Absent
A2) Spherical segmental opacities	5	Absent
A3) Linear opacity	6.5	Absent
A4) Structural failure		
a) Translucent subcortical band	0.5	Present
b) Collapse of articular cortex	2	Present
c) Sequestration	3	Present
A5) Osteoarthritis	5	Present
B) Head, neck and shaft		
B1) Dense areas	20.5	Absent
B2) Irregular calcification	40	Absent
B3) Translucencies and cysts	2	Absent

[a] 334 men with 820 positive lesions.
[b] M. R. C. Decompression Sickness Registry, July, 1972.

TABLE II

NUMBER OF MEN WITH LESIONS OF TOTAL OF 1,694 MEN EXAMINED[a,b]

334 men had definite osteonecrosis
187 men had disabling or potential disability at joint surface (36% of these were bilateral)
57 men had structural failure with joint symptoms

[a] 229 men had suspected osteonecrosis; of these, 75 men had to be reclassified as having definite osteonecrosis on followup over a period of 5-6 years.
[b] M. R. C. Decompression Sickness Registry, July, 1972.

sufficiently good. So far 75 of these suspected cases have become definitely positive. The policy of following up doubtful lesions is difficult because the population is itinerant. In fact, it has been possible to re-examine only 16% of the suspected cases and much less on a third or fourth occasion.

Radiographs were made of 197 divers who had been to depths between 100 feet and 600 feet; four have been found to have definite lesions of osteonecrosis and eight are suspected to have them.

The importance of obtaining radiographs of the highest quality showing good trabecular detail cannot be overemphasised. Positioning of the patient correctly is also important. The full survey examination consists of an anteroposterior projection of each shoulder and each hip, and a lateral projection of each knee joint. For the shoulder the patient lies supine with the trunk rotated at an angle of 45° to bring the shoulder in contact with the x-ray table, the arm being partially abducted and elbow flexed. The object is to show a clear joint space

FIG. 7. This series shows radiographic faults.

8.9.64. Radiograph is underpenetrated. Trabecular detail not shown. Suggestion of increased density superiorly adjacent to the articular cortex.

25.4.67. Good penetration—trabecular detail seen, definite areas of increased density adjacent to an intact cortex.

7.4.72. Overpenetration; trabecular detail not seen. Bad positioning—the coracoid process overlies the femoral head. Increased density is present, adjacent to the intact articular cortex.

and to avoid the acromion overlapping the head of the humerus. The centering point is one inch below the coracoid process of the scapula and the field is coned to show the head and shaft and proximal shaft of the humerus. For the hip the centering point is taken as one inch below the midpoint of the line joining the anterior superior iliac spine and the upper border of the pubic symphysis. The foot should be at 90° to the tabletop. *Gonad protection is essential.* For the knee the field should include the distal femur from a point proximal to its midpoint and the proximal tibia and fibula to their midpoints or just beyond.

Further projections may include a "frog lateral" of the femoral head; this projection is particularly useful in revealing a translucent subcortical band. Tomography may be required for improved definition. The commonest fault is insufficient penetration (Fig. 7), due to the fact that the increase in bone bulk resulting from the revascularisation requires an increased kilo-voltage (up to 10 or more) for satisfactory results. Another fault is that the head of the humerus is allowed to rotate so that the earliest features of osteonecrosis may be missed.

ACKNOWLEDGMENT

The editor of the *Journal of Bone and Joint Surgery* kindly gave permission to reproduce Figure 5A and B. The data has been drawn from the MRC Decompression Sickness Registry, 21 Claremont Place, Newcastle upon Tyne.

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THE ETIOLOGY AND PATHOGENESIS OF DECOMPRESSION SICKNESS: RADIOGRAPHIC, HEMATOLOGIC AND HISTOLOGIC STUDIES IN SWINE

P. J. Stegall and K. H. Smith

Aseptic bone necrosis (dysbaric osteonecrosis) has been produced in miniature swine exposed to compression-decompression profiles. Corroborating evidence is presented to support the hypothesis that dysbaric osteonecrosis is the sequela to an occlusion of bone blood flow due to bubble-induced coagulation of blood (ischemic infarction).

Destructive bone lesions have long been recognized as a latent problem associated with exposure to compressed gas atmospheres (2,32). While the lesion has been recognized, the etiology and pathogenesis have remained unknown (8) despite interest, concern and study during the last half century. Numerous attempts have been made to reproduce this condition in laboratory animals, but until now no conclusive evidence has been presented to demonstrate a lesion comparable to that found in man (6,7,11,16,23).

Methods

A colony of eight miniature swine was exposed daily in a hyperbaric chamber to 60 feet of sea water (fsw) for 6 hours. Decompression was continuous at a rate of 30 feet per minute (fpm).

Control radiographs were taken of each animal prior to its first hyperbaric exposure and were compared to monthly followup radiographs. Bi-weekly blood samples were compared to pre-study values, with each pig serving as his own control. A Model S Coulter Counter was used to run a complete blood count which included RBC, WBC, Hct, and Hgb. Smears of whole, EDTA anticoagulated blood were counterstained with Wright's stain, and reticulocytes were counted. Citrated blood diluted with Rees-Ecker solution was used for platelet counts by the direct microscopic method. Replicates were run through the Coulter Counter to check microscopic platelet count accuracy. Platelet adhesiveness to glass was determined using fresh uncoagulated blood as described by Bowie et al. (4) and Salzmann (24), in which platelet counts were performed before and after the passage of blood through a standard column of glass beads at a controlled rate.

Thrombin time, partial thromboplastin time (Fibrolet method), prothrombin time (Quick's method) and quantitative fibrinogen (Clauss method) were done on serum samples

from each pig. Chemistries run on a Technicon Auto-Analyzer included LDH (Amador-Dorfman-Wacker method), calcium (Kressler-Wolfman), uric acid (Hawk-Oser), alkaline phosphatase (modified Messey-Lowry), and phosphorus (Goldberg-Fernandez). A specially modified Barron-Moline procedure was used to determine total serum lipids (triglycerides, free cholesterol, cholesterol esters, and phospholipids). Other procedures which were modified included tests for SGOT (Karman) and CPK (Tanzer-Gilvarg).

Silastic catheters were surgically placed in the jugular vein and passed into the anterior vena cava of pigs to facilitate routine blood sampling. The extravascular portion of the catheter was covered with Dacron felt, allowing tissue ingrowth to help assure successful implant of the catheter which traveled subcutaneously to exit on the dorsal side of the neck. Firm tissue ingrowth decreased the incidence of bacterial pocket formation where the catheter surfaced. A catheter removed from a pig 4 weeks post-surgery showed that the tissue had penetrated the Dacron felt and a firm bond was achieved. Catheters usually remained patent with every-other-day maintenance which included clearing with physiologic saline and a 1% heparinized saline lock to maintain patency. It was suggested that platelet contact with catheter surfaces may significantly reduce survival times (26). Investigations with labeled platelets were carried out to determine if the silastic catheters used in this study had that effect before values relating to survival times were considered valid.

When indicated, biopsy specimens were obtained. The femur of the anesthetized animal was surgically exposed through a lateral incision. After radiographically localizing the area of suspected necrosis, samples of medullary ossium, cancellous bone and cortex were obtained.

Four pigs, who were either sacrificed or died during the study, were necropsied and specimens were obtained. To identify microscopic changes in bone and soft tissues of these animals, histologic preparations were stained with H&E for routine screening, as well as osmic acid for fat differentiation, PTAH for fibrin, Landing's method for lipids, Van Geisen's method for collagen fibers, and Manuel's method for reticulum identification. To reduce tissue shrinkage in the preparation process, after decalcification, the bone specimens were imbedded in celloidin; routine paraffin sections were done on soft tissue specimens. Uncut bones were radiographed on a Faxitron for gross inspection prior to imbedding.

Results

Hematology

No significant changes were observed in WBC, RBC, Hgb or Hct in routine blood sampling of pigs exposed to daily compression-decompression stress. In seven of eight pigs, however, platelet counts were decreased, sometimes as much as 50% (28). One pig, whose platelet counts were monitored hourly at depth throughout a 50 fsw for 6 hours dive with slow decompression, showed little change during the dive but did show a 16% decrease 12 hours after surfacing.

Platelet adhesiveness rose an average of 30% following hyperbaric exposure, with all samples showing marked clumping of platelets. Pre-dive adhesiveness in one pig was 38% while its post-dive sample drawn immediately after surfacing showed platelet adhesiveness of 49%.

Preliminary studies using Cr^{51}-labeled platelets, I^{131}-labeled fibrinogen and I^{125}-

labeled plasminogen, were employed to monitor coagulation function in two human divers subjected to 150 ft no-stop decompression dives for 10 minutes and 15 minutes on 2 consecutive days. Platelet survivals of 5.34 and 5.45 days, respectively, were found during the 4 days of observation following the exposures (normal platelet survival is 9.5 ± 0.6 days). A transient fall in platelet count occurred lasting for 24 hours following the dive. In one diver with bubble formation documented by a Doppler bubble detector, plasminogen and fibrinogen survivals were modestly shortened to 1.4 and 3.78 days, respectively; normal plasminogen survival time is 2.2 ± 0.2 days; fibrinogen 5.4 ± 0.3 days. In the second diver, in whom bubble formation was not detected, plasminogen and fibrinogen levels remained normal. Based on this information, expanded investigations in this area have been planned.

In the study of changes occurring in the clotting mechanisms of blood after decompression in miniature swine, an increase in thrombin times has been observed. From an average control value of 10-13 seconds, thrombin times increased to over 60 seconds, with no clot forming at all in over 2 minutes in three cases. Reported normal thrombin times are 10-16.5 seconds for miniature pigs, with an average spread of 1.4 seconds for individual animals (5).

There was an upward trend in fibrinogen levels following decompression in all exposures measured, with the greatest increase occurring after fast rates. There were no observed changes in partial thromboplastin and prothrombin times.

In the chemical analyses of blood samples drawn during the study, calcium, uric acid, alkaline phosphatase and cholesterol levels showed little variance from their control levels. Reported normal values for SGOT in miniature swine are 23 U/L (5). All of this experimental colony had SGOT values within the 95% tolerance limits of 8-38 U/L even after fast decompression; however, there was a consistent rise above baseline levels after stress:

Sidney	Pre-dive	15.3 U/L
	Post-dive	37.8 U/L
Simone	Pre-dive	15.2 U/L
	Post-dive	23.0 U/L
Stanley	Pre-dive	14.0 U/L
	Post-dive	24.0 U/L
Seymour	Pre-dive	12.0 U/L
	Post-dive	26.2 U/L

Mean LDH values reported for miniature pigs are 332 U/L with normal limits of 130-534 (3). Again, while the values for this group of animals showed a general trend upward when each pig's control value was taken as its reference point, the post-dive values still fell within the normal 95% tolerance limits established in the literature:

Sidney	Pre-dive	174 U/L
	Post-dive	224 U/L
Simone	Pre-dive	130 U/L
	Post-dive	301 U/L
Stanley	Pre-dive	102 U/L
	Post-dive	210 U/L

CPK levels rose above baselines also after animals were subjected to stress. Typical changes observed were:

Sidney	Pre-dive	6.6 U/L
	Post-dive	15.5 U/L
Simone	Pre-dive	1.39 U/L
	Post-dive	10.98 U/L
Stanley	Pre-dive	2.78 U/L
	Post-dive	8.78 U/L

Radiology

A 3-year-old TRF-strain miniature swine (S.J.—a cross between a true miniature from the Yucatan Peninsula and a Labco miniature) underwent a series of 35 compression-decompression exposures. Radiographs of its shoulders and pelvis prior to its initial dive established that these areas were within normal limits. A complete series of hematologic tests as outlined in the methods section were reported within normal limits.

Its initial diving profile was 6 hours of bottom time at 50 fsw with decompression at 20 fpm to 10 fsw and 10 to 0 feet at 1 fpm. It was asymptomatic after five dives but exhibited signs of right foreleg pain after the sixth dive. It continued to exhibit signs of leg pain and eventually, 10 days after onset of the signs, it was unable to support weight on the leg. Following its 10th dive, there was an episode of respiratory difficulty for 1 hour following decompression but it was not recompressed. Blood tests run on samples after its 12th dive showed a platelet count of 470,000 (pre-dive value: 434,000) and a reticulocyte count of 1.6% (pre-dive value: 0.2%). There was a pronounced rise in fibrinogen level from 80 mg% to 427 mg%. While partial thromboplastin time decreased from 33 seconds to 20 seconds, thrombin time increased from 12 seconds to 2 minutes. Total serum lipids rose from 127 mg% to 234 mg%.

On the 15th dive the decompression rate was changed to a continuous 30 fpm which the pig appeared to tolerate well. A blood sample drawn after this dive, however, revealed a marked drop in platelet count to 196,000/mm^3 and a further decrease in partial thromboplastin time to 13 seconds. There had been a gradual appearance of schistocytes—burrlike RBCs—in previous samples, but in the 24-hour sample 90% of its red cells were crenated. Platelet counts done on a third sample drawn 48 hours after this fast decompression episode revealed a further drop to 153,000/mm^3.

Since the main goal of these exposures was the production of bone lesions, efforts were made to stress the animal as much as possible without causing death. Therefore, the dive depth was increased to 60 fsw for 6 hours on the 25th dive; the rate of decompression remained at 30 fpm. After surfacing from its 26th dive, the animal experienced marked decompression sickness for the first time. It responded well to treatment which included a fast recompression to 70 fsw and a continuous decompression rate of 1 fpm. Some stumbling and sluggishness were observed after treatment but all vital signs appeared within normal limits.

After a 3-week rest period, the animal was again recompressed on the same 60 fsw for 6 hours profile. It exhibited signs of bends within 20 minutes after surfacing. Its respiratory

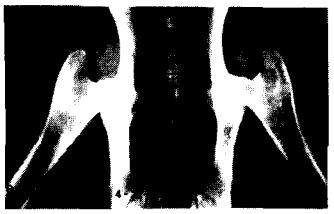

FIG. 1. Small suspicious radiolucency was picked up 2½ months after this miniature pig was first exposed to compression-decompression stress. Arrow points to irregularly marginated lucency in the subtrochanteric area of left femur.

rate rose to 80 per minute and it was unable to stand. Recompression treatment was successful. Although its routine decompression rate was slowed to 2 fpm on its 31st dive, it again experienced severe decompression sickness, necessitating treatment. The decompression rate was further slowed on the 32nd and 33rd dives to 0.75 fpm but it continued to experience marked respiratory difficulty, ataxia and environmental disorientation following decompression. The profile on its 34th dive was shortened to 3 hours of bottom time with a 0.75 fpm ascent rate; the animal again manifested signs of severe bends and required treatment.

After a rest of 12 weeks, it experienced severe decompression sickness after surfacing from a 60 fsw for 6 hours dive with a 1.33 fpm decompression rate. Despite a 4-hour recompression treatment, its respirations were rapid and it was unable to stand. It was recompressed to 30 fsw and remained in the chamber at this depth for 12 hours. After being brought to the surface in a 6-hour decompression, it was retired from any further compression exposure.

Radiographs taken periodically throughout the course of its series of pressure exposures demonstrated a small suspicious area (Fig. 1) in one femoral shaft 2½ months after it was first compressed. Five months later, followup radiographs (Fig. 2) showed progression in the radiolucency and the appearance of a second radiolucency in the other proximal shaft. Nine months after its initial dive, radiographs showed bilateral mixed radiolucent and sclerotic irregularities (Fig. 3), thought to be bone infarcts. Films of the upper extremities (Fig. 4) then revealed sclerotic irregular deformities in the proximal humeral shafts. Two months later, films revealed still further progression in the sclerotic bony infarcts.

Specimens removed from one femur at biopsy contained nonviable cancellous and cortical bone with fat necrosis (Figs. 5-7). A diagnosis of aseptic bone necrosis was made.

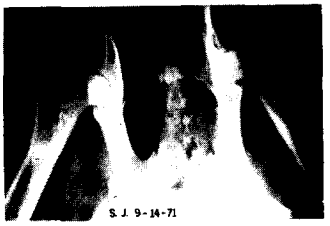

FIG. 2. Progression in the lesion in the left femur as well as the appearance of a second lesion bilaterally was seen in this followup film taken 5 months after the one seen in Fig. 1.

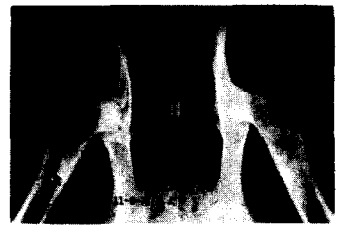

FIG. 3. Further progression in the sclerotic bony infarcts is seen 7 months later as well as the biopsy site (arrow) done 2 months earlier.

Two other animals developed radiographic evidence of bony changes, consistent with infarct following hyperbaric exposure, as evidenced by mixed sclerotic and radiolucent patterns in femurs and humeri within 3 months after repeated daily exposure. Numerous dense, almost striated bony trabeculae seen in both the humeri and femurs of another pig have subsequently disappeared during a 3-month rest period, and the bony pattern has now become normal. The animal has resumed diving and has exhibited signs of leg pain after each dive; it will be watched closely for the return of abnormalities.

Only one animal, exposed at the same time to all the same profiles, failed to exhibit any clinical signs of decompression sickness or hematologic changes in 59 dives with fast decompression rates. However, it now has a distinct area of linear trabecular concentration in the left humerus.

HISTOPATHOLOGY

Histologic slides have been obtained from specimens of pigs who had either experienced or succumbed to severe decompression sickness. Normal bone tissue is well supplied with osteocytes (cells) within the lacunae of the matrix (Fig. 5). Examination of sections of cortical bone and curettings of cancellous bone taken at biopsy revealed large areas of acellularity (Fig. 6). New apositional bone formation was seen to occur on nonviable bone structure with a definite line of demarcation (Fig. 8) in a specimen from one pig which had no radiographic evidence of bone changes. Absence or degeneration of vessels within

FIG. 4. Consistent with the aseptic bone necrosis developing bilaterally in the femurs, irregular sclerotic deformities are now seen in the proximal humeri.

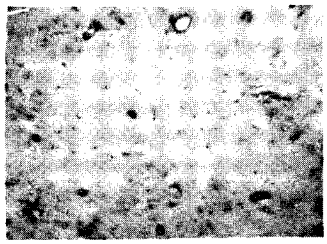

FIG. 5. Small black dots are osteocytes within the lacunae of the bony matrix seen in the normal femoral specimen taken at biopsy.

Haversian canals was observed. Along with normal fat marrow, patches of dense fibrous tissue were seen replacing normal tissue and compressed small vessels in the marrow (Fig. 7). Early and later stages of fat necrosis were present. These findings are compatible with aseptic bone necrosis.

In addition to bubblelike formations found intravascularly, large amounts of dense fibrin (Fig. 9 **A** and **B**) were seen in pooled serum. Frequently what appeared to be clumps of platelets were also seen (Fig. 10). Large areas of lymphocytic infiltration were often noted in bone marrow undergoing necrotic changes, i.e. cellular thickening which takes place in the early stages, lymphocytic grouping around degenerating tissue, and finally the dense fibrotic tissue which replaces normal fatty marrow (Fig. 7). The appearance of endothelial breakdown in vessel walls was also observed as well as some abnormal collagen patterns, but there was no conclusive evidence that this was decompression-related. Intra- and extravascular vacuoles which did not stain positive for fat (Fig. 11) were seen in many tissue specimens; the possibility that these are gas bubbles is considered.

Discussion

The intent in this study was to induce aseptic bone necrosis in miniature swine through repeated exposure to compressed gas atmospheres for periods of time calculated to saturate bone tissue with gas. Evidence of the successful fulfillment of this goal has been presented, although conclusive statements concerning the etiology or etiologies and pathogenesis of the induced disease cannot be offered.

Bornstein and Plate, as early as 1912, connected this form of osteonecrosis with bubble

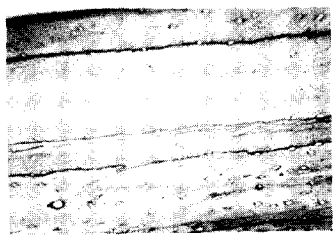

FIG. 6. White spaces seen in the femoral specimen are lacunae devoid of osteocytes, a picture consistent with the appearance of aseptic bone necrosis. Larger white spaces are Haversian canal systems, the vessels of which have degenerated.

FIG. 7. Fat marrow necrosis is seen in this specimen removed from a pig following multiple decompression episodes. Lymphocytic infiltration is seen (1) in the early stage of fibrosis with compression of a small vessel (2) by the dense tissue. Degenerating fat cells form lakes of fat (3).

formation but the etiology and/or pathogenesis were not unequivocally demonstrated (3). The bubble has continued through the years to play a dominant role in the hypothetical etiologies. However, fat embolism and even the act of compression itself have also been implied as significant mechanisms in the chain of events which leads to death of the bone cells. Jacobs and Stewart (14) in 1943 reported the interaction of decompression-related bubbles and blood when they observed platelet aggregates around intravascular bubbles in decompressed rats. Sludging of blood and infarction had been reported prior to that time (9,27,31), but the blood-gas interface was not recognized as being an initiator of the process.

The role of hemostatic abnormalities triggered by decompression stimuli is being increasingly investigated. Philp et al. (21) demonstrated platelet and red cell aggregates in the fine vasculature of the lungs of rats which had decompression sickness. Similar aggregates and thrombi were seen in the lung vessels of rabbits infused with air intravenously. These rabbits also showed a sharp decrease in circulating platelet counts during the air infusion, which was further exaggerated by agents known to aggregate platelets. Philp has advanced the hypothesis that with intravascular bubble formation, microthrombi involving platelet aggregates may occur in a manner similar to that seen in disseminated intravascular coagulation. Holland noted that disseminated intravascular coagulation is a coexisting factor in some cases of decompression sickness (13).

Several recent works have suggested the findings that platelets are maximally depressed 48-72 hours after decompression, although the reason for the delayed reaction remains unclear. Martin and Nichols (18) followed the circulating platelet count of divers for several days after exposure of 60 minutes to compressed air at a simulated sea water depth of 100

FIG. 8. New apositional bone formation is seen occurring on nonviable bone structure with a definite line (arrow) of demarcation. This animal had no radiographic evidence of necrosis.

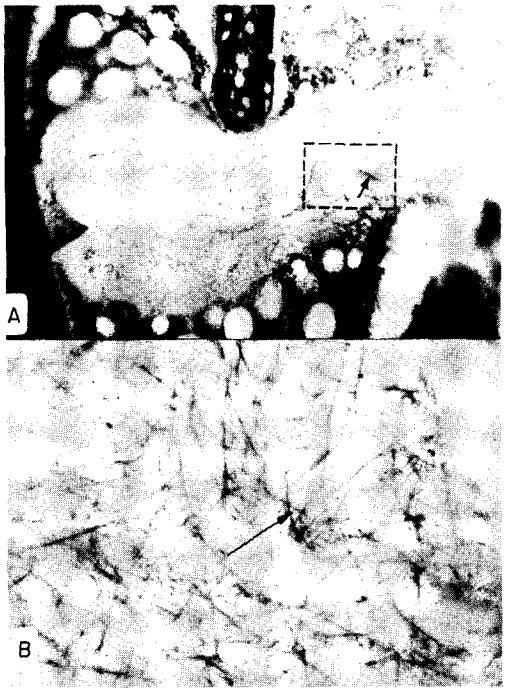

FIG. 9. Seen as hairy fibers (arrows) in **A** and magnified in **B** are large amounts of fibrin found in pooled serum in necropsy specimens of a pig who died of acute decompression sickness.

fsw. Following decompression they observed a progressive fall in circulating platelets which was greatest 48-72 hours post-dive. In a later study, Philp et al. (21) demonstrated that six of seven subjects lost a significant number of circulating platelets from 24-96 hours post-dive with the greatest depression occurring at 72 hours. With electron photomicrography they saw that platelets were lined up at the blood-gas interface of an intravascular bubble, and aggregates of platelets formed interstitial membranes between gas bubbles.

Further evidence supporting blood-gas interaction in vivo was shown by Adebahr and Kupffer (1) in rabbits injected intravenously with air. Circulating bubbles were surrounded by lines of platelets, and the blood contained large and small aggregates of platelets and leukocytes.

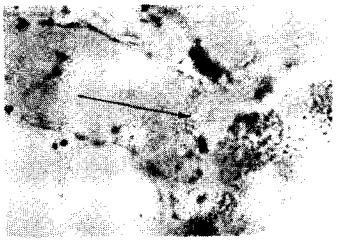

FIG. 10. Platelets (arrow) are found clumped in pooled serum in this specimen taken from an animal who died of decompression sickness.

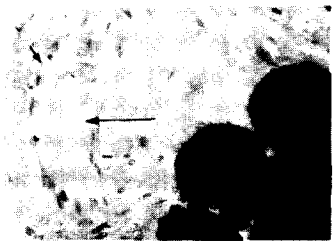

FIG. 11. Arrows point to intravascular vacuoles found in a pig who died of decompression sickness 1 hour after a fast decompression from 60 fsw for 6 hours.

Fat emboli have also been shown to be present in the circulatory system following decompression, and plasma lipids have been the subject of investigation (12,15,19,20,22). Philp et al. (21) have indicated the importance and connection of lipids, platelets and clotting. According to Everts (10), a lipid-mobilizing hormone may be liberated after tissue injury, and this tissue factor may act to increase serum lipids, coalesce small chylomicrons and thus induce fat emboli. However, he felt that the finding of fat emboli in the lungs may be of less significance since investigators have found such emboli in normal animals who had ingested a fatty meal and in other nontraumatic conditions. LeQuire et al. (17) felt that it was the catecholamines released following tissue injury which sent an overload of fatty acids to the liver, triggering a metabolic block and causing the release of lipoproteins—mostly cholesterol—into the serum. These lipoproteins themselves, they said, were responsible for the aggregation and clotting alterations seen. Finally, Schmid et al. (25) presented confirming evidence that embolized fat particles are not important etiologic factors in the development of idiopathic femoral head necrosis, however important they may be in coagulation changes. These studies do not directly define the relevance of fat emboli in decompression sickness but do relate to their possible pathogenic role.

Several explanations for platelet depression have been considered: decreased platelet production, active organ sequestration of "hurt" platelets, platelet involvement (and thus removal from circulation) in repair of endothelial surfaces damaged by foreign surfaces such as bubbles, or plugs of platelets at blood-gas interfaces. Intravascular bubbles, circulating lipids or circulating endothelial cells may all cause an instability of the platelet membrane with increased adhesiveness and subsequent aggregation, resulting in thrombosis of vital vessels, tissue anoxia and cellular death. Based on these studies, a course of pathophysiologic phenomena is postulated which has probable relevance. Presented in simple, general form (a scheme which closely follows Philp et al. [21]), they are believed to encompass the problem:

1) Decompression results in tissue supersaturation.
2) Supersaturation plus other factors lead to intra- and extravascular bubble formation.
3) Bubbles are foreign surfaces to the body and may damage tissues.
4) Damaged tissue and the presence of foreign surfaces may cause:

Platelet aggregation
↓
Microembolization of platelet clumps
↓ ↓
Thrombosis — Clearing by washing action with little resultant damage
↓
Tissue anoxia and death

Evidence exists now to demonstrate the unequivocal presence of "silent" bubbles (29,30); other investigators (21) have demonstrated "silent" reductions in platelet counts. In this study, osteonecrosis was induced in those animals which exhibited signs of decompression sickness as well as in those which remained free of outward manifestations. A decompressed diver may therefore possibly undergo hematologic alterations and be asymptomatic. It seems appropriate then to coin a new phrase, "silent decompression sickness."

The most profound effect of long-term exposure to repeated hyperbaric profiles is obviously aseptic bone necrosis, as graphically and dramatically illustrated in this study. It seems reasonable to assume that there are other concomitant side effects of such severe hematologic alterations that may come to light only through long-range observation. Future studies, attempting to solve the critical pathogenesis riddle, need to examine the effect of fat emboli as infarcting agents and circulating entities, in order to answer such questions as: Is the coalescence of chylomicrons to form fat emboli a pathogenic phenomenon? What is the role of fat in the syndrome of decompression sickness? Are the reported coagulation changes seen in decompression sickness a result of the presence of bubbles or the result of fat emboli and the release of unstable beta-lipoproteins (cholesterol) into the serum? Or is there a multiple, simultaneous or chain-reaction, direct or indirect, interaction of bubbles, fat and other tissue or cellular factors?

Whether the osteonecrosis experimentally induced in this study was the result of the obstruction of small nutrient vessels by platelet plugs as the result of blood-gas interfaces, or of the obstruction of these end vessels by the bubbles themselves which may become covered with a lipoprotein skin and sit quietly and indefinitely, or of fat emboli which derange the coagulation system and favor thrombogenesis, or of a combination of these etiologies, cannot be concluded by this study. It can be concluded that dysbaric osteonecrosis is a decompression-related phenomenon and that the problems of protection and prevention cannot be adequately approached until additional gaps in the knowledge of the short and long-term effects of repeated exposure are closed.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Ms. Dianna Durboraw who assisted in the many phases of the project.

This work was supported by the Department of the Navy, Office of Naval Research, Contract No. N00014-71-C-0273.

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BONE DENSITY CHANGES AND DECOMPRESSION SICKNESS

G. M. Adams, G. P. Vose and S. J. Norton

Aseptic bone necrosis has been described as a long-term consequence of decompression sickness that may be related to a single decompression insult (8). For divers, data suggesting a direct relationship between aseptic bone necrosis and decompression sickness have been assembled (3). However, animal studies have not consistently supported these observations when minor decompression sickness was induced (12). Blood or urinary calcium should be one of the principal biochemical constituents to reflect a bone response to decompression sickness. Biochemical studies, to date, have indicated that urinary calcium levels do not reflect a decompression insult in man (13) and that serum total calcium levels do not correlate with the occurrence of decompression sickness in animals (G. M. Adams, unpublished observation). Thus calcium studies might not consistently provide the sensitive indicator necessary to correlate an immediate bone change with a decompression sickness insult.

Bone density can be evaluated by numerous noninvasive methods, of which x-ray radiography using a calibration density wedge is one reliable technique for these determinations (5). Slight changes in mineral matrix have been evaluated by this technique in osteoporosis pursuant to weightlessness from space flights (7). If a bone density shift could be correlated with a single decompression sickness insult, a potential basis for a relationship between decompression sickness and the eventual occurrence of aseptic bone necrosis might be established.

Materials and Methods

In this study mature, male Sprague-Dawley rats weighing 250 ± 10 gm were used as a separated pair in each hyperbaric exposure. The oxygen partial pressure was maintained between 0.2 and 0.5 atmospheres throughout the dive while the gas temperature was regulated at 90°F ± 2°F. A continuous vent was employed to eliminate gas contaminants. The paired animals were used according to one of the schedules depicted in Fig. 1. Pre-mixed gases were switched at the indicated points to effect the oxygen partial pressure control. The total bottom time for all dives was 80 minutes. The dive depth was 267 p.s.i.g. or 598 feet of sea water (simulated). Schedule A contained a rapid linear decompression profile with a total decompression time of 26 minutes and resulted in the death of 99% of the tested animals at or near the surface. Schedule B contained a four-stop decompression

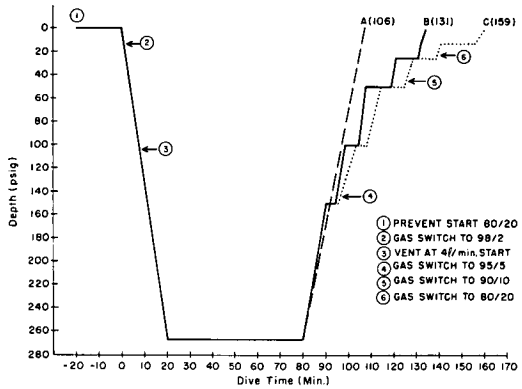

FIG. 1. Dive schedules. Circled numbers indicate points of gas manipulation according to the following. Input gases were analyzed and correct to ± 0.5%. Compression rate—13 p.s.i.g./min. Total bottom time—80 minutes. Gas temperature throughout exposure—90° ± 2°F. Three decompression schedules (A, B and C) were used with total decompression time in minutes in parentheses beside appropriate letter. The breathing gases were helium/oxygen mixtures.

profile with a total decompression time of 51 minutes, and schedule C contained a five-stop decompression profile with a total decompression time of 79 minutes. Schedules B and C were used to induce nonlethal bends and produce symptom-free animals after the dive. When possible, all dived animals were observed for 1 hour after each dive on slow moving, flatbed treadmills. Signs of decompression sickness were subjectively evaluated according to the categories depicted in Table I. Light respiration consisted primarily of excessive, visible rate changes. Heavy respiration appeared as a rapid increase, then a dramatic slowing of rate with frequent gasping for breath. Uncoordinated movement was recognized by the animal's inability to track straight and/or seeming inability to control direction of movement. Minor paralysis in the hind legs included drop foot and/or the partial dragging of a foot with its occasional usage. Major paralysis of the hind legs appeared as flaccid paralysis with both legs extended, although spastic paralysis could not be wholly excluded. Paralysis in three or four limbs was generally spastic in nature. No attempt at objective definition for these subjective signs has been made.

TABLE I

SUBJECTIVE SIGNS OF DECOMPRESSION SICKNESS

Decompression Sickness Signs	Abbreviation
No symptoms	Ns
Light respiratory rate changes	Lr
Heavy respiratory changes, gasping, etc.	Hr
Discoordinate movement, vertigo, etc.	Disc
Minor paralysis, hind leg(s)	Min
Major paralysis, hind leg(s)	Maj
Paralysis, foreleg and hind leg	Para
Death	Death

TABLE II

RESPONSE OF CONTROL RATS TO DIVE SCHEDULE B

Signs (Abbrev.)	All Signs[a]		Major Signs Only[b]	
	No. Rats	%	No. Rats	%
Ns	2	4.3	11	23.9
Lr	7	15.2	N/A[c]	—
Hr	33	71.7	N/A	—
Disc	10	21.7	7	15.2
Min	6	13.0	3	6.5
Maj	28	60.9	13	28.3
Para	8	17.4	7	15.2
Death	5	10.9	5	10.9

[a] All signs tabulated from each animal.
[b] Only the most severe signs tabulated for each animal.
[c] N/A—not applicable classifications. Total of 46 animals in control population.

Forty-six animals were evaluated as a control dive population with schedule B, the four-stop decompression profile. When all signs were equally weighted and observed for their occurrence in each animal, the distribution in the left column of Table II was observed. No trend in the time of occurrence of the various signs could be determined. If respiratory changes are not considered and the most severe limb signs are tabulated as the only signs observed—as has been suggested as a means of tabulation (10)—then the distribution in the right hand column of Table II was observed. Regardless of the tabulation method used, schedule B induced decompression sickness signs in the majority of all the dived animals, was essentially nonlethal and permitted the animal's full recovery from signs within 1 hour in most cases. Once dived, an animal was not resubjected to a pressure exposure.

Approximately 1 hour prior to the dive and within 30 minutes after the dive, animals were lightly anesthetized with ether and x-rayed alongside a density wedge on Eastman Kodak type AA film. The same calibration wedge was used in all x-ray films. Figure 2 shows a typical x ray obtained with a control animal. Extreme care was taken to assure that all animals were reproducibly positioned for the x-ray films. The films were densitometrically scanned at the following sites: a lateral scan through the distal femoral shaft at one-third distance of the total femur length; an axial scan path through the tibial epiphysis; an axial scan through the tibial diaphysis; and a lateral scan through the tibial shaft halfway between the proximal epiphyseal edge and the tibia-fibula junction. Each site was scanned at least twice to assure reproducible readings. Only the results of the tibial diaphysis scan will be discussed. After scanning, an analog computer calculated the bone density, as corrected by the aluminum alloy density wedge, and presented the results for evaluation.

Results

Table III details the results obtained from axial scans through the tibial diaphysis. The

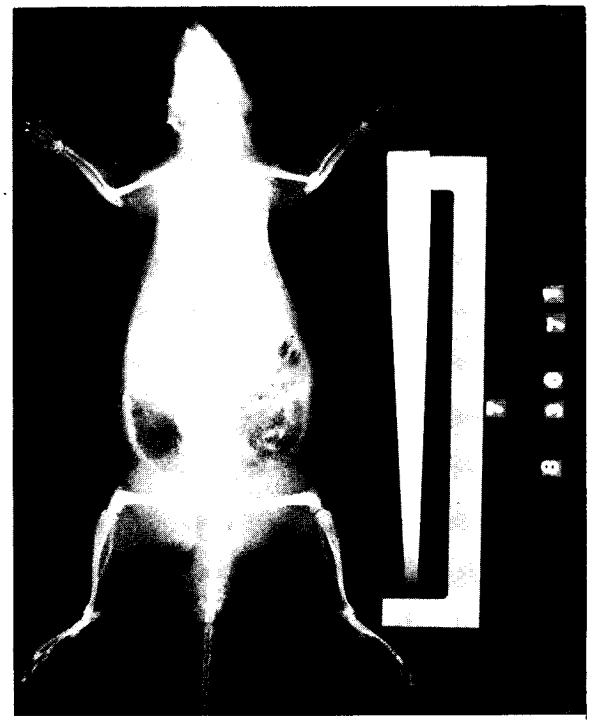

FIG. 2. Typical radiograph for bone density evaluation. Calibration density wedge included beside the prostrate, anesthetized animal.

TABLE III

BONE DENSITY CHANGES FROM SCHEDULE B

	Rat No.	ΔDensity[a]	% Change[b]
Control rats	15	−0.005	− 1.9
	18	+0.005	+ 1.4
	19	+0.007	+ 2.7
Dive rats, schedule B[c]	2	+0.014	+ 4.3
	3	−0.018	− 6.5
	4	−0.034	−12.7
	5	−0.037	−14.0

[a] Density units in x-ray aluminum equivalency values in terms of mm aluminum/mm bone.

[b] Percent change calculated as described in text.

[c] The four-stop decompression schedule.

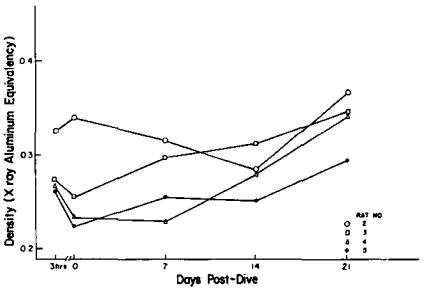

FIG. 3. Bone density response profile. Changes pursuant to exposure on schedule B with initial alterations noted in Table III. Density times 10 plotted on the ordinate.

actual density changes indicate the magnitude of the measured density alterations. The percent change represents the density change occurring after a dive relative to the pre-dive value or equivalent time differences for nondived, control animals. Control animals exhibited a ± 2% change which demonstrates the accuracy of this technique when applied to these animals. The dived animals exhibited a much greater bone density fluctuation when decompressed on schedule B which induced signs of decompression sickness in each animal. An immediate and significant density decrease occurred in all of the dived animals with the exception of rat 2. While this animal's response did not reflect an immediate density decrease, a slow decrease to an equally low level did occur over the next 14 days as can be seen in Fig. 3. All the animals seemed to recover from the density loss, but each exhibited an individual recovery profile as seen in Fig. 3.

To further explore these results, animals were dived on schedule C, the five-stop decompression profile which induced only very minor signs of decompression sickness in some animals. Table IV details the results obtained from the diaphysis scans. In two animals, rats 11 and 13, no density changes were observed and the signs of decompression sickness were not apparent. Rats 10 and 12 did demonstrate minor signs of decompression sickness and exhibited the previously observed decrease in bone density.

TABLE IV

BONE DENSITY CHANGES FROM SCHEDULE C[a]

	Rat No.	ΔDensity	% Change
Control rats	15	−0.005	− 1.9
	18	+0.005	+ 1.4
	19	+0.007	+ 2.7
Dive rats, schedule C	10	−0.045	−15.0
	11	0.000	—
	12	−0.037	−11.7
	13	+0.003	+ 1.0

[a] The control values repeated from Table III. Schedule C contained the five-stop decompression profile (see text for further description).

A decrease in bone density suggests that a mineral loss from the bone matrix has occurred. To explore this possibility, exogenous porcine calcitonin was administered to a group of rats. Calcitonin, a physiologically active peptide hormone, enhances bone mineral accretion at the expense of serum calcium levels (11). Table V details the results obtained with this treatment. Two Medical Research Council (MRC) units of porcine calcitonin were administered subcutaneously in 16% gelatin to each rat. In one nondived control animal an obvious enhancement of density was observed. The dived animals exhibited significant changes. The density decrease observed in untreated dived animals was reversed in all of the treated animals, and two dived animals exhibited significant density increases as might be expected with this treatment. Thus, calcitonin appeared to reverse the bone density patterns observed in nontreated animals from the same dive profile.

The results of the calcitonin treatment on bone density prompted an evaluation of this hormone on the incidence of decompression sickness. An eight-animal treatment group was evaluated using schedule B. The results observed in this evaluation are shown in Table VI. While the treatment population size is not large, enumeration statistics (14) determined that the increase in sign-free animals was significant at the 0.05 level when compared to the control population, irrespective of the method used to tabulate decompression sickness signs. The 25% or 50% sign-free animals are compared to the 4.3%-23.9% observed in the nontreated, dived population. Serum total calcium levels were decreased about 25-30% 1 hour after the dive in each of the calcitonin-treated animals.

Discussion

The bone density decrease observed in the untreated, dived animals suggests a potential bone mineral loss related to the decompression sickness insult. Blood calcium levels should increase to reflect the bone loss; however, blood calcium measured 1 hour after the dive did not demonstrate an alteration from control values. Calcium is a highly labile constituent of blood (2). A shift of plasma calcium to nonplasma fluids or tissues, concomitant with or

TABLE V

BONE DENSITY CHANGES IN CALCITONIN-TREATED RATS

	Rat No.	ΔDensity[b]	% Change[c]
Control rats, calcitonin injection[a]	20	+0.025	+ 7.3
	21	+0.007	+ 2.1
	20	—0.007	— 2.0
Dive rats, schedule B[d], calcitonin injection[a]	24	+0.034	+13.3
	25	+0.042	+15.2
	26	—0.010	— 3.2

[a] Two MRC units S.C. in 16% gelatin.
[b] Actual density change in x-ray aluminum equivalency units.
[c] Percent change calculated as described in text.
[d] Contained the four-stop decompression profile.

TABLE VI

RESPONSE OF CALCITONIN-TREATED RATS TO DIVE SCHEDULE B

Signs (Abbrev.)	All Signs [a]		Major Signs Only [b]	
	No. Rats	%	No. Rats	%
Ns	2 [c]	25.0	4	50.0
Lr	2	25.0	N/A	...
Hr	4	50.0	N/A	...
Disc	1	12.5	0	...
Min	2	25.0	2	25.0
Maj	2	25.0	2	25.0
Para	0	...	...	...
Death	0	...	...	...

[a] All signs tabulated from each animal.
[b] Only the most severe sign tabulated from each animal.
[c] Significant to 0.05 level. (See text for statistical details.)

shortly after a decrease in bone density, could result in the observed normal blood calcium levels. Hemoconcentration is known to occur in decompression sickness, and altered membrane permeabilities have been discussed to explain this apparent intravascular fluid loss (1). Increased calcium fluxes into tissue beds should occur at this time, and the resultant localized hypercalcemias may contribute to the overt signs of decompression sickness.

Calcitonin was observed to reverse the bone density decrease found pursuant to decompression sickness and increase the percentage of sign-free animals. One hour after the dive the serum calcium level in calcitonin-treated, dived animals was 25-30% below the level observed in nontreated dived and nontreated control animals. The increased bone density observed with this treatment reflects the bone mineral accretion occurring with the simultaneous reduction in plasma calcium levels consistent with the mechanism for calcitonin's action. However, this does not explain the protective effects of calcitonin in decompression sickness unless the reduction in blood calcium level is the contributing factor.

Heparin has been described as a drug that reduces the incidence of decompression sickness (9). The mechanism for heparin's protective action is thought to be its activation of lipoprotein lipase to reduce the level of circulating plasma lipids. The reduction of circulating lipids by this mechanism must result in an increase in plasma free fatty acids (6). This mechanism has been suggested for the reduction in blood calcium affected by heparin administration (4). Thus heparin and calcitonin, two seemingly unrelated, biologically-derived substituents, might have the same course of action in reducing the incidence of decompression sickness: the reduction of circulating calcium levels.

Summary and Conclusion

The observations presented in this study indicate that a bone density decrease results from

a decompression sickness insult and suggest that the bone calcium loss, which must occur concomitant with a density decrease, contributes to the overt signs of decompression sickness. Exogenous calcitonin has been suggested as a potentially protective drug in decompression sickness therapy, and one possible course for its action has been described. A relationship between the observed bone density decrease and the potential occurrence of aseptic bone necrosis has not been described; however, current research is directed toward this goal.

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A STUDY OF AVASCULAR BONE NECROSIS IN SHEEP

D. B. Coltman and D. N. Walder

A great deal is known about the radiological appearances and considerable additional information is being gathered concerning the histology of bone lesions in compressed air workers and divers. Unfortunately the information derived from these two sources is in conflict and therefore is not helpful in elucidating the aetiology of this condition. For example, histological examination always indicates a much greater area of bone necrosis than can be detected from the radiograph (1). It therefore seems important, if indeed bone lesions result from embolization, to delineate the nature and extent of the vascular blockage required to result in a lesion of the type seen in caisson disease of bone.

The purpose of this work was to find out what changes, if any, occurred when the blood supply, via most of the vessels in the femoral neck of a large animal, was interrupted. The animals were followed up by regular radiological examinations and eventually were killed in order that the bones could be examined.

Method

The animals used for this study were adult sheep weighing about 200 lbs. These animals were selected because they have femoral heads which are about one-third the diameter of a human femoral head, unlike common laboratory animals such as rats, rabbits, dogs and cats in which the head is much smaller. Also, sheep are readily obtainable in Great Britain and are easy and cheap to maintain.

Twenty-one animals were successfully operated upon. Three of these were killed immediately post-operatively for vascular studies and 18 were followed up on a long-term basis. Before operation standard anteroposterior and lateral control radiographs were obtained. The operative procedure was carried out under general anaesthesia on one hip. The approach to the hip joint was similar to the Southern approach used on man. A complete capsulotomy was carried out, and the femoral neck at the margin of the articular cartilage was cut from above with an osteotome in such a way that the head was supported by only a thin sliver of bone (Fig. 1). The wound was then closed. No attempt was made to interfere with the ligamentum teres.

In no animal did wound infection develop, and all animals were put out to graze within

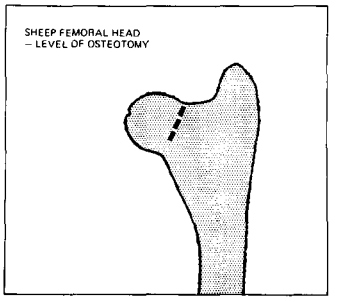

FIG. 1. Diagram shows level of osteotomy used so that head of the femur is supported by only a thin sliver of bone.

24 hours. At various intervals after operation, the animals were again anaesthetised and followup radiographs of the hip were taken.

All animals, including those killed immediately post-operatively, were heparinized before being killed by an overdose of Nembutal. They were then immediately infused via the abdominal aorta or the carotid artery with a barium sulphate suspension (Micropaque) mixed with a blue dye at a pressure of 200 mm Hg for about 4½ hours. An average of 12 litres of injection medium was needed.

Both femora were then removed from the carcass and cleaned so that the gross appearance of the femoral heads could be noted. Radiographs of both bones were obtained, after which they were placed in 10% formalin solution and prepared for histological study.

Coronal sections of the head and neck (including the site of trauma and the ligamentum teres) were cut; from these, thin slices were obtained which were decalcified and then stained with haematoxylin and eosin. A number of the coronal sections measuring 3-5 mm in thickness were also examined radiographically, using a special microfocal source which gives a magnified image.

All radiographs were examined by Dr. Griffiths, of the British M.R.C. Decompression Sickness Central Registry, who has wide experience with the radiological appearances of caisson disease of bone. The radiographs were classified as: 1) normal, 2) containing a suspected lesion, or 3) containing a positive lesion.

Results

The hip joints of the 18 long-term surviving animals were radiographed at intervals during the 9 months following operation (Fig. 2). Suspect or positive lesions were detected in 15 of them. The earliest was seen at 55 days, but the interval from operation to the first radiological change was usually about 3 months.

In 5 of these 15 animals, the bone lesions progressed. All the lesions were located in the neck of the femora, none being found within the heads (Fig. 3). The animals were not killed until some 8-16 months post-operatively.

When conventional radiographs of the excised, cleaned, injected bones from both sides were examined, it was found that there were no signs of bone lesions of the femora in any animal. The only change to be seen was a small notch in the superior cortex of the neck of the femur in some cases; this notch was at the site of operation, and the heads appeared to

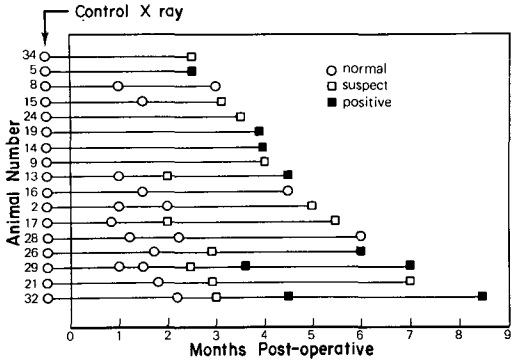

FIG. 2. Occurrence of bone lesions in hip joints of 17 sheep. Post-operative radiological examinations. [Course of animal number 11 (not shown) was same as number 32].

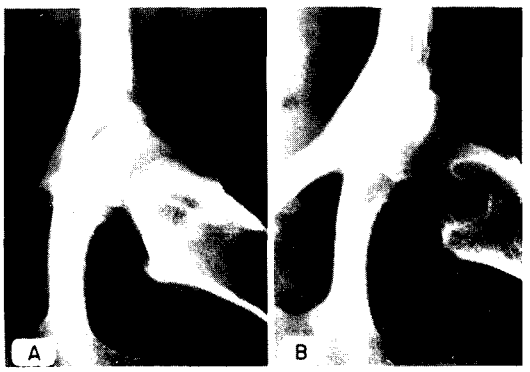

FIG. 3. Radiographs show **A**, normal femur and **B**, femur with lesion in neck region.

be quite normal. However, on those sections radiographically examined, using a special microfocal x-ray source, lesions could easily be seen within the neck of the bone although there was no detectable change within the head (Fig. 4).

The injected specimens showed a number of important differences between the blood

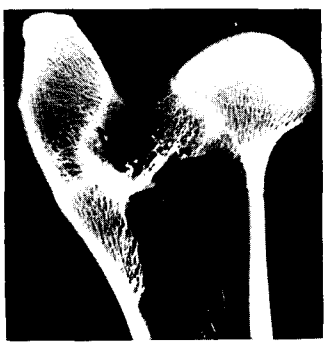

FIG. 4. *Lesions are evident within neck of the femur.* (Radiographic examination with special microfocal x-ray source.)

FIG 5. Blood supply to femoral head as seen in injected specimen shows branches extending from nutrient artery branches in marrow cavity (x2).

supply to the femoral head of the sheep and that of man. Perhaps the most important was the presence of large vessels arising from the nutrient artery branches in the marrow cavity and running up to the head, mainly in the inferior margin of the neck (Fig. 5). There are also substantial inferior retinacular vessels which anastomose freely with the lateral epiphyseal vessels, as can be seen in the radiographs of an animal in which an osteotomy was carried out, but only a partial capsulotomy (Fig. 6).

As in the human, the ligamentum teres contained vessels in some of the animals, but in no animal could vessels be shown to enter the bone. The differences between the blood supply to the head of the femur in man and in sheep are illustrated in Fig. 7.

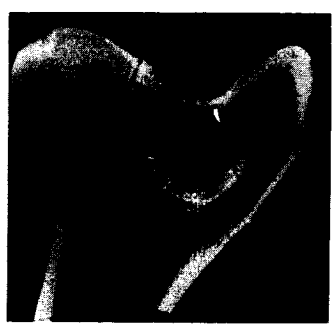

FIG 6. Radiograph from animal in which osteotomy performed, but only partial capsulotomy, shows inferior retinacular vessels anastomosing with lateral epiphyseal vessels.

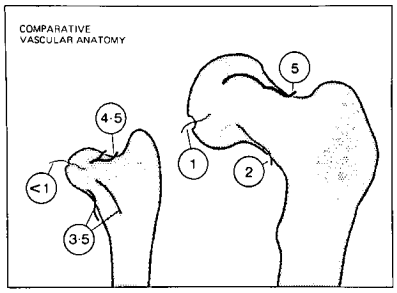

FIG. 7. Number of blood vessels typically seen supplying the head of the femur in sheep (left) and man (right).

On the magnified radiographs taken with the special microfocal x rays, there was little evidence of vessels crossing the line of the osteotomy (Fig. 8), but retrograde filling of the lateral epiphyseal vessels may be seen. It is important to remember that since a complete capsulotomy had been carried out in this series, retrograde filling must have been by vessels other than the inferior retinacular.

The histological examination confirmed that there were no lesions within the heads of the femora (Fig. 9), but it could be seen that the trabeculae in the line of the osteotomy were slightly thicker than those on the head side, indicating new bone formation on dead trabeculae. In some sections there was also evidence of active osteoblastic activity along this line.

Discussion

It is clear then that the method employed did not produce avascular necrosis of the *head* of the femur. The radiological changes which were found in 15 of the 18 long survival animals probably represent those found after any fracture and are consistent with an intact blood supply on either side of the site of trauma. This means that, despite the absence of vessels to the femoral head via the ligamentum teres, sufficient vessels are present in the inferior rim of the neck in the sheep to prevent the occurrence of necrosis—even when all other major vessels are divided.

FIG. 8. A magnified radiograph (2X), using special microfocal x rays, shows little evidence of blood vessels crossing line of osteotomy. Retrograde filling of lateral epiphyseal vessels may be seen.

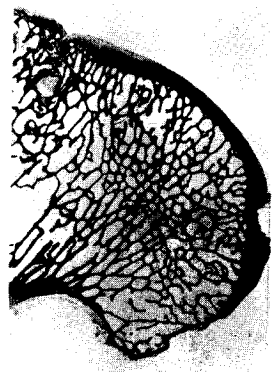

FIG. 9. Histological preparation shows no lesions within head of femur (2X).

On the basis of Sevitt's work on the human femur (2) in which, as in this study, he divided most of the neck on post-mortem specimens, evidence of developing necrosis was expected in at least some of these animals. The fact that this was not seen suggests that the blood supply to the femoral head in this species is considerably better than it is in man. The inability to produce convincing evidence of dysbaric osteonecrosis in animals may be due to the fact that most laboratory animals, like the sheep in this study, have a better blood supply to the femoral head than man.

The evidence nevertheless suggests that bone damage due to vascular interference in animals may be transient and, therefore, could easily be missed by conventional long-interval radiographic examination.

If bone lesions in compressed air workers and divers are caused by bubbles of gas within the vascular tree, then these results would suggest that either: 1) blockage of the lateral epiphyseal vessels in man cannot be compensated for by an increased circulation from the inferior retinacular vessels or via any vessels in the ligamentum teres; or more probably, 2) the site of blockage of the vessels in the head of the femur must lie in the distal arterial tree beyond the arcades of the lateral epiphyseal vessel. This latter suggestion would be in accord with the evidence from the histological examination of lesions in men in which the whole head of the femur or humerus plus most of the neck are seen to be necrosed.

Summary

An attempt was made to produce avascular necrosis of the head of the femur in adult sheep by severing most of the vessels to the head. No permanent bone lesions of either head or neck were produced on conventional radiographs, though transient radiological changes were seen within the neck.

A possible explanation to account for the inability to produce dysbaric osteonecrosis in most laboratory animals is given.

It is postulated that the level of vascular block which results in bone necrosis in divers and compressed air workers is probably at the distal limit of the arterial tree.

ACKNOWLEDGMENTS

This work was supported by a grant from the Scientific & Research Committee of the Newcastle Regional Hospital Board.

We are indebted for technical assistance to Mr. E. P. Jennings, senior technician, Department of Pathology, Dental School, University of Newcastle upon Tyne; Mr. D. Wilson, technician, Department of Surgery; Mr. V. B. Thickett, administrative assistant (research), M.R.C. Decompression Sickness Central Registry, Newcastle upon Tyne; and Dr. W. M. Park, radiologist, Oswestry.

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INVOLVEMENT OF CO_2 AND CALCIUM STORES IN DECOMPRESSION SICKNESS

K. E. Schaefer

In a previous communication Schaefer et al. (17) reported that symptoms of decompression sickness manifested in poorly localized muscle aches and stiffness, which did not respond to recompression and O_2 treatment, appeared to be associated with fluid and electrolyte shifts and a large CO_2 excretion in the urine.

Further analysis of urine electrolyte data revealed that in these cases the CO_2 tide was preceded or followed by a calcium tide in the urine. These observations, which point to the bone with its CO_2 and calcium sink as a target organ in decompression sickness, are presented in this report.

Methods

Four subjects participated in saturation-excursion dives to pressures equivalent to 800 and 1,000 feet of sea water (fsw). Details of these experiments and decompression schedules have been described previously (17).

Twenty-four-hour urine specimens were collected in polyethylene bottles. A cover of liquid silicone* was used to prevent CO_2 from escaping from the urine into the chamber atmosphere. Aliquots were frozen until analyzed. Total urine CO_2 was determined by the manometric method of Van Slyke. Urine pH was measured with the Beckman pH meter.

Inorganic phosphorus in the urine was determined by the method of Fiske and SubbaRow as modified by Roe and Whitmore (15). Calcium in the urine was measured according to the method of Clark and Collip (4), a modification of the procedure of Kramer and Tisdall.

Results

Data obtained during the saturation-excursion dive to a depth equivalent to 800 fsw are shown in Figs. 1 and 2.

The dive profile is presented in the lower part of the figures. CO_2 excretion and calcium excretion are plotted together. In the first case the diver developed bends during the 800 fsw

*Dow Corning 200.

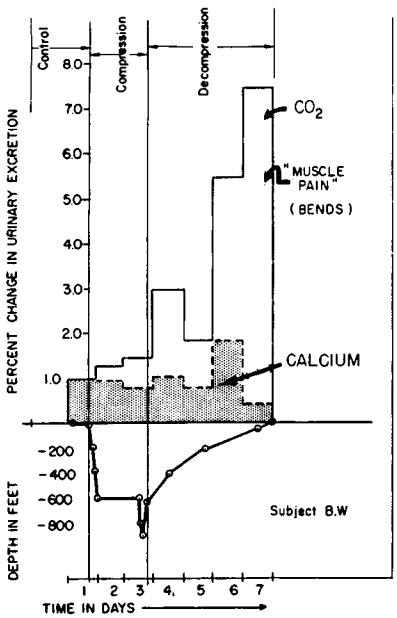

FIG. 1. Carbon dioxide and calcium excretion in the urine during saturation-excursion dive to 800 fsw (expressed in percent change from control values). Subject B. W.

saturation-excursion dive. During the same day the CO_2 excretion rose to a peak six times above the initial value, while the calcium excretion rose to a level twice normal during the subsequent day (Fig. 1). The other diver who did not experience bends during the decompression from 800 feet showed smaller elevations of CO_2 excretion and calcium elimination during the decompression period (Fig. 2).

In the second saturation-excursion dive to 1,000 fsw the two divers experienced symptoms of decompression sickness during decompression at 30 fsw. Recompression to 60 fsw relieved the symptoms in one diver but not in the other. Subsequent recompression to 165 fsw and 527 fsw also had no success in relieving the symptoms in the second diver. After staying 2 hours at the depth of 521 feet, the diver received one tablet of Bufferin which had an immediate effect. The diver could sleep without pain and the subsequent decompression was uneventful.

Figure 3 shows the CO_2 and calcium excretion of the diver who experienced severe bends during the decompression from 1,000 fsw saturation-excursion dive.

CO_2 excretion is increased sevenfold during the 24 hours preceding the appearance of bends in which the divers were decompressed from 200 to about 50 fsw and shows only a small elevation during the day on which the bends occurred. However, calcium excretion exhibits a peak, three times above normal, during the day the symptoms of decompression sickness occurred and the massive recompression to 527 fsw was instituted.

The second diver, who had only temporary intermittent pain in both knees between 50 and 40 fsw, exhibited throughout the decompression period at intervals of approximately 2 days large increases in CO_2 excretion; only one time was such a large peak in CO_2 excretion preceded by a marked rise in urinary calcium reaching a level three times higher than initial

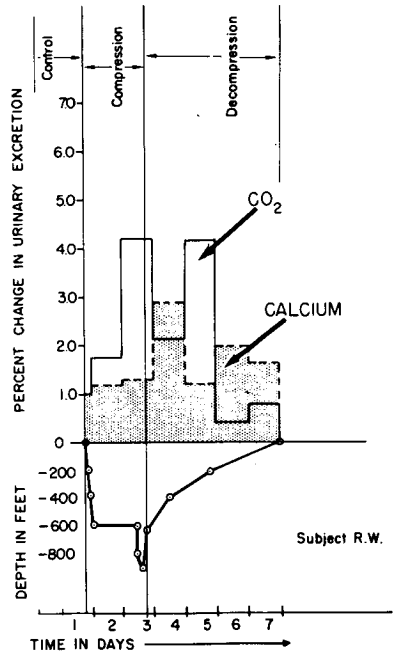

FIG. 2. Carbon dioxide and calcium excretion in the urine during saturation-excursion dive to 800 fsw (expressed in percent change from control values). Subject R. W.

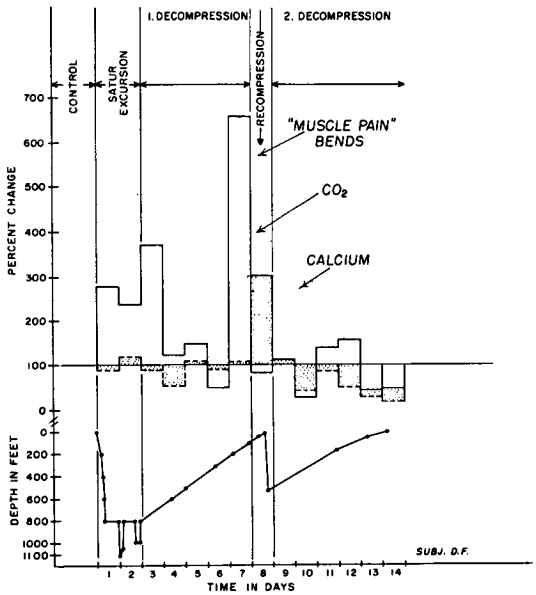

FIG. 3. Carbon dioxide and calcium excretion in the urine during saturation-excursion dive to 1,000 fsw (expressed in percent change from control values). Subject D. F.

values. This occurred on the second day of the decompression period following the recompression to 527 fsw (Fig. 4).

The calcium tides in the urine observed in association with large CO_2 excretion during decompression were never found to have any correlation with alterations in the excretion of inorganic phosphate as seen in Figs. 5 and 6 for the two divers participating in the 1,000 fsw dive.

The excretion of inorganic phosphate generally increased during compression and tended to decrease during decompression. A similar behavior was found in the two divers during the 800 fsw saturation-excursion dive.

Discussion

The observations made in this study point to the bone with large CO_2 and calcium sinks as a target organ in decompression sickness, but this would involve rapidly exchangeable calcium and CO_2 pools in the bone.

Recent findings of Bursaux (3) and Neumann and Ramp (11), demonstrating that over 40% of the bone CO_2 store consists of rapidly exchanging bicarbonate, provide a better understanding of the interaction of bone CO_2 and calcium stores.

It was originally shown by Neumann and Mulryan (10) that a significant amount of bone CO_2 is released by heating the bone. After a single injection of ^{14}C bicarbonate it was found that the specific activity of CO_2 lost on heating the bones was more than twice that of the remaining CO_2. They concluded that the rapidly exchangeable bone CO_2 pool was mainly represented by bicarbonates.

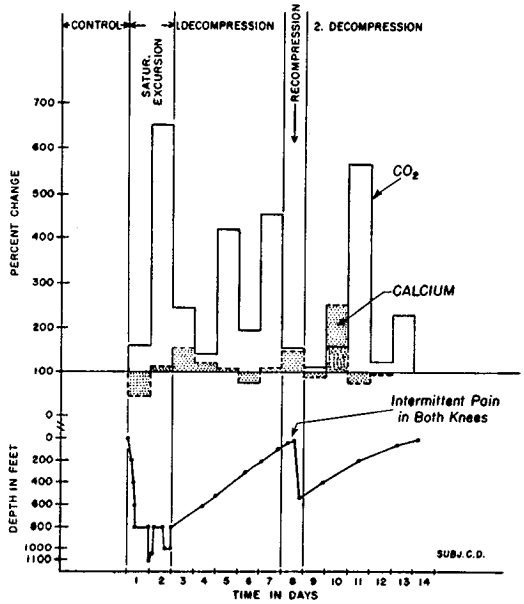

FIG. 4. Carbon dioxide and calcium excretion in the urine during saturation-excursion dive to 1,000 fsw (expressed in percent change from control values). Subject C. D.

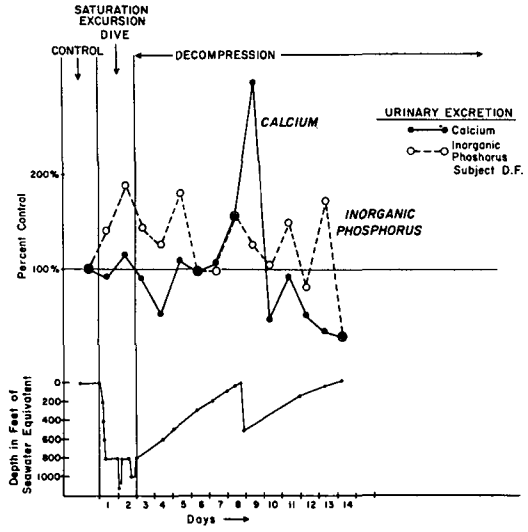

FIG. 5. Calcium and inorganic phosphorus excretion during saturation-excursion dive to 1,000 fsw (expressed in percent change from control values). Subject D. F.

Bursaux and Poyart (3) investigated the exchangeability and relation between blood bicarbonate and bone bicarbonate by ventilating mechanically paired rats for 1 hour with air and different CO_2 mixtures. In this way he produced hyper- and hypocapnia of 1 hour's duration. He found a good correlation between CO_2 content of mixed venous blood obtained with a catheter and bone CO_2 content. The average slope was 1.27 m CO_2/kg bone/mm P_{CO_2}.

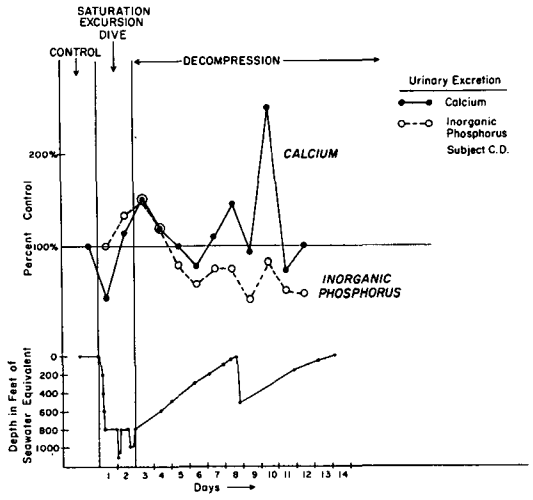

FIG. 6. Calcium and inorganic phosphorus excretion during saturation-excursion dive to 1,000 fsw (expressed in percent change from control values). Subject C. D.

This rapidly exchangeable CO_2 pool in the bone must play a major role in the maintenance of acid-base balance. At any rate the bicarbonate pool in the bone is large enough and can apparently be released fast enough to account for the large CO_2-bicarbonate excretion observed during decompression.

What is the evidence for a coupling of CO_2 and calcium exchange in the bone necessary to explain the calcium tides following the bicarbonate tides?

From the 1953 studies of Freeman and Fenn (7) on the effects of chronic hypercapnia and hypocapnia, it is known that the accumulation of bone CO_2 during chronic hypercapnia produced by exposure to 10% CO_2 is accompanied by an increment in bone calcium. Moreover, studies by Schaefer et al. (16) of calcium metabolism in chronic hypercapnia in men exposed to 1.5% CO_2 for 42 days indicated that calcium and CO_2 are stored in bone during acclimatization to CO_2, and bone calcium and CO_2 are released from bone on de-acclimatization.

During exposure the pH showed a biphasic response, a decrease lasting for 23 days during the period of uncompensated respiratory acidosis and a return to normal levels during the subsequent period of 24-42 days of compensatory respiratory acidosis. Pulmonary and urinary CO_2 excretion showed corresponding biphasic changes. The plasma calcium mirrored the pH changes. Plasma inorganic phosphorus levels were markedly increased during the initial 23 days of uncompensated respiratory acidosis. The behavior of the plasma inorganic phosphorus level may have had a particular significance for calcium storage. Recent studies of Nichols et al. (12) have demonstrated that increased phosphate concentrations in the fluid medium of bone cells cause an increase in calcium influx into the bone cells—or block parathyroid hormone (PTH)-stimulated calcium movement out of the cell. This effect of increased phosphate concentration has also been observed by Nichols et al. in bone cells obtained from clinical cases. Based on these findings, the elevated phosphate concentrations during the first 23 days of exposure to 1.5% CO_2 could have contributed to the calcium storage by blocking the outflux of calcium.

During the recovery on air following 42 days of CO_2 exposure, a calcium tide in the blood occurred after 8 days, associated with a peak pulmonary CO_2 excretion. These findings lead to the conclusion that storage of CO_2 and calcium in the bone and release from the bone are correlated.

In studies of Gray et al. (8), with prolonged exposure of submariners to 1% CO_2, the findings of Schaefer et al. (16) on calcium phosphorus changes were confirmed.

The evidence cited so far for the coupling of CO_2 and calcium exchange in bones involves the slow CO_2 compartment and the slow calcium compartment. At present there are no known studies in which the fast exchangeable CO_2 store bicarbonate in bone and the fast exchangeable calcium fraction were simultaneously measured under conditions of acid-base alterations or hyperbaric conditions.

It is assumed that the fast exchangeable CO_2 and calcium fractions have a relationship similar to what seems to exist for the slow exchangeable compartments of CO_2 and calcium.

These studies of saturation-excursion dives to 800 and 1,000 fsw produced evidence showing a release of CO_2 and calcium during decompression which appeared to be correlated in time. In subjects with symptoms of decompression sickness the peaks in urinary CO_2 and calcium excretion were more pronounced.

Taking into account the findings of Bennett and Gray (2) and Radomsky and Bennett (14) on calcium retention during compression and increased calcium excretion during

decompression from saturation dives to 1,500 fsw in human subjects, of Adams et al. (1) showing a decrease in bone density in rats following decompression from 600 fsw simulated dives, and of Hills and Straley (9) demonstrating an increased bone blood flow during compression and a decreased bone blood flow during decompression, the following hypothesis is suggested:

1) During compression there is a greater influx of calcium and CO_2 into the fast exchanging bone CO_2 and calcium stores related to an increased bone blood flow.
2) During decompression there is a greater efflux of CO_2 and calcium corresponding with a decreased blood flow in the bones.
3) Symptoms of decompression sickness which do not respond immediately to recompression and oxygen therapy occur when the efflux of CO_2 and calcium is accelerated.
4) Other factors may influence this hypothetical mechanism in various ways.

Two considerations led to the presentation of this hypothesis of involvement of CO_2 and calcium stores in decompression sickness even if the evidence is scanty at present. First, a largely taxonomic descriptive approach to the problem of decompression sickness and aseptic bone necrosis—as any survey of literature can show—still exists. There are great difficulties in establishing adequate experimental models, and there is not a real understanding of the physiological mechanism underlying the development of decompression sickness and aseptic bone necrosis.

The second consideration is related to some newer aspects in bone physiology which provided the framework for the proposed hypothesis. Bone is the major CO_2 store of the body comprising 80% of the total CO_2 store or 110 L out of 130 L in a 70 kg man (13). Bone is also a major reservoir for electrolytes and plays a very important part in the maintenance of normal acid-base balance. Recent findings of Neumann et al. (10,11) and Bursaux (3) have demonstrated that about 40% of the bone is present in the form of rapidly exchangeable bicarbonate. A rapid turnover of CO_2 in the bone would require rapid changes in bone blood flow. It was found by Shim and Patterson (18) that the metabolic control mechanism of bone blood flow is the most potent one.

Breathing mixtures of increased CO_2 or low O_2 increased bone blood flow by about 20% (6,18). Acid metabolites had similar effects. The bone blood flow is about 10% of the total cardiac output in younger people and 6-8% in older people. In bone diseases, such as Recklinghausen's disease and Paget's disease, an enormous increase in cardiac output has been observed (5).

The metabolic control of bone blood flow might play a role in the recently reported increases in bone blood flow during compression and decreases during decompression found by Hills and Straley (9). Moreover, increasing evidence indicating that bone has a membrane function [Neumann and Ramp (11)] gives further support for rapid exchanges of calcium and CO_2. The potassium distribution between bone fluid, 140 mEq/L, and serum, 4 mEq/L, provides good proof for the compartmentalization of bone (3, 11). Large fluxes of ionic calcium into and out of the skeleton are, according to Neumann and Ramp (11), continuously maintained and are several orders of magnitude greater than the fluxes related to resorption that are under the control of PTH.

Recent studies of acetazolamide action on calcium metabolism in rats suggest a role of carbonic anhydrase and thereby of CO_2 in bone demineralization (19).

ACKNOWLEDGMENTS

The contributions of Mrs. G. A. Cadwell and Mr. A. Messier for the determinations of urine calcium, inorganic phosphorus and carbon dioxide are gratefully acknowledged.

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STRONTIUM SCANNING IN CAISSON DISEASE OF BONE

P. T. Cox and D. N. Walder

The diagnosis of caisson disease of bone is at present made by radiological examination of the major joints. The changes that are detected early in the course of the disease are principally those due to the formation of new bone surrounding and invading the necrotic lesion. It is reasonable to assume that considerable new bone formation must occur before a radiological change may be detected. According to Davidson (5), the degree of new bone development necessary to show on a radiograph takes at least 3½ months to occur.

It is recognised that strontium scanning will detect metastatic neoplastic deposits in bone which are not visible on routine radiological examination (3, 8). There is also some evidence that areas of bone necrosis may be revealed by scanning before the development of radiological changes (2, 4).

The aim of this study has been to investigate the use of strontium scanning in detecting caisson disease of bone, with particular reference to the early lesion. Two series of investigations were carried out—the first on humans and the second on animals.

A group of 10 compressed air workers with radiologically detected bone lesions were examined by a strontium scan. The age of the men at the commencement of compressed air work, the duration of work, the maximum working pressures, history of decompression sickness, and the interval between the men's last exposure to compressed air and the strontium scan are shown in Table I.

One hundred microcuries of ^{85}Sr (half-life of 64 days) were administered in seven cases and 3 millicuries of ^{87M}Sr (half-life of 2.8 hours) in the remaining three cases. The short half-life of ^{87M}Sr enabled a larger dose to be given safely.

Scanning of both hips and shoulders was carried out in most cases. In addition, scans of the appropriate parts of the shafts of the long bones were carried out in three men whose radiological examinations revealed bone lesions at these sites. Scanning was carried out with a Picascanner and the results were presented in the form of a colour scan.

Serial scans following the injection of ^{85}Sr were carried out daily for 7 days in one compressed air worker and also one normal volunteer who had never been exposed to a hyperbaric environment. These revealed a maximum uptake of Sr after 24 hours followed by a gradual clearance over the following 6 days.

TABLE I

CASE HISTORIES—GROUP OF TEN COMPRESSED AIR WORKERS

	Age (yrs.)	Duration of Work	Maximum Working Pressure (p.s.i.g.)	Reported Attacks Decompression Sickness	Interval—Compressed Air to Strontium Scan (yrs.)
1.	33	13 months	35	1	5
2.	29	13 months	33	0	5
3.	22	18 months	42	3	5
4.	38	30 months	30	4	5
5.	33	24 months	42	3	6
6.	40	34 months	42	0	5
7.	25	13 months	33	2	7
8.	45	4 months	35	1	7
9.	41	6 weeks	19	2	7
10.	35	12 months	38	3	24

The results of the scans are shown in Tables II and III. Out of a total of 18 femoral heads, eight had positive radiological lesions and seven had positive scans. In 15 cases there was correlation between a scan—either positive or negative—and the radiograph. In one case there was a positive scan and a negative radiograph and in two cases, a positive radiograph and a negative scan. Of a total of 18 humeral heads (Table III), nine had positive radiological lesions and 12 had positive scans. In 13 cases there was correlation between a scan—either positive or negative—and the radiograph. In four cases there was a positive scan and a negative radiograph and in one case a positive radiograph and a negative scan. Three radiologically diagnosed shaft lesions in the lower femora showed no abnormality on scanning.

One femoral head, which was removed at operation, was studied in great detail. Radiologically, it showed collapse and destruction of the articular surface with secondary osteoarthritis. The strontium scan was positive. Following resection of the head 12 days after the administration of ^{85}Sr, the specimen was examined by normal and point source radiography, autoradiography and histology, including thin decalcified sections stained throughout with haematoxylin and eosin and thicker undecalcified sections stained with toluidine blue on one surface only. Examination of the gross specimen and blocks of the

TABLE II

RESULTS OF STRONTIUM SCANS OF 18 FEMORAL HEADS

	Radiograph Positive	Radiograph Negative	Total
Scan positive	6	1	7
Scan negative	2	9	11
TOTAL	8	10	18

TABLE III

RESULTS OF STRONTIUM SCANS OF 18 HUMERAL HEADS

	Radiograph Positive	Radiograph Negative	Total
Scan positive	8	4	12
Scan negative	1	5	6
TOTAL	9	9	18

undecalcified sections was carried out with ultraviolet light, to detect fluorescence from tetracycline administered to the patient 48 hours before surgery. Figure 1 shows an anteroposterior radiograph of a slab of the specimen and the autoradiograph produced by placing the same specimen against a fast x-ray film for 2 weeks. The scalloped area of bone

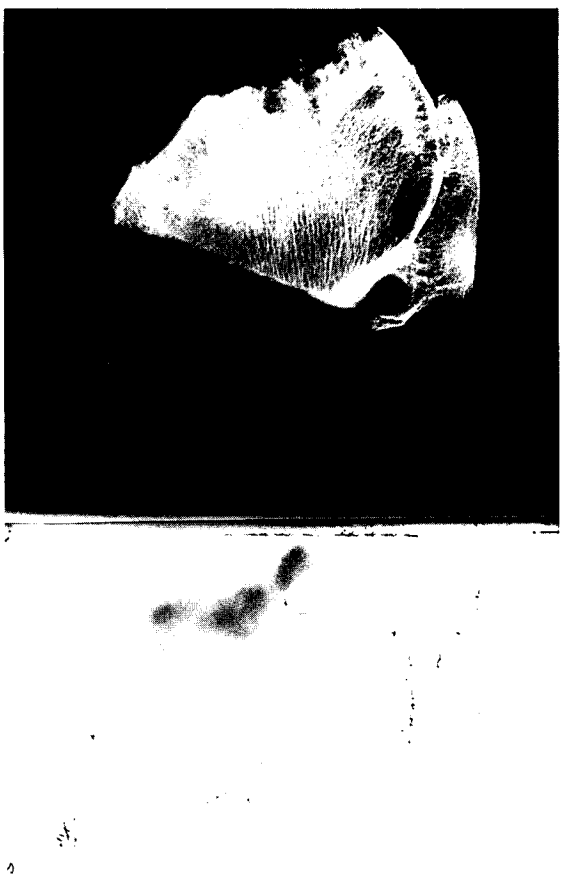

FIG. 1. Anteroposterior radiograph and autoradiograph of slab of femoral head.

destruction is situated at the upper part of the femoral head. A large osteophyte is closely apposed to the medial side of the head. The autoradiograph shows increased uptake of Sr at the margin of the roughened area in a position similar to the area of maximum tetracycline fluorescence shown by examination with ultraviolet light. These results indicate the margin of the roughened area to be the site of new bone formation.

Histological examination showed some unexpected changes. There were degenerative changes in the original articular cartilage, and elsewhere a new surface of less hyaline cartilage had formed. In the latter were embedded some bone fragments. The new surface was undergoing enchondral ossification in places. All the appearances suggested that the necrotic fragments had been completely ground away and that the base of the rough area, seen to be the site of increased bone formation, was the reaction front that had surrounded the necrotic lesion.

The second series of investigations was carried out on rabbits. Aseptic necrotic bone lesions were produced by using an intra-arterial injection of small lead glass spheres or Ballotini to infarct the femora of adult and immature New Zealand white rabbits. Three series of experiments were carried out using Ballotini of 120 μ, 70 μ and below 45 μ in diameter. A single nonlethal bolus of the smaller sizes of Ballotini was injected into the left external iliac artery at laporotomy. Kistler demonstrated in 1933 (7) that most of the arterial supply of the head of the rabbit femur, via the ligamentum teres, comes from the profunda branch of the femoral artery. The 120 μ Ballotini were injected retrograde into the lower aorta via a narrow gauge polythene cannula inserted into the left external iliac artery. From the illustration (Fig. 2), it can be seen that the Ballotini reached the circulation to the bone. Radiographs were taken every 2–4 weeks and the animals were killed at intervals of up to 5 months after the injection. Out of a total of 30 rabbits in the three series, 10 developed bone

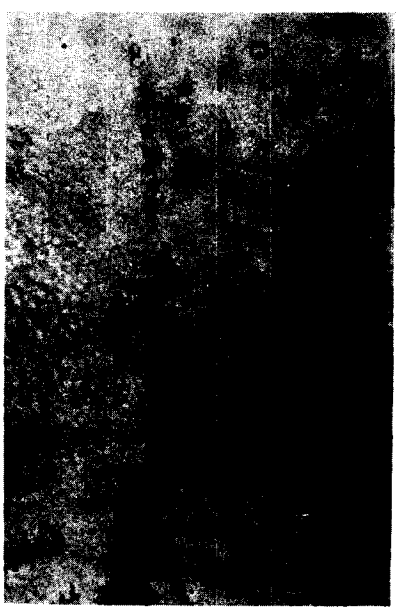

FIG. 2. Ballotini impacted in an arteriole in bone marrow, surrounded by stained fat cells.

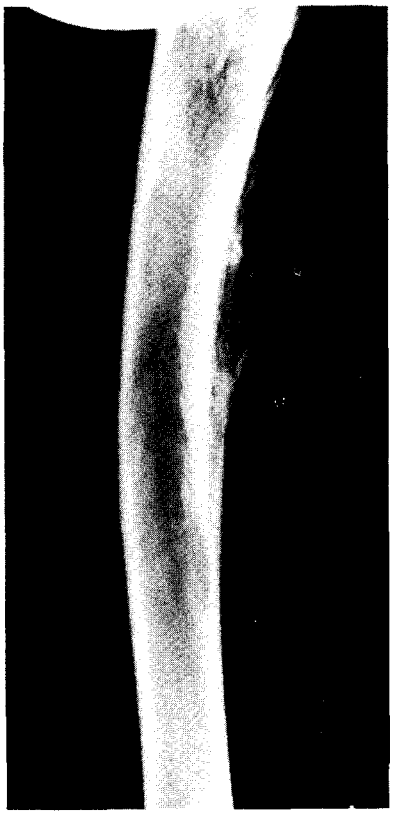

FIG. 3. Lateral point source radiograph of a rabbit femoral shaft showing a lesion.

lesions. All of these animals were from the series receiving injections of Ballotini of 70 μ and below 45 μ in diameter. None of the animals receiving injections of 120 μ Ballotini developed lesions. The bone changes were situated mainly in the femoral shaft; Fig. 3 shows a typical lesion. Histologically, the lesions consisted of necrotic areas in the cortex and marrow with extensive subperiosteal and some endosteal new bone formation (Fig. 4). Interestingly the dead cortex was removed by osteoclastic activity on both the subperiosteal and endosteal surfaces. Subperiosteal osteoclasis is a rarely occurring process.

Three cases also showed avascular necrosis of the femoral head (Fig. 5). Histological examination of the head showed necrotic bone trabeculae with appositional new bone (Fig. 6). Three animals with shaft lesions produced by Ballotini of below 45 μ in diameter received an intravenous injection of ^{87M}Sr and ^{32}P a few hours before death. After death, the bodies were scanned, and the femora excised for autoradiography. The three scans showed a slight but definite increase in the Sr uptake over the left femur which contained the lesions. Figure 7 shows the autoradiographical appearance of a normal femur on the left and a femur with a single radiologically detected lesion at the middle of the shaft on the right. Increased ^{32}P uptake is visible at this site, in the region of the greater trochanter, and

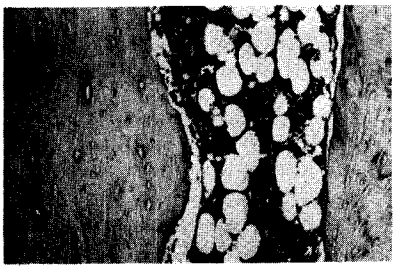

FIG. 4. Histological appearance of shaft lesion, shown in Fig. 3, with necrotic bone cortex on the left and subperiosteal new bone on the right (X 150).

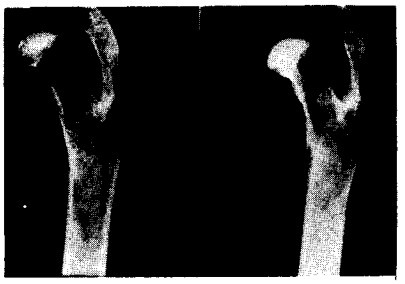

FIG. 5. Point source radiograph showing aseptic necrosis of a rabbit femoral head (right) with increased density of the head. A normal is shown for comparison (left).

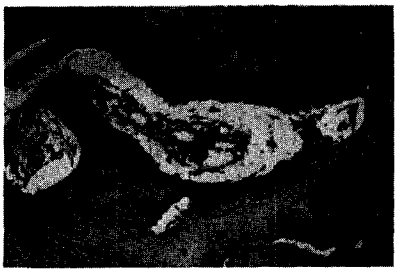

FIG. 6. Histological appearance of the femoral head (shown in Fig. 5) showing appositional new bone (X 150).

FIG. 7. ^{32}P autoradiograph of a bisected rabbit bone, with lesions (right). A normal is shown for comparison (left).

at the lower end of the bone. Histological examination of the upper end showed cancellous bone necrosis in the greater trochanter and in the femoral head.

It has been shown in the past that increased ^{32}P uptake (1) and presumably increased Sr uptake, as has been shown with the resected femoral head, occurs at the active, viable front around an area of necrosis related to the increased new bone formation. It is logical to assume that this is the reason for correlation between the positive radiographs and positive scans in this group of compressed air workers. In those cases with a negative radiograph and a positive scan, there was no evidence of other joint disease, e.g., osteoarthritis. Johnson (6) has said that the increased activity around a lesion may last for a long time, possibly indefinitely. Strontium scans carried out up to 9 years after fractures of the shafts of long bones in men (9) have been shown to be positive even when there is no residual radiological evidence of the injury. It is reasonable to assume that minor degrees of bone injury may occur in compressed air workers that cannot be recognised by radiological examination and that the increased activity could persist to give an abnormal scan for many years. In the opposite situation, a negative scan and a positive radiograph may be due to the fact that, for some reason in the minority of cases, the increased activity around a necrotic lesion has ceased and new bone formation has returned to normal.

In conclusion, there is evidence from both human and animal experiments that strontium scanning may be especially useful in the detection of the minor and early changes of caisson disease of bone. This should be of great importance when the aetiology and treatment of the condition is finally known.

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GULF COAST COMMERCIAL DIVER DYSBARIC OSTEONECROSIS SURVEY

C. J. Fagan and E. L. Beckman

It has been estimated that there are approximately 500 commercial divers in the Gulf Coast area consisting of the coastlines of Florida, Alabama, Mississippi, Louisiana and Texas. Most of these divers are concentrated in the coastal region between New Orleans, Louisiana and Corpus Christi, Texas. In an attempt to ascertain what the incidence of osteonecrosis might be in these workers, a sample survey was conducted on the worker population of a local company employed in underwater operations of various types.

This report is based on the roentgenologic observations of 30 commercial divers. These men, from 24 to 53 years old, were surveyed during a 6-month period. All of the divers were submitted to a conventional radiologic bone survey; 12 had additional studies: six underwent a radioisotope bone scan and six had a bone survey utilizing a xeroradiographic method of imaging.

The radiological bone survey for osteonecrosis was tailored after the British protocol (6). Specifically, the following roentgenograms were obtained on each subject: 1) anteroposterior projections of each shoulder on 10 × 12 films with the patient rotated 45 degrees to the table, permitting the shoulder to be in contact with the tabletop; 2) anteroposterior, 10 × 12 projections of each hip; and 3) anteroposterior and lateral projections of each knee to include the distal two-thirds of the thigh and proximal one-third of the legs on separate 14 × 17 films. A Bucky grid was used in the exposure of all films. Initially, the frogleg position of the hips was utilized when small lesions were suspected in the anterior portion of the femoral head. It was soon realized that, in some cases, this projection was the only one that allowed visualization of the narrow, crescent-shaped, translucent, subcortical band typical of osteonecrosis (5). As a result, the frogleg projection of each hip has replaced the anteroposterior projection described above as a routine projection of the hips in this survey.

The xeroradiographic survey for osteonecrosis consisted of the same projections; however, the xerograph technique of recording x-ray images was employed. The basic elements of this technique are described on page 190. Radioisotope bone scans utilizing fluorine-18 consisted of anterior and posterior total body, rapid survey scans starting approximately 2½ hours after the intravenous administration of the isotope. The usual isotope dose was 2.25 to 3.25 millicuries (mc). Anterior and posterior rectilinear scans of suspect regions with a slower scan speed and a 1:1 image ratio were also obtained.

Results

Roentgenographic Findings

The classification and terminology, formulated by the British Medical Research Council Decompression Sickness Panel, were used as diagnostic parameters (7). This classification is as follows:

A. Juxta-articular lesions:
   1. Dense areas, with intact articular cortex.
   2. Spherical segmental opacities.
   3. Linear opacity.
   4. Structural failure.
      a. Translucent subcortical band.
      b. Collapse of articular cortex.
      c. Sequestration of cortex.
   5. Osteoarthritis.

B. Medullary lesions of the head, neck and shafts:
   1. Dense areas.
   2. Irregular calcified areas.
   3. Translucent areas and cysts.

Case Examples

Case 1, R. N., age 50, had 20 years' diving experience. Figure 1**A** and **B** shows very subtle rarefactions with surrounding sclerosis in the humeri (arrows)—examples of B3, medullary shaft lesions of osteonecrosis. Spherical dense areas (horizontal arrows) in the proximal shaft of the left tibia are shown in Fig. 1**C** and **D**. In view of the findings in the shoulders, this lesion is believed to represent an area of osteonecrosis as opposed to a "bone island"—it represents a B1-medullary shaft lesion. Old traumatic changes are indicated by the vertical arrow.

Case 2, J. H., age 32 had diving experience of 10 years. In Fig. 2**A** the juxta-articular dense area (arrow) with an intact articular cortex in the right humeral head is seen, an example of an A1 lesion. Figure 2**B** shows subtle translucent areas in the proximal left humeral shaft (arrows), additional examples of a B3 lesion.

Case 3, T. H., age 26, had 8 years' diving experience. A normal right shoulder is shown in Fig. 3**A**. Juxta-articular areas of density with an intact articular cortex in the left humeral head, an early snow cap lesion (Fig. 3**B**) can be compared with Fig. 3**A**. A translucent area with sclerotic margin (arrows) abuts the old epiphyseal plate line, B3 area of osteonecrosis (Fig. 3**C**). The lateral film (Fig. 3**D**) also shows the lesion (arrow).

Case 4, R. L., age 32 had diving experience of 8 years. Figure 4**A** shows subtle area of sclerosis of humeral head with intact cortex. Tomograms more clearly define a juxta-articular, snow cap lesion or A1 area of osteonecrosis (Fig. 4**B**).

Case 5, E. A. S., is 40 years old with 30 years of diving experience. Extensive juxta-articular changes consist of subchondral fragmentation and depression (Fig. 5**A**). Osteoarthritic changes involve the glenoid fossa as well as the humeral head. These are A4 and A5 areas of osteonecrosis, respectively. In Fig. 5**B**, patchy areas of sclerosis (arrows) in the

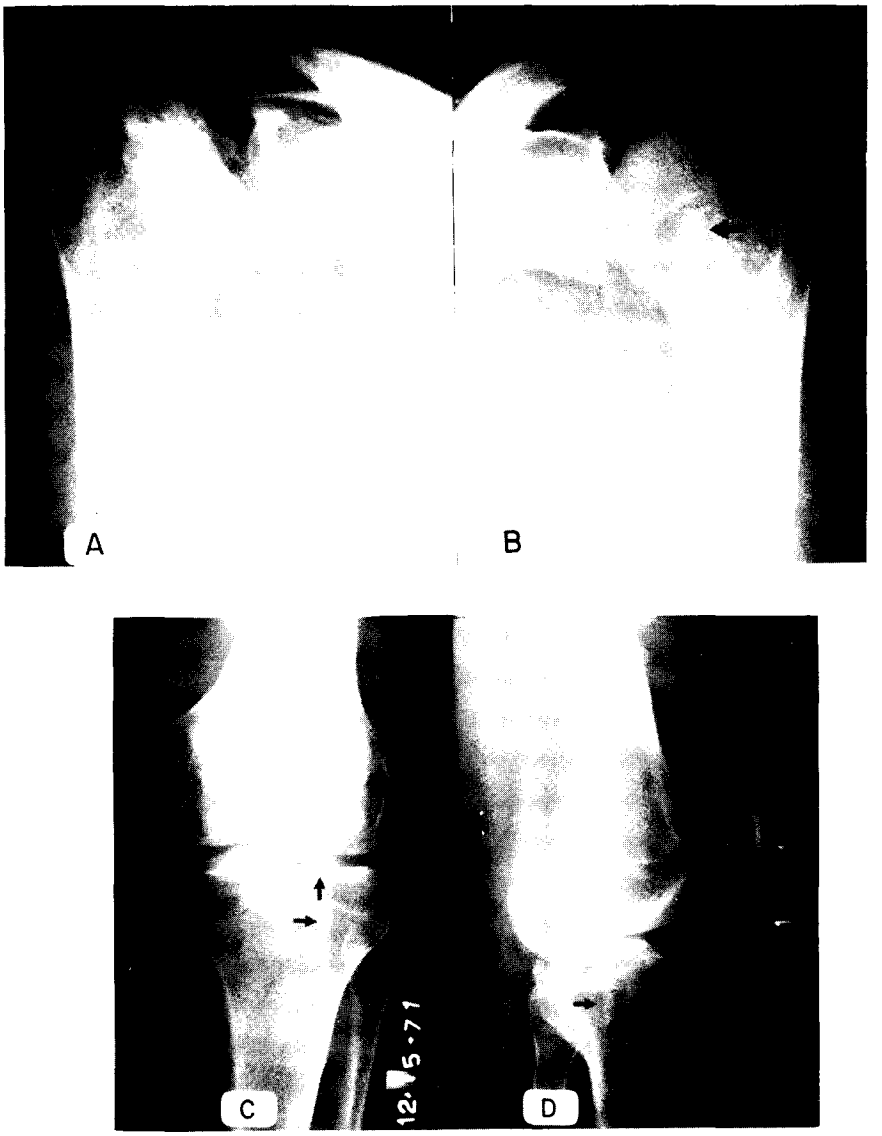

FIG. 1. **A, B, C, D.** Case 1.

medullary portion of the left humeral shaft are examples of B1 type of lesions. Small focal dense areas (arrows, Fig. 5**C**) in the medial condyle of the distal femur and proximal tibial shaft look like small "bone islands" but in view of the evidence of osteonecrosis in the shoulders, these findings probably represent additional areas of osteonecrosis. A normal knee is shown in Fig. 5**D** for comparison.

Case 6, W. W. V., aged 53 had 20 years of diving experience. Obvious cystic rarefaction with sclerotic margin in the anteriomedial surface of the right humeral head (arrow) and

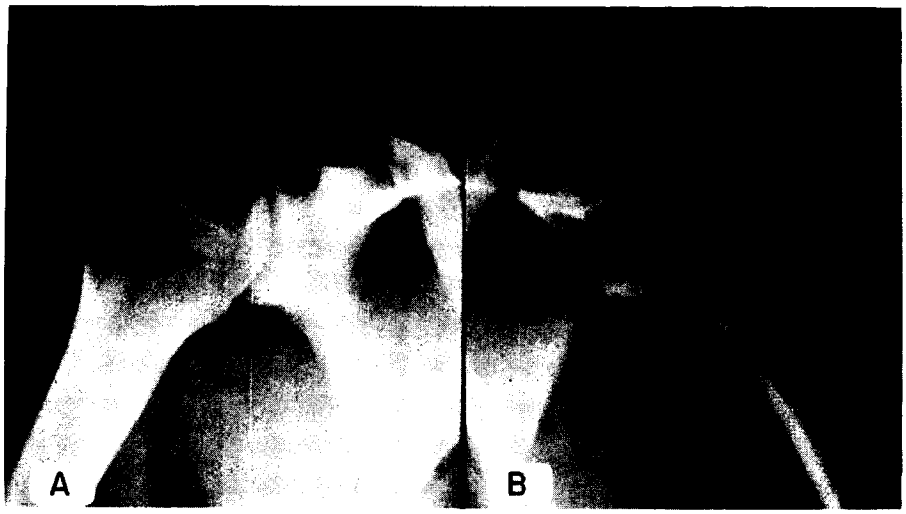

FIG. 2. A, B. Case 2.

translucent as well as patchy areas in the neck of the right humerus are shown in Fig. 6A; these are examples of A1 and B3 lesions of osteonecrosis. Similar B3 lesions are seen in the left humeral shaft (Fig. 6B). Frontal and lateral films of the left knee demonstrate small localized rarefactions with some sclerosis in the distal femoral shaft and proximal tibial shaft (horizontal arrows, Fig. 6C, D). Extra-epiphyseal center, the fabella, is shown by vertical arrow.

Case 7, J. B. G., age 47, had diving experience of 22 years. Juxta-articular lesions are manifested by dense areas as well as spherical segmental opacities (arrows, Fig. 7A). In addition, there is a dense area in the humeral shaft. In the contralateral shoulder, marked juxta-articular changes including collapse of articular cortex (arrow, Fig. 7B) reveal an A4 type lesion. A normal right hip is shown in Fig. 7C and, in contrast, Fig. 7D shows an extensively involved left hip with extensive structural failure (A4 lesion) and complicating osteoarthritis (A5 lesions). A sequence of films demonstrating progression of changes in the left hip in the same patient is shown in Fig. 8: A, 1961, B, 1963, and C, 1972. In Fig. 9 (the same patient), the value of the frogleg projection of the hip is demonstrated. Compare the anteroposterior (frontal) film (Fig. 9A) with the frogleg projection (Fig. 9B). Note a fracture, manifested by a subcortical translucent band, is evident only on the frogleg projection.

Case 8, W. S., age 30, had diving experience of 11 years. Figure 10 shows classic medullary shaft, B2, area of osteonecrosis.

Of the 30 divers surveyed, eight presented objective evidence of osteonecrosis. The sites of involvement are depicted in Table 1. Note the prevalence of the lesions in the shoulders—a finding common to other osteonecrosis surveys in divers (2, 3). Juxta-articular knee involvement was not observed and has, to our knowledge, been reported only once in other surveys (7). Table II compares the incidence of osteonecrosis in previous diver surveys with these results. The sample survey disclosed a 27% occurrence of osteonecrosis in Gulf Coast commercial divers.

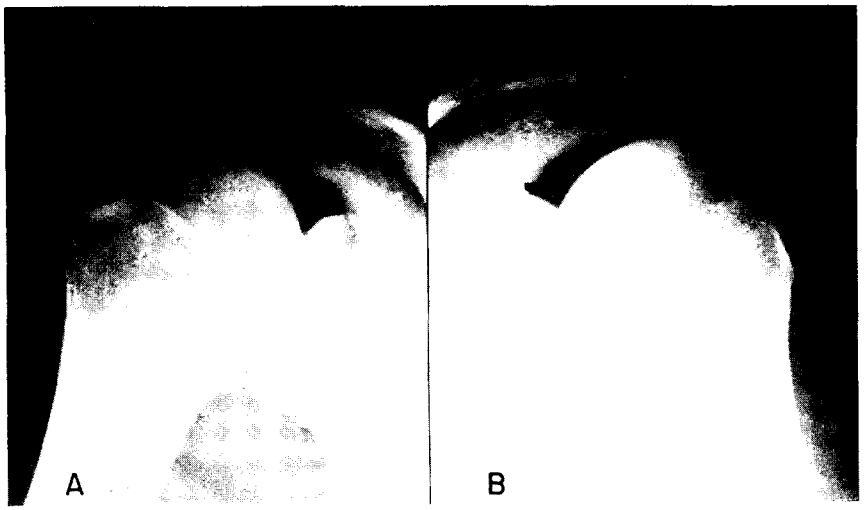

FIG. 3. **A, B, C, D.** Case 3.

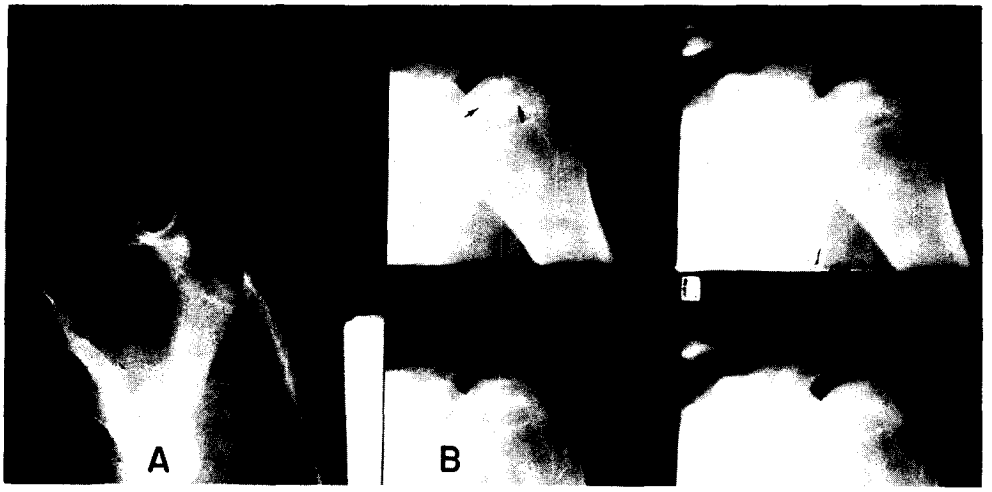

FIG. 4. A, B. Case 4.

Xeroradiography

Xeroradiography is a technique using photoconductors and electrostatic charges as a method of recording x-ray images. A selenium-coated and positively charged metal plate is used instead of film. The x-ray beam is attenuated as it passes through the part of the body being exposed. The attenuated rays discharge the area of the plate which they strike in proportion to their intensity and form a latent image. This image, which is similar to that seen on the conventional radiograph, is preserved by transferring it to paper by an adhesive technique or by photographing it.

Although reports by some observers that there is actually loss of fine detail and low contrast when skeletal parts are xeroradiographed were known, it was decided to use this technique to survey a few of the divers in this study.

Figures 11, 12 and 13 compare xeroradiographic images with those obtained on conventional film. Although this type of comparison was only made in six divers, no definite advantage of xeroradiographic imaging over plain conventional films was noted; it was felt that the additional exposure incurred from the xeroradiography (specifically, there is a fivefold increase in exposure of the shoulder, a sevenfold increase in exposure of the hip area, and a twofold increase in exposure of the knee) did not justify its routine use as a survey technique (7).

Radioisotope Scanning

Skeletal system scanning provides a sensitive method of studying bone pathology. In spite of its poor specificity and some of its technical and logistic problems, the bone scan often yields a fairly accurate estimate of a disease process. As a result, fluorine-18 bone scans were performed on six of the divers. This particular isotope was selected because of its short half-life, its adequate photon energy that allows one to use small dosing (making the radia-

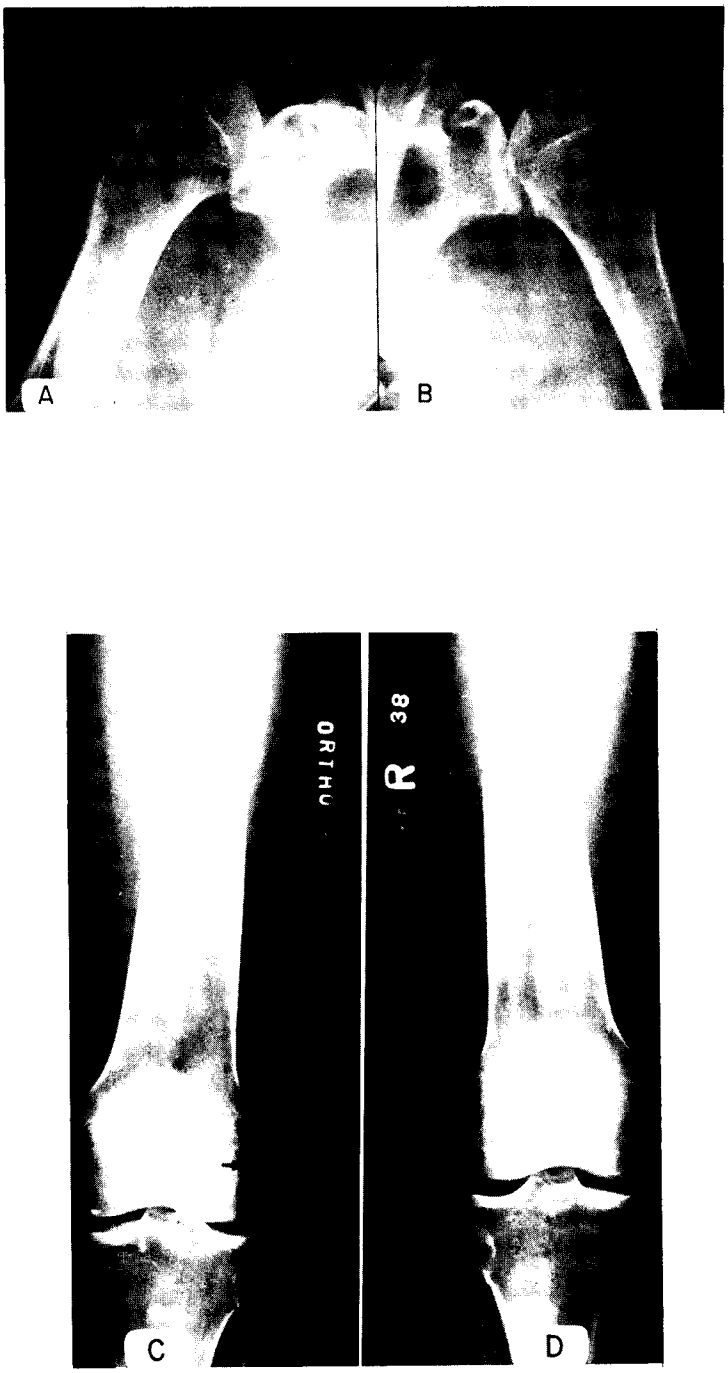

FIG. 5, **A, B, C, D.** Case 5.

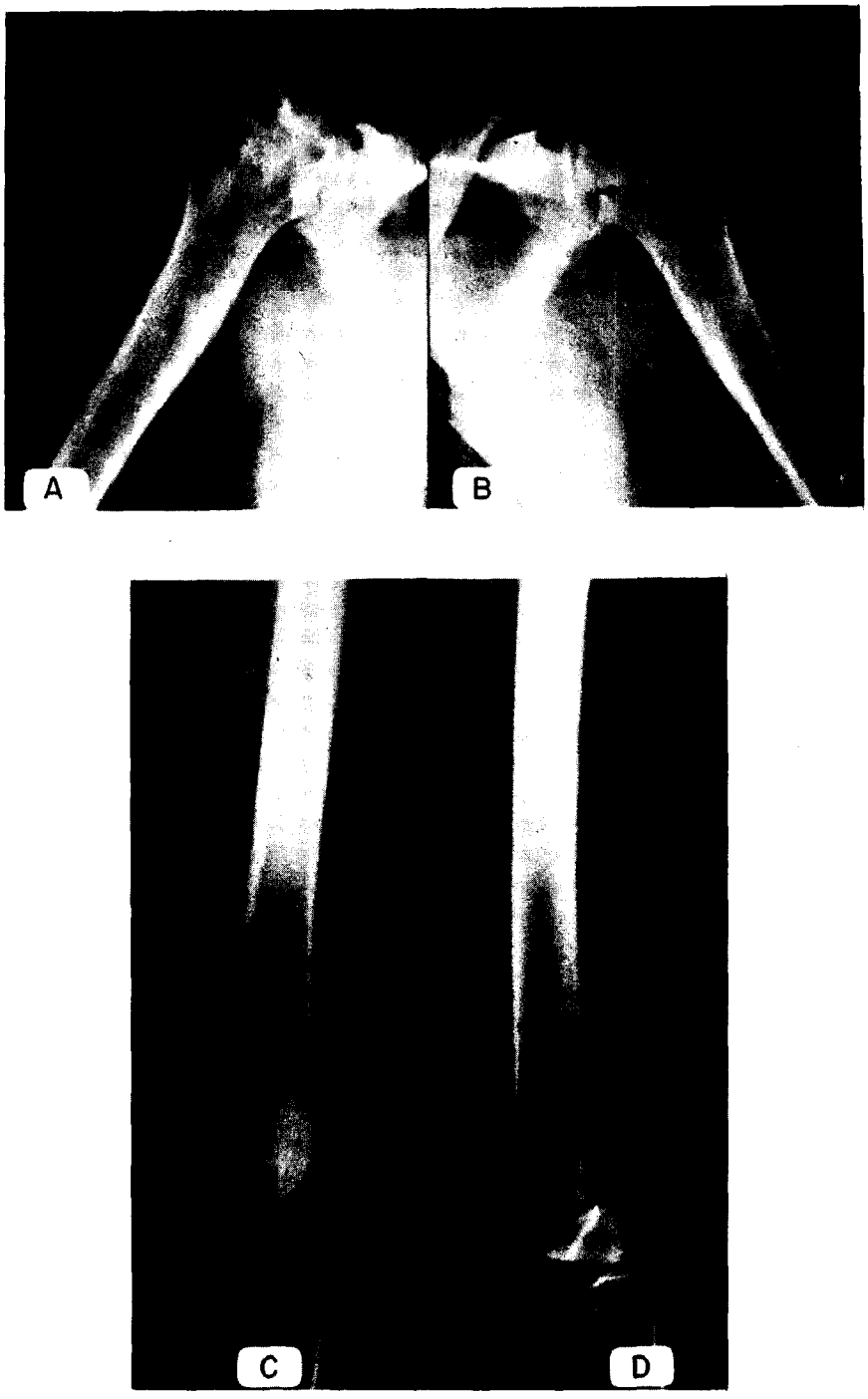

FIG. 6. **A, B, C, D.** Case 6.

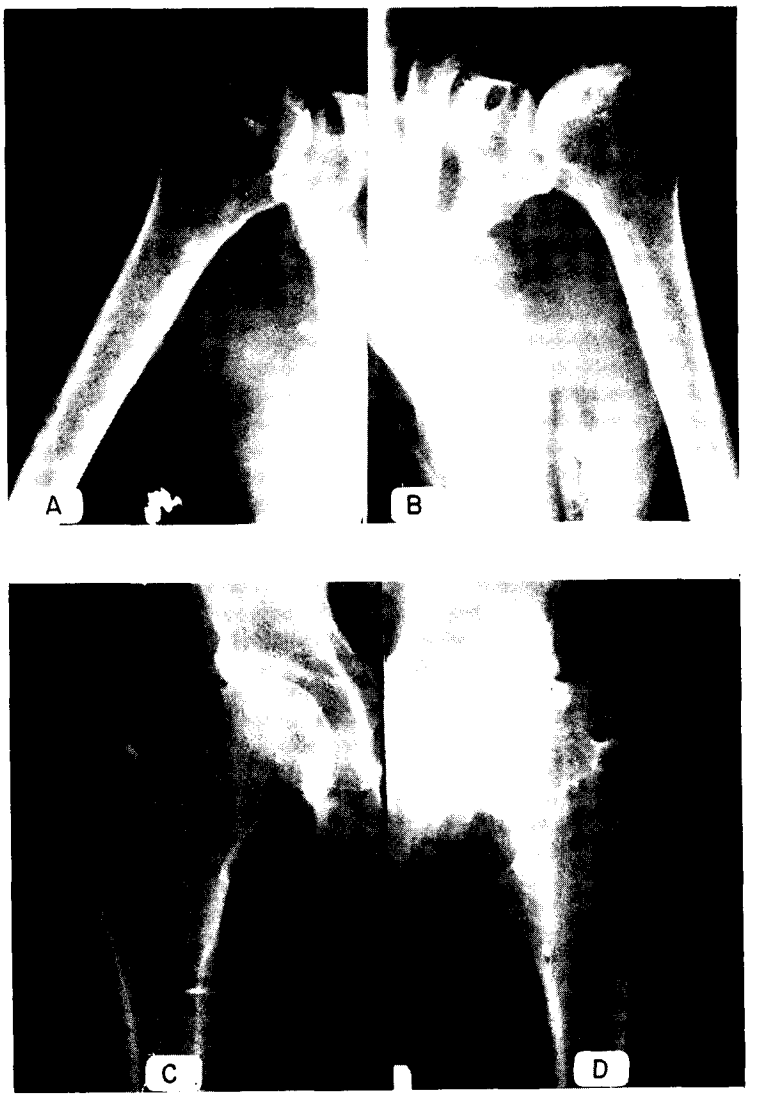

FIG. 7. **A, B, C, D.** Case 7.

tion hazard negligible), and its rapid blood clearance manifested by a high target over background activity ratio—permitting patients to be scanned within 2 to 4 hours after the intravenous injection of the isotope.

Figures 14 and 15 show anterior and posterior total body scans of two divers who had positive findings from photoroentgenographic studies.

In all, six fluorine-18 scans were done. In one instance, both the isotope scan and plain film study were negative. In another case, the isotope scan was positive and the plain film study only questionably positive. In still another instance, the isotope scan was questionably

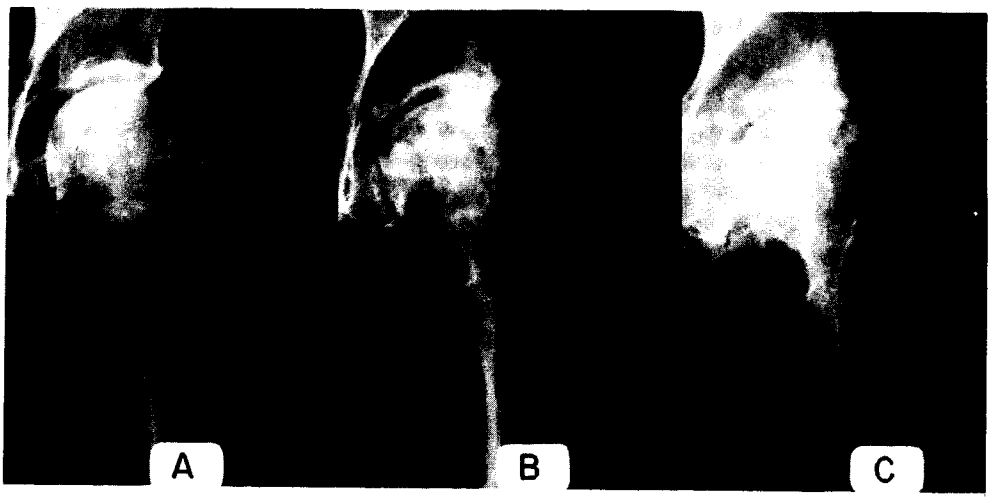

FIG. 8. **A, B, C.** Case 7.

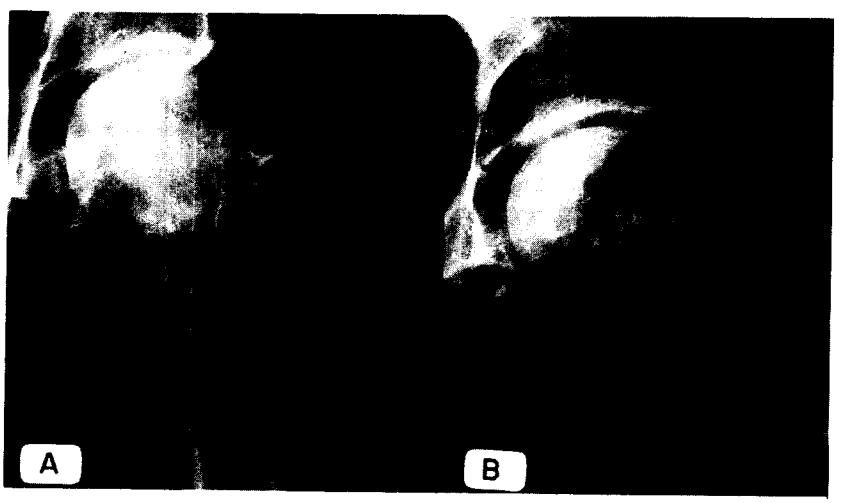

FIG. 9. **A, B.** Case 7.

positive while the plain film study was decidedly positive. In the remaining cases, the findings on the plain film survey and the scans coincided.

There are probably three reasons why the plain film surveys and isotope scans did not coincide in each case. First, it certainly is conceivable that the plain film survey can be incorrectly interpreted, resulting in a false positive. Secondly, for unknown reasons, bone scans do not always detect all of the lesions that are present. In this regard, occasionally one sees a skeleton riddled with metastatic disease on conventional films while the isotope scan demonstrates many but not all of the lesions. Finally, while one would expect a bone scan to be positive during the revascularization and reossification stages of bone repair and

FIG. 10.

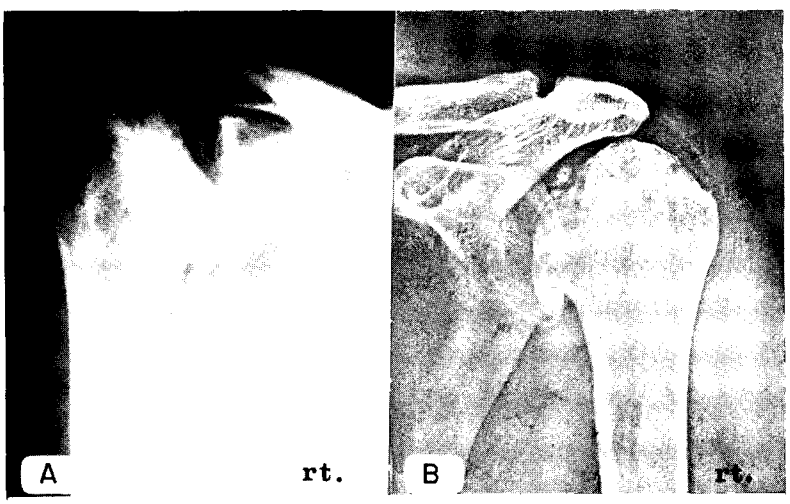

FIG. 11. Extensive changes involving the right humerus, described previously, are identified on conventional film (**A**) and on a xeroradiograph (**B**). The findings are equally evident on the two studies so that the additional exposure resulting from the xeroradiographic technique is not justified.

TABLE I

DISTRIBUTION OF DYSBARIC OSTEONECROSIS

	Number of Patients		
Sites	Right	Left	Total
Shoulder	10	12	22
Hip	0	3	3
Knees	1	5	6

FIG. 12. This diver's left shoulder is depicted on the xeroradiograph (A) and a conventional film (B). Once again, the image on the xeroradiograph does not add any additional information over that obtained on the conventional film.

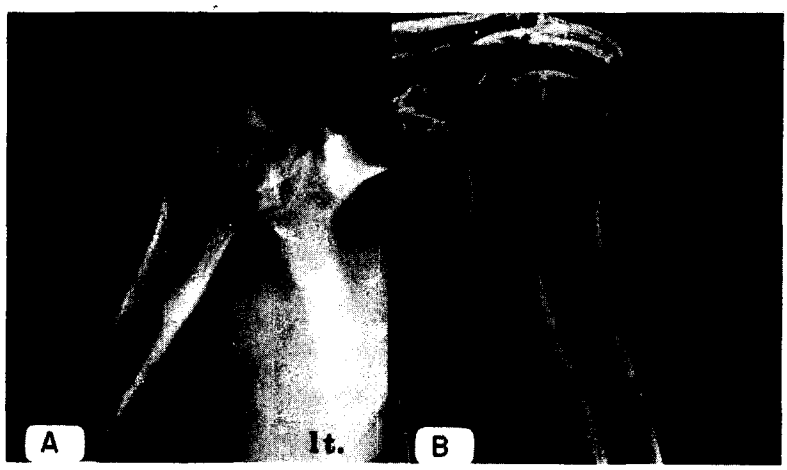

FIG. 13. The fairly extensive left humeral and proximal shaft lesions seen on the conventional films (A) are also identified on the xeroradiograph (B) without any improvement in detail of the lesion.

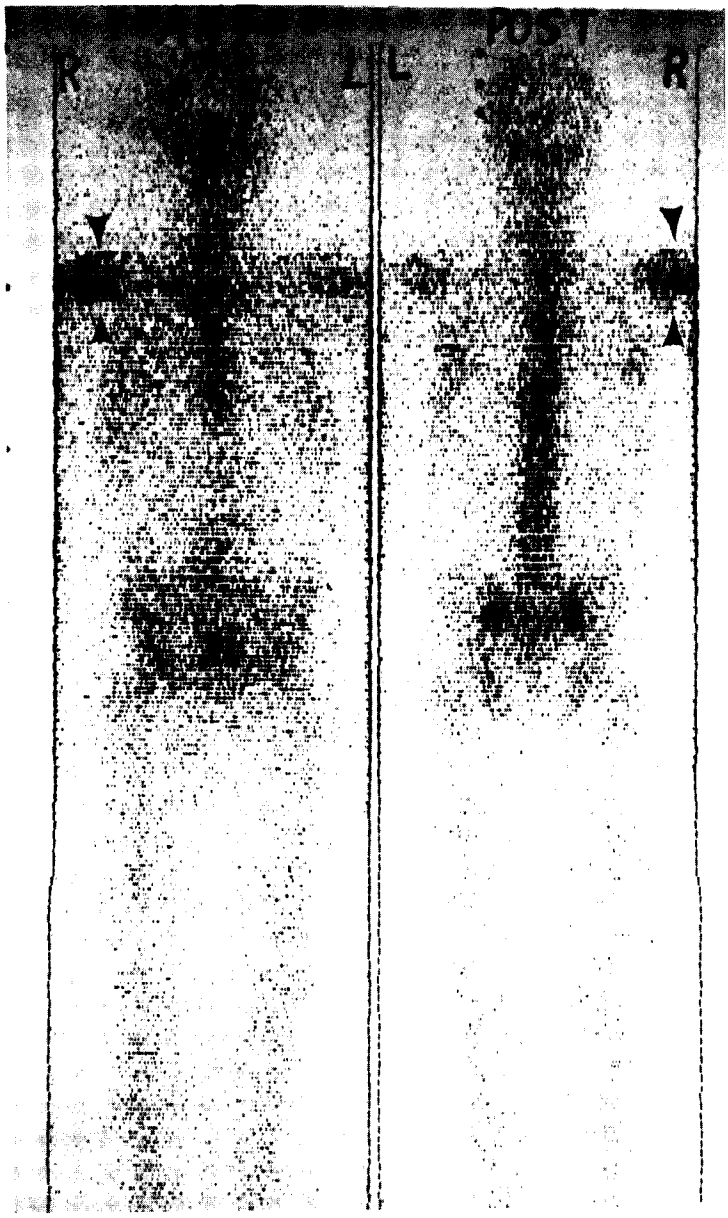

FIG. 14. Anterior and posterior total body rapid survey bone scans were performed starting 2 hours after the intravenous administration of 2.6 millicuries (mc) of fluorine-18. These scans revealed abnormally increased areas of tracer concentration in most of the right shoulder region (arrows). The areas of increased activity over the sternum, spine, sacroiliac joints, and urinary bladder are anticipated and not representative of an abnormality. No additional abnormalities were detected. The x-ray bone series demonstrated lesions in both shoulders.

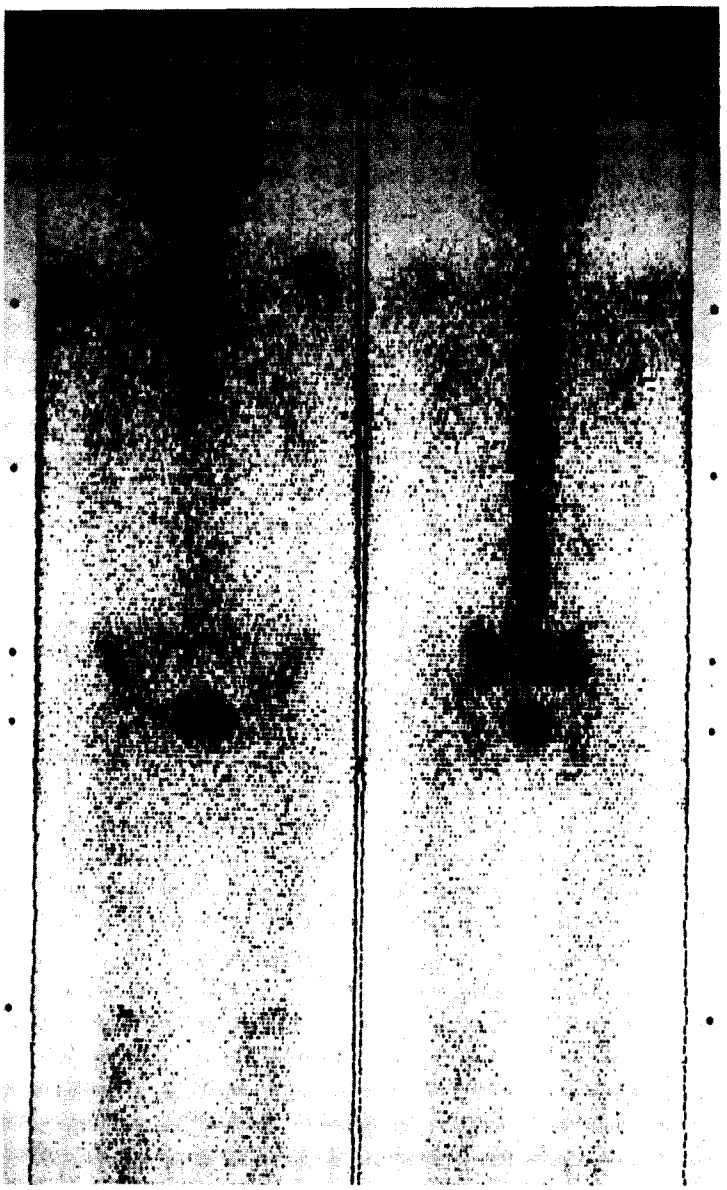

FIG. 15. This patient was given 3.2 millicuries (mc) of fluorine-18. Two hours later anterior and posterior total body rapid survey scans were begun. A slight difference in the tracer concentration in the two shoulders was detected. The plain film examination demonstrated definite areas of osteonecrosis in both shoulders and the left knee.

TABLE II

INCIDENCE OF BONE LESIONS IN PREVIOUS SURVEYS OF DIVERS

Surveys	Divers	Divers with Lesions	Incidence (%)
Hergert	47	13	34
Hergert	90	29	32
Slordahl	13	3	23
Alnor	131	72	55
Ohta	301	152	50
British Navy	250	13	6
Gulf Coast	30	5	27

osteonecrosis, the scan may well be non-revealing after an area of osteonecrosis has healed or associated with secondary osteoarthritis. In this regard, bone scans using fluorine-18 are usually negative over areas of osteoarthritis in the general population. Perhaps then, the bone scan coupled with the plain film survey may be revealing something about the stage of the illness. It is suspected that fluorine-18 scans are a sensitive study in detecting new or active areas of osteonecrosis, and more observation using fluorine-18 bone scans to detect areas of osteonecrosis are indicated and justified.

Summary

Dysbaric osteonecrosis of bone is not an uncommon disease among Gulf Coast divers. A sample survey consisting of a small number of commercial divers yielded a high incidence (27%) of roentgenographic findings. As a result of this initial work, an attempt is being made to expand the survey.

A comparison of plain film imaging with xeroradiographic imaging, in an admittedly limited number of divers, has not sufficiently justified the additional radiation exposure inherent in the xeroradiographic technique.

Fluorine-18 bone scanning probably represents a reasonable and practical modality in an osteonecrosis survey. The bone scan probably relates to the osteogenic activity of the disease process and, therefore, may be helpful in detecting early or active areas of osteonecrosis.

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PREVENTION OF OSSEOUS AVASCULAR NECROSIS IN COMPRESSED-AIR WORKERS

A. R. Behnke

Over 65,000 feet of soft-ground tunneling was completed on the 75-mile San Francisco Bay Area Rapid Transit (BART) project from November, 1967 through May, 1969. This major industrial operation included about 15,000 feet of machine-shield tunneling in compressed air under hazardous obstacle and soil conditions. There have been 80,360 man decompressions at pressures from 9 to 36 p.s.i., and a total of 135 cases of decompression sickness involving 85 men in a group of approximately 400 compressed air workers. As of November, 1972 no clinical or radiographic evidence of osteonecrosis had been reported.

Over 17,000 skeletal roentgenograms were taken on more than 2000 workers. During the pre-employment examinations, 33 lesions were detected in long bones of 15 workers who were rejected for work in compressed air. Eleven of these men had had potentially disabling juxta-articular lesions which affected principally the humeral head. Two of the rejected workers had previously been exposed to only 16 and 18 p.s.i., respectively, but both showed evidence of chronic alcoholism. The remaining rejected workmen had been exposed to pressures in excess of 30 p.s.i. The absence of pathology in the long bones of the 1985 workers who had not been employed in compressed air was noteworthy.

Preliminary survey indicates that disabling avascular bone necrosis can be prevented by 1) employment of unique engineering principles designed to minimize exposure to pressure; 2) comprehensive physical examination which includes initial and followup radiologic survey; and 3) extended decompression in air as stipulated by the Washington State tables in their present or modified form (25). In efforts to solve the overall problem—namely, prevention of gas-phase separation from tissues in the form of nascent intrasvascular bubbles and complete abrogation of decompression sickness—it is essential to resort to oxygen decompression under conditions which favor isobaric nitrogen transport from tissues. Undoubtedly, establishment of a compressed air habitat, for periodic and extended sojourn in the hyperbaric environment, would constitute the definitive and conclusive preventive measure.

Nature of Avascular Bone Necrosis

The following are some of the systemic conditions associated with nontraumatic bone necrosis in adults (11): decompression syndromes, hemoglobinopathies, hypercortisonism,

alcoholism, pancreatitis, Gaucher's disease, and arteriosclerosis. The necrosis often involves multiple bones with symmetrical bilaterality. Despite the diversity of the types of exciting agents (bubbles, fat globules, coalesced lipids, sickle cells, pancreatic enzymes), the radiographic-viewed pathology is remarkably similar. However, the decompression syndrome shows a striking predilection for the head of the humerus in contrast to the nonbubble agents of other entities which are pathophilic for the head of the femur (Table I). Also the distal femur and the proximal tibia are frequently involved. The distal tibia, body of the talus, carpal scaphoid and lunate, and humeral capitellum are less frequently affected. Although epiphyseal lesions often produce pain and stiffness, the metadiaphyseal lesions are usually asymptomatic since no cortical or periosteal damage is present. Patients with systemic conditions predisposing to avascular necrosis, who develop bone pain and tenderness in an area commonly involved by this process, may not initially show x-ray evidence of necrosis (11).

In decompression syndromes, only radiologic lesions classified as juxta-articular may lead to pain and limitation of movement; other lesions in the head, neck, and shaft of the humerus and femur are usually symptomless. Remarkably, joint involvement is confined to the shoulder and hip, despite the fact that the knee is the most common site of bends.

The initiating event is probably obliteration of blood supply by any of several possible mechanisms, e.g., embolism, venous stasis, or compression of vessels. If loss of osseous vascularity is sufficient to cause necrosis, the radiologic and histologic appearances of the reparative processes are similar regardless of the systemic condition known to be associated with nontraumatic necrosis in adults. The precise mechanism, however, which underlies osseous ischemia and results in infarction, remains obscure. Nevertheless, predisposing etiologic factors are strikingly manifest in the various systemic conditions associated with avascular bone necrosis. These factors may be grossly designated as the following agents with embolic potential: intravascular bubbles, frank fat globules or coalesced lipids, sickled erythrocytes, Gaucher's cells engorged with cerebrosides, platelet-RBC aggregates, and activation of fibrin clotting. In pancreatitis, minute pancreatic cell emboli may serve to release lipolytic enzymes destructive of marrow fat.

With reference to alcoholism, hypercortisonism, and possibly other conditions, Jones and Sakovich (12) investigated the hypothesis that intravenous fat embolism might be the initiating event in producing avascular necrosis. Infusions of the radiopaque substance, Lipiodol, in rabbits produced intraosseous fat emboli during a 5-week period following the infusion. After this interval, subchondral arterioles and capillaries of the femoral head were obstructed with resulting focal anemic infarctions in the metaphyseal and epiphyseal regions. Although bone marrow components had completely regenerated by 17 weeks, focal osteocytic death persisted. These results suggest that, although one episode of fat embolization may not result in gross or radiologic evidence of necrosis, multiple bombardments of fat emboli over periods of several months or years may produce irreversible vascular necrosis (11). This conclusion may well apply to all agents with embolic potential and associated complications.

Medical Monitoring of Compressed Air Workers

Tunnel workers may be divided into miners who engage in manual labor during shifts of specified duration, supervisory personnel, and those with special skills who are usually non-

TABLE I

PREDOMINANCE OF LESIONS IN THE FEMORAL HEAD IN ALCOHOLISM AND HYPERCORTISONISM IN CONTRAST WITH PREDOMINANT LESIONS OF DIVERS AND CAISSON WORKERS IN THE HUMERAL HEAD

Author	Condition or Activity (Number of Patients, Individuals)	Bone Lesions							
		Humeral Head		Femoral Head		Distal Femur		Proximal Tibia	
		R	L	R	L	R	L	R	L
Jones (11)	Alcoholism (N = 30)	4	5	21	22	6	4	6	6
	Hypercortisonism (N = 32)	5	6	25	20	3	3	—	—
Alnor[a]	Divers (N = 72)	58	57	10	8	20	18	2	2
Ohta[a]	Divers (N = 152)	66	60	39	37	50	50	9	
Royal Navy[a]	Divers (N = 13)	4	2	3	1	4	6	4	5
Walder et al. (17)	Compressed-air workers (N = 29)	17	17	7	5	9	13	5	2

[a]From Elliott and Harrison (8).

shift employees. The examination criterion for miners is fitness to engage in strenuous labor in compressed air; there was no difficulty in enforcing stringent criteria with reference to age, weight, and freedom from pulmonary pathology, designed to protect them against decompression sickness and aeroembolism. The difficult problem is disposition of supervisors over the age of 40 years. The demand for the services of these men, who are highly conversant with pressurized tunnel construction, is insistent. However, many of these men harbor the usual impediments of an age group long subjected to job and recreational stress. If their sojourn in compressed air was extensive, there is great likelihood of necrosis in the medullary shafts of long bones as well as juxta-articular involvement of humeral and femoral heads. Furthermore, a condition of fat-enlarged liver as a complication of alcoholism may be the source of embolic lipid extrusion to bone and other organs. Obstructive pulmonary disease, if subclinical, may be undetected and conducive to aeroembolism. The predominant impediments militating against work in compressed air are those associated with age and obesity. An outline of the comprehensive medical examination for BART tunnel workers has been reported (4). Pertinent to the prevention of avascular bone necrosis are disqualifications for asymptomatic bone necrosis as a result of radiologic survey, for metabolic disorders (including enlarged liver and impaired liver function), sickle cell hemoglobinopathy, and for cardiovascular disorders (5).

In monitoring the decompression procedure and specifically patients afflicted with decompression sickness, surveillance was limited chiefly to physiologic parameters evaluated in the pre-employment examination (4). These included highly useful equilibrium tests (from the Pensacola test battery), tests of pulmonary function and of cardiovascular fitness. The response of the 135 cases of decompression sickness (seven of which were type II) to oxygen recompression alone was so favorable that only an occasional blood examination was made of Hct, RBC and WBC counts.

It was an omission not to have examined systematically prothrombin time, platelet-lipid levels, and the secondary effects of bubbles which derange formed elements and lead to intravascular stasis and coagulation (7, 19). Biochemical assay of 5-hydroxytryptamine (serotonin), which is amenable to highly quantitative analysis, may well have shed some light on pulmonary symptomatology and the remarkable malaise which has been ascribed to the presence of "silent" bubbles.

Relation of Osseous Necrosis and Decompression Sickness to Decompression Practice

The Decompression Sickness Panel of the Medical Research Council (G.B.) reported that bone lesions are related directly to the number of times a man has been decompressed, to the height of the pressure at which he has worked, and to the number of attacks of bends for which treatment was given (16). The basic problem, however, transcends prevention of decompression sickness (a prime objective) to formulation of decompression schedules which circumvent intravascular bubble formation. Thus, nascent bubbles in the circulation, whether "silent" or clinically active, are clearly identified with such secondary phenomena, common to "foreign" material, as platelet aggregation, cell clumping, lipid coalescence, settling of blood masses, hemoconcentration, and circulatory stasis (19).

In the BART compressed-air operations, decompression was in accord with Washington State tables (1963) which provide extended-stage decompression for single daily work shifts (25).

The experience with these tables (adopted by California, New York, and Wisconsin) was that crippling bone disability had not been reported as of November, 1972, following trials in the Lake City Tunnel (Seattle, Washington, 1964-1967) and in the BART project (1967-1969). The incidence of decompression sickness, however, was about the same as that in previous compressed-air operations with greatly reduced decompression schedules.

Two proposals directed to prevention of intravascular bubble evolution, decompression sickness, and bone necrosis will be outlined: 1) oxygen inhalation during decompression to provide inert gas transport from tissues under isobaric conditions; and 2) periodic sojourn in a compressed air habitat similar in principle to that employed in saturation diving operations. Prior to presentation of specific regimens implementing these projections, it is appropriate to examine earlier decompression experience which has been firmly linked with avascular necrosis of bone.

New York State Decompression Practice

Prior to 1912. In 1909, F. L. Keays, Medical Director for the contractor in charge of construction of the East River Tunnels for the Pennsylvania Railroad, reported on 3692 cases of decompression sickness emanating from 557,000 man-decompressions. There were 20 deaths. At gage pressures of 32 p.s.i., men worked 8 out of 24 hours, taking one-half hour for lunch either at the working or at slightly reduced pressure. At pressures higher than 32 p.s.i., the workshifts were two 3-hour exposures with a 3-hour rest interval. The working pressure never exceeded 42 p.s.i. Keays preferred a 6-hour continuous shift to two 3-hour shifts "as it exposes the man to the risks of only one decompression instead of two" (13).

Some remarkable feats of work in compressed air were accomplished at the time. In 23,000 decompressions from working levels of 40 to 42 p.s.i., there were no serious or fatal cases. For 36 days, 330 men were employed in a two-shift daily schedule that called for 3 hours on shift with a 3-hour rest interval between shifts *at normal pressure.* The total decompression time for each shift was 48 minutes, as follows: 1) pressure was lowered from 40 to 29 p.s.i. in 5 minutes, and the men then walked 1000 feet during the course of 10 minutes to a second lock; 2) pressure was lowered from 29 to 12.5 p.s.i. in 8 minutes, and the men spent another 10 minutes in walking to a third lock; 3) pressure was lowered from 12.5 p.s.i. to 0 p.s.i. in 15 minutes. These details emphasize that considerable time was spent at relatively high pressure levels during the course of decompression, and that the workers engaged in light exercise (walking). Reporting on 8510 of these decompressions, Keays recorded 1.6% minor cases (presumably type I, decompression sickness). Since only seasoned men were employed, the phenomenon of acclimatization or acquired resistance to decompression sickness, subsequently confirmed by systematic data of British medical authority, is remarkable.

One of the paradoxes of decompression practice observed over the years is that decrease in incidence of bends (type I, decompression sickness) is not commensurate with augmented decompression time beyond a minimal requirement. The workshift of 3 hours at 40 p.s.i. followed by 48 minutes decompression, would entail 98 minutes (British tables) (17), 162 minutes (U.S. Navy stipulation for exceptional exposure) (24) and 183 minutes (Washington State tables) (25). A pertinent consideration is the probability of extensive bubble evolution during the initial (first stage) drop in pressure to one-half of the absolute or gage pressure level. If such

a condition supervenes, the pressure head implementing gas transport would be greatly reduced, and the benefit of moderate increase in decompression time would not be apparent. It is possible that 48 minutes decompression time (13) at higher pressure levels is as effective as the two- or threefold increase in decompression time at lower levels, following the large initial abrupt decrements. A quantitative approach to this problem in the U.S. Navy was interrupted at the time of World War II. Independently, Hills (9) has analyzed this problem.

Practice since 1912. Progressively, hours of work were decreased and the interval between shifts lengthened. The 1922 revised New York State table (26) seemed liberal compared with earlier stringent stipulations (Table II). Nevertheless, despite curtailed hours of work and somewhat longer intervals in open air, serious disability occurred. Of 300 cases of decompression sickness reported by Thorne (23) in connection with the Queens Midtown Tunnel (1938), 25 were type II cases with paralytic nervous system involvement, 15 were cases with cardiopulmonary (chokes) symptoms, and 30 were type II cases with vertigo (the "staggers").

Bone Necrosis Relative to the 1922 Tables

In 1942, Bell, Edson and Hornick (6) reported a radiologic survey of 32 compressed-air workers in New York, none of whom had symptoms or gross signs indicative of bone lesions. The men had worked from 3 to 33 years intermittently in compressed air. The shortest continuous employment was 10 months, the longest, 36 months. Fourteen men gave a history of decompression sickness, but 18 men stated that they had not had attacks of bends. Only 8 of the 32 workers were free from radiologic evidence of bone lesions. The puzzling aspect of avascular bone necrosis was recognized at the time: namely, individual variation in response to similar conditions of decompression and employment. Taylor (22) considered that individuals with an inadequate circulatory tree who work under increased pressures may develop bone and joint lesions with the ordinary so-called adequate decompression; even in the absence of an acute attack of caisson disease, agglutination of red cells may play a part.

In October 1963, tunnel workers made 63 claims for disability based on radiologic evidence of bone lesions. Paradoxically, these claims were subsequent to the Lincoln Tunnel (third tube) operations in 1955-57, conducted in accord with "enlightened" tables which stipulated minimal hours of work and maximal decompression, in accord with U.S. Navy practice (27). Excerpts from these economically prohibitive tables (formulated by Naval medical officers) are shown in Table III. Although Kooperstein and Schuman (14) reported only 44

TABLE II

The 1922 Revised New York State Diving Table [abstracted from (26)]

P.s.i.	1st Shift (hrs)	Interval (hrs)	2nd Shift (hrs)	Shift Decompression[a] (min)
To 18	4.0	0.5	4.0	5
Over 18 to 26	3.0	1.0	3.0	12
Over 26 to 33	2.0	2.0	2.0	21
Over 33 to 38	1.5	3.0	1.5	23
Over 38 to 43	1.0	4.0	1.0	27

[a] Decompression time for maximal pressure in each category (26).

TABLE III

THE 1965 REVISED NEW YORK STATE DIVING TABLE [ABSTRACTED FROM (27)]

Pressure (p.s.i.)	1st Shift (hrs)	Interval (hrs)	2nd Shift (hrs)	Decompression[a] (min)	
				1st Shift	2nd Shift
Over 22 to 30	2.0	3.5	2.0	22	37
Over 30 to 35	1.5	4.0	1.5	25	42
Over 35 to 40	1.0	4.5	1.0	29	49

[a] Decompression for maximal pressure in each category (27).

cases of decompression sickness out of 138,034 decompressions (3.18 cases/10,000) in the Lincoln Tunnel operation, it cannot be stated with assurance that there were no bone lesions complicating this specific operation. Pre-employment radiologic survey was interdicted by union ruling. It is highly probable that the 63 processed disability claims were referrable to lesions incurred as a result of inadequacies of the earlier 1922 tables.

BRITISH DECOMPRESSION EXPERIENCE

In the construction of the Tyne Road Tunnel, 641 men worked in compressed air over a period of 31 months. The maximum pressure was 42 p.s.i. and the overall rate for decompression sickness (at pressures of 18 p.s.i. and higher), was 2 percent. Decompression data extracted from the Report of the Decompression Sickness Panel (Medical Research Council) affirm the high percentage of decompression sickness with increased exposures over 4 hours and at higher pressures (Table IV). The modified statutory decompression table applied to this tunnel project inexplicably does not provide for increased decompression time when work shifts exceed 4 hours (17). The rigorous British work schedule is comparable to New York practice in the early part of the century. In an earlier report, Paton and Walder (18) reported that increase in decompression time, stipulated in the statutory tables, did not diminish decompression sickness. A possible explanation of this circumstance is the lack of sensitivity of any decompression schedule, which is in effect a "treatment" table applicable to control of bubble size rather than to elimination of inert gas held in supersaturation.

During construction of the Tyne Tunnel, radiologic examinations were made on 171 men with the finding of bone necrosis in 44 men (26% of examinees). Although most of the men were symptomless 3 years after termination of compressed-air exposure, four men were partially disabled and refractory to surgical intervention (17).

Table IV shows that work shifts of less than 4 hours were accompanied by minimal complications. When the shifts were 8 hours in duration or longer, the incidence of type I decompression sickness (bends) increased fivefold; in addition, there were 16 cases of type II disability.

Total decompression times for the usual range of tunnel pressures relative to shift duration are given in Table V, with the much greater decompression time stipulated in the Washington State tables shown in parentheses. In the British tables, the absolute pressure is halved during rapid descent to the first stop. Doppler ultrasonic technique has substantiated an earlier hypothesis that bubbles form in the circulation during this initial stage of decompression. The conclusion of British authority is that currently enforced decompression

TABLE IV

INCIDENCE OF DECOMPRESSION SICKNESS RELATIVE TO TUNNEL PRESSURE AND SHIFT DURATION, TYNE ROAD TUNNEL[a]

Pressure (p.s.i.)	Shift Duration			
	Under 4 hrs	4 to 6 hrs	6 to 8 hrs	8 hrs and over
14–19				
Number of man-decompressions	7625	1504	2332	8698
Decompression sickness, type I (bends)/1000 man-decompressions	0.53	0	5.58	5.98
20–29				
Number of man-decompressions	5262	1073	1617	9367
Decompression sickness, type I (bends)/1000 man-decompressions	4.6	27.0	33.4	20.8
30–36				
Number of man-decompressions	1547	359	404	2977
Decompression sickness, type I (bends)/1000 man-decompressions	8.5	61.3	40.4	53.4
37–41				
Number of man-decompressions	495	105	38	564
Decompression sickness, type I (bends)/1000 man-decompressions	18.2	—	—	67.4
TOTAL				
Number of man-decompressions	14,929	3041	4391	25,606
Decompression sickness, type I (bends)/1000 man-decompressions	3.35	23.4	19.1	17.1
Decompression sickness, type II	1	3	3	16

[a]Abstracted from Walder et al. (17).

procedure in civil engineering practice does not prevent avascular necrosis of bone in compressed air-workers (16).

WASHINGTON STATE TABLES, 1963

In 1961, Dr. J. Leon Sealey (20), Medical Consultant to the Municipality of Seattle and Metropolitan Engineers, organized a committee to revise decompression tables for compressed-air workers. Objectives were guided by two concepts: 1) a single daily work shift

TABLE V

DECOMPRESSION TIME (MIN) RELATIVE TO SHIFT DURATION AND PRESSURE, TYNE ROAD TUNNEL[a]

Pressure (p.s.i.)	Shift Duration (hrs) 1 to 2	2 to 3	3 to 4	over 4[c]	(6[b])
18[b]–20	8 (8)[b]	10 (11)	12 (17)	17.5	(63)
24[b]–26	17 (27)	27 (52)	37 (92)	51.0	(122)
28[b]–30	30 (41)	51 (98)	58 (127)	70.0	(153)
34[b]–36	52 (98)	72 (151)	82 (178)	98.5	(218)
38[b]–40	62 (128)	86 (178)	98 (203)	117.5	(238)

[a]Abstracted from Walder et al. (17).

[b]The numbers in parentheses are the decompression times from the Washington State tables for the underlined pressure.

[c]In the U.K. Statutory Code, there is no increase in decompression time for exposures over 4 hours.

with stage decompression limited to a ΔP of 16 p.s.i. (assumed pressure head for gas transport) relative to tissues with half-times of 30, 60 and 120 minutes; and 2) a potential 8-hour exposure in compressed air apportioned between work at tunnel pressure and tabular decompression time. A 6-hour shift, for example, at 22 p.s.i. requires 103 minutes decompression; total time under pressure is 7 hours and 43 minutes. A 5-hour shift at 32 p.s.i. is accorded 178 minutes decompression; total time under pressure is 7 hours and 58 minutes. The extended time stipulated for decompression in the usual range of tunnel pressures (0–35 p.s.i.) is more than twice the time allocated for the same work shifts in England and even exceeds decompression time for exceptional air exposures in the U.S. Navy diving tables.

INCIDENCE OF DECOMPRESSION SICKNESS RELATIVE TO EXTENDED DECOMPRESSION TIME

The above-outlined innovations in decompression practice have been in force throughout the Seattle project (1964-67), the BART project (1967-69), and recently in Milwaukee (1971-72). Unexpectedly, the incidence of decompression sickness was not diminished (from previous 1–2% of man-decompressions) by the greatly extended decompression time, much of which was expended during the last stage from 4 to 0 p.s.i. The exceptionally high incidence of decompression sickness in the BART operation at 29.5 and 35.5 p.s.i., relative to

the modest duration of the work shifts (in contrast with British practice), may be due in part to lack of acclimatization of workers involved.

The response of the 345 patients with decompression sickness (Seattle-BART projects) to "low-pressure" oxygen therapy (in accord with U.S. Navy treatment Tables 5 and 6) was highly favorable; there were no residual complications. Moreover, in the BART project the average interval between the onset of symptoms and recompression therapy (5) was about 5 hours. In a congested metropolitan area in which some of the decompression sickness were "pool" drivers, it may be surmised that the extended decompression time ameliorated symptomatology.

Absence of Disabling Bone Lesions

The major benefit from the single shift, prolonged decompression schedule, is absence of disabling juxta-articular necrosis. Limited opportunity for radiologic follow-up precludes a conclusive statement as to ultimate benefits, but the reduced number of work shifts above 26 p.s.i. (as well as the relatively short duration of 3–4 hours) does not predispose to osseous complications.

Inherent and Extraneous Impediments to Decompression

In the Washington State tables, the ΔP of 16 p.s.i. (between work pressure and the first stage of decompression) is effective at the end of 3 minutes. At 30 p.s.i. (tunnel pressure) following a work shift of 6 hours, the rapid decrease in pressure to 14 p.s.i. is substantially below the calculated P_{N_2} (approximately 18 p.s.i.) in a 120-minute half-time tissue. The pressure is then reduced from 14 to 4 p.s.i. in 35 minutes, and from 4 to 0 p.s.i. in 130 minutes. The substantial part of decompression, therefore, is expended under conditions of diminishing potential for N_2 transport, i.e., a decreasing size of the O_2 window. On the assumption (verified by Doppler ultrasonic technique applied to U.S. Navy tables) that bubbles have formed during the initial stage of decompression, the Washington State schedule is, in effect, a "treatment regimen." A systematic test procedure is required to provide for maintenance of N_2 transport under isobaric conditions.

Two undesirable physical effects of rapid drop in pressure are chilling and fog formation in the chamber lock. Workers—who previously may have been hot and sweating from exposure to high-temperature, automated machinery—are subjected to chill. The consequent peripheral vasoconstriction serves to impair blood flow and favor bubble evolution in subcutaneous vessels.

Various physiologic factors impair gas transport: dehydration (in the hot atmosphere at the face of the tunnel), disruption of circadian rhythms due to periodic rotation of shifts, and deficient blood perfusion of tissues attending alcoholism, nascent bubbles, cell clumping, increased blood viscosity and stasis. During the course of long decompressions, the relative immobility of workers, engaged in playing cards, obviously restricts circulation in the lower extremities.

Oxygen in Decompression and Recompression Practice

Isobaric (Oxygen Window) Principle (2)

In the 1930s at the U.S. Navy Experimental Diving Unit, credence was accorded the concept that a transfer of O_2 from blood to tissues renders an equivalent amount of inert gas

available for transport from tissues. At normal pressure during the inhalation of air, arterial P_{O_2} (100 mm Hg) falls to an average level of about 40 mm Hg in passage through capillaries. The small O_2 window (60 mm Hg) can be greatly enlarged by inhalation of oxygen at increased pressure. Thus, if O_2 pressure is raised to 2 ata (15 p.s.i. gage), then an arterial P_{O_2} of some 1500 mm Hg will fall to 500 mm Hg or less in the capillary bed. Individual tissue variation, of course, is large. The prime merit of the "oxygen window" principle is that during the course of decompression from hyperbaric atmospheres, inert gas can be transported from tissues via a "window" at a pressure isobaric with ambient pressure.

Applications of Oxygen Inhalation

Oxygen has not been routinely employed for decompression of tunnel workers, chiefly because a regimen has not been developed to control the fire hazard. In 1939, divers who participated in the *U.S.S. Squalus* salvage operations were brought rapidly to the surface and then recompressed on oxygen at pressures between 26.7 p.s.i. (60 feet) and 17.8 p.s.i. (40 feet). Since then, oxygen decompression of divers and the treatment of decompression sickness with oxygen (interspersed with intervals on air to augment O_2 tolerance) is routine in the U.S. Navy.

Sealey (21) found that minimal recompression, hyperbaric oxygen treatment of decompression sickness (in accord with U.S. Navy treatment Tables 5 and 6) was convincingly effective in his management of 210 cases (Lake City Tunnel, Seattle). The favorable experience with oxygen treatment of 135 cases in the BART project—despite the long interval between onset of symptoms and recompression—has been referred to. In no case was adjuvant therapy required. With few exceptions, treated patients (90 to 220 minutes of O_2 inhalation) resumed their next regular work shift (5). The application of U.S. Navy diving routine is a simplification and augmentation of O_2 administration of an earlier era. Essentially, during the course of treatment, two O_2 pressure levels were utilized, one at 25–27 p.s.i. the other at 15 p.s.i.; at the termination of O_2 inhalation at this level, pressure was lowered to 0 p.s.i. within a period of 5 minutes.

Oxygen Decompression of Tunnel Workers

Oxygen inhalation during decompression of tunnel workers would not only reduce decompression time (Washington State tables) conservatively by a factor of two, but this measure holds the firmest promise (since excess N_2 is eliminated from tissues under isobaric conditions) for prevention of decompression sickness and bone necrosis. In addition, longer daily work shifts are safe.

Table VI incorporates values for isobaric decompressions utilizing O_2 inhalation for the following work shifts relative to gage pressure: 6 hours at 20, 25, 30, 35 p.s.i. and 4 hours at 40 p.s.i. The calculations are referrable to a 120-minute half-time tissue, presumably adequate for bone marrow as well as lipid in adipose tissue of a lean man (i.e., 10% body fat in adipose tissue). The values in the column "N_2 to lose" represent the difference in tissue pressure (absolute) at the beginning of decompression and the allowable pressure of 18 p.s.i.a. at the end of decompression. Permissible excess N_2 at the termination of decompression is about 6 p.s.i. above normal pressure.

In Table VI, the size of the O_2 window must be equal to total P_{N_2} to assure isobaric transport of nitrogen. At 35 p.s.i.g., for example, decompression time is allocated to stages at 25, 20 and 15 p.s.i. for 20′ (x), 40′ (2 x) and 60′ (3 x), respectively; the average size of

TABLE VI

PROTOTYPE OXYGEN DECOMPRESSION TABLE FOR TUNNEL WORKERS

Pressure (p.s.i.)	Work Shift (hrs)	N_2[a] (p.s.i.a.)	N_2 to Lose (p.s.i.)	Calculated Decompression Time[b] (min)	
				Total	Per Hr Work[c]
20	6	26.0	8.0	64	11
25	6	29.5	11.5	86	15
30	6	33.0	15.0	105	18
35	6	35.7	17.7	120	20
40	4	36.0	18.0	120	30

[a] In a 120-minute half-time tissue, e.g., white bone marrow.
[b] Calculated from *N_2 to lose/N_2* for a 120-min half-time tissue.
[c] For any fraction of a work shift stipulated in column 2.

this "O_2 window" is 20 p.s.i. (30 p.s.i.a.) which is approximately the value of P_{N_2} at the start of decompression. The tabular data is conservative and permits total O_2 decompression time to be fractionated on the basis of per hour work shift.

In tests of oxygen decompression on a "worst possible" case (overage, overweight, elevated blood sugar), the following schedule (Table VII) was well-tolerated over a period of several months, despite adverse conditions (voluntarily imposed) of an unventilated, high humidity chamber for "work shifts" up to 6 hours followed by O_2 inhalation. Oxygen was inhaled continuously at pressures no higher than 30 p.s.i., for short periods, following the 40 p.s.i. exposures, and no lower than 15 p.s.i. Admittedly, if decompression is isobaric, the pressure level for O_2 inhalation could be reduced commensurate with N_2 transport from the 120-minute tissue. That is to say, O_2 decompression under isobaric conditions of N_2 transport can be treated in a manner differently from O_2 recompression and elimination of N_2 from bubbles. The O_2 apparatus consisted of a closed (CO_2-absorbent) system, periodically rinsed from a "demand" regulator, which (with a comfortable mask or mouthpiece over the mouth and not in contact with gingival tissue) afforded inhalation of humidified O_2, interposed with some desirable resistance to expiration. The continuous O_2 breathing against some expiratory resistance and punctuated by periodic deep breaths was well-

TABLE VII

OXYGEN DECOMPRESSION OF TUNNEL WORKERS (MIN/HR OF WORK) FOR PRESSURE LEVELS OF 20, 30, AND 40 P.S.I.

Pressure (p.s.i.)	Duration (hrs)	O_2 Decompression (min/hr of work)
20	1-6	10
30	1-6	20
40	1-4	30

tolerated for 2-hour periods. At the termination of O_2 inhalation at 15 p.s.i., the subject was decompressed on air to normal pressure over a period of 5 minutes.

Oxygen inhalation at 15 p.s.i. (2 ata) is compatible with vigorous exercise (1). Some activity to improve circulation appears desirable during decompression at this level. Old tests, however, demonstrated that O_2 inhalation was unfavorable for even low-grade exercise at 3 ata. The current problem centers on tolerance for daily O_2 inhalation at therapeutic levels. Tests by Jacobs et al. (10), which revealed unexpected benefits in elderly persons afflicted with senile impairments, demonstrated that O_2 was well-tolerated for 90-minute periods (twice daily with a 12-hour interval) at 2.5 ata. There were 150–435 repetitive exposures 7 days a week, with only an occasional break. A prime need is systematic daily exposure of healthy men to the O_2 regimen following work shifts of 6 hours duration (Table VI).

TABLE VIII

PROJECTED EXCURSION WORK SHIFTS OF 6 TO 2 HOURS' DURATION AT PRESSURES OF 25 TO 40 P.S.I. FOR WORKERS LIVING IN A HOLDING FACILITY PRESSURIZED TO 15 P.S.I.

Pressure in Holding Facility (p.s.i.)	Work Shift (p.s.i.)	Work Time (hrs)
15	25	6
15	30	4
15	35	3
15	40	2

Periodic Sojourn in Compressed Air

It has been shown repeatedly that habitation in dry chambers and undersea habitats is feasible for many weeks in air atmospheres at depths to 50 feet (22.2 p.s.i.). Larsen and Mazzone (15) have reported "no-decompression" excursions to simulated depths of 100 feet (44.5 p.s.i.) for a sojourn of 4 hours from a saturation (storage) pressure of 15.6 p.s.i. (35 feet).

The schedule applicable to tunnel workers remains to be developed in detail and established by a test program. Table VIII indicates the scope of this innovation for "no-decompression" work shifts. At the higher pressures, men could work two shifts daily with appropriate rest intervals supplemented by O_2 inhalation. If the pressure in the habitat were raised to an upper level of 22.2 p.s.i. (50 feet), work could be carried out at tunnel pressures up to 32 p.s.i. for indefinite periods under conditions of constant isobaric N_2 pressure in tissues. At higher pressures, O_2 could be inhaled for short periods at habitat pressure level.

Proposal of a pressurized habitat for tunnel workmen usually encounters resistance. If, however, as much as 1000 feet of pressurized tubing is available for daily work, it should be no problem to provide accommodations for sleep and rest.

Summary

Osseous avascular necrosis is a major hazard of compressed-air (caisson) workers employed in civil engineering projects. The bone lesions have been associated with inade-

quate decompression and with liberation of intravascular bubbles. Important also are "foreign" body phenomena induced by the presence of nascent bubbles which give rise to platelet aggregation, cellular clumping, release of lipids with embolic potential, hemoconcentration, and blood stasis. Predisposing factors reported by British authority are the number of times a worker has been decompressed, the pressure level at which he has worked, and the number of attacks of decompression sickness he has sustained.

During the past 50 years steps have been taken in New York to shorten hours of work in the effort to reduce decompression sickness and the chronic complication of bone necrosis. In both New York and England, it has not been possible to circumvent these hazards.

Tables, compiled by the State of Washington, greatly extend decompression time. Experience with these tables (Seattle, 1964-67; San Francisco, 1967-69) has not shown a reduction of decompression sickness. Yet to date (1972) there has been no report of disabling bone necrosis.

In order to eliminate future injury, two positive procedures have been outlined: 1) oxygen decompression with isobaric transport of N_2 from tissues; and 2) residence in compressed air habitats with pressure adjusted to tunnel work level. These procedures are routine in diving practice and await implementation in compressed air tunnel operations.

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PART III. PATHOPHYSIOLOGY OF BONE*

DISCUSSION

D. N. Walder, Chairman

Dr. Figarola: I would like to ask Dr. Behnke and also the panel about use of hyperbaric oxygen to increase the safe duration of the surface interval after air diving. I ask this question because we have divers who work at 40 feet of depth, and we have had four cases of bends lately, even though the exposures are within the no-decompression limits. The divers work around five hours, but they exert a lot of physical effort.

We are considering putting these men—during the two hours' interval between dives—at 30 feet of depth, breathing oxygen all the way down, after they have surfaced first; we would keep them there for 30 minutes and follow this with a 30-minute ascent to the surface, still breathing oxygen. Would this be an intelligent thing to do?

Dr. Behnke: That is an intelligent thing to do. Perhaps a more appropriate thing to do would be to keep them at 30 feet. There is no reason for them to come back to atmospheric pressure. Let them eliminate the inert gas at 2 atmospheres pressure rather than at 1 atmosphere.

Dr. Figarola: We have to surface them from the water.

Dr. Behnke: Then keep them in the water—that is to say, to 30 feet of depth—until decompression is complete, and then bring them out for 5 minutes.

Dr. Idicula: Dr. Smith, I would like to raise a question about the reality of the vacuoles which you saw in the kidney.

Dr. Smith: About the vacuoles, we have been very careful in our sectioning techniques, using different stains, to try to show that these were not bubbles and to try to get them in our normal control samples. We have been unable to show that they are not bubbles by a variety of techniques.

Now let me go one step further and say that these gas vacuoles that are in the body are most likely surrounded with a liberal protein membrane because they are a foreign surface, and so whether the gas is there or not you still have the protein structure that helps keep the gas in a single position. I do not say definitely that those are bubbles.

Dr. Philip: I think I would support Dr. Smith that those are bubbles since the picture is almost totally interchangeable with one which we published on a similar situation in lungs of rabbits infused intravenously with air. I am delighted that he agrees that bubbles and platelets may interact and we have photomicrographs of what we think are probably the first electron micrographs of intravascular bubbles fixed in situ in decompressed experimental animals. The magnification is of up to 350 thousand. What you see is a layer of material surrounding the gas interface, which is approximately 200 angstroms in thickness and which we have reason to believe is fibrinogen, and lying on that are numerous platelets undergoing various stages of degranulation and release and aggregation. I am quite convinced that what is seen encapsulating these vacuoles is a mass of platelets and fibrinogen and possibly lipids as well.

Dr. Bonfiglio: Similar observations have been made for fat globules in which platelets and fibrinogen produce a thrombus. This is in relation to the increased thrombosis in patients with hyperlipidemia.

I think this is a good example of how many diverse theories and etiologies have been proposed. I think we may find a common thread connecting multiple factors involved—and that has to do with an interruption to the blood supply by a variety of means.

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* *Panelists:* G. M. Adams, M. Bonfiglio, D. B. Coltman, P. T. Cox, J. K. Davidson, C. J. Fagan, K. E. Schaefer, K. Smith.

Dr. Holland: Dr. Bonfiglio, how well established is alcoholism as a factor in the etiology of bone necrosis? Can you elaborate on any association of alcoholism with exposures to pressure?

Dr. Bonfiglio: In a recent study of 50 patients, we had a high percentage of these people with alcohol intake. The percentage was well over 50%. In these patients with high alcohol intake there were a number of associated problems. Hyperuricemia is one of them. Hyperuricemia has been reported to increase platelet adhesiveness. Patients with high alcohol intake do have sludging of blood. They develop an initial thrombocytopenia and then have a rebound thrombocytosis, either end of which in this spectrum of the bleeding-clotting mechanism could affect the nutrition to anatomic sites at risk. And indeed in reports other than our own—except for the one patient that I showed with a necrosis in the shaft of the femur—other sites are involved in the systemic nontraumatic diseases.

Dr. Walder: Dr. Bonfiglio, could you please define what you mean by a high alcohol intake?

Dr. Bonfiglio: We have considered 6 ounces of 80-100 proof spirits at any one time as being sufficient to produce sludging of blood.

Dr. Walder: But there are large numbers of workmen who take alcohol. In fact, Dr. Behnke has told us today of his 2,000 men doing compressed air work, men that we subsequently call compressed air workers—amongst 2,000 of them only those who had previous experience with compressed air in fact showed bone lesions. And we have our control series in Great Britain of men taken on to do compressed air work (150 of them) amongst whom none showed any bone lesions. I think the alcohol story has yet to be substantiated.

Dr. Bonfiglio: I agree. One does not know in patients who are known alcoholics what the incidence of necrosis is. Such epidemiologic studies are needed and are underway. We are doing one currently.

Dr. Elliott: I wonder what evidence Dr. Smith has for extending the evidence of platelet aggregation to include other sites, like the central nervous system? Has study of effects in the CNS been done?

Dr. Smith: Yes, we have done many soft tissues, including the central nervous system—not including the spinal cord, but a cerebellar area and the cerebral area. We have found beautiful areas of infarction in several of our pigs in the cerebral hemispheres as a result of these decompressions.

Dr. Osterella: Dr. Adams, in your study you suggested that the loss of bone density in the experimental rats was due to a loss of calcium, although I do not believe you were able to show an increased calcium level in the blood 1 hour after the rats were exposed to decompression. I wonder if there are other possible explanations for this loss of density besides removal of calcium from the bone?

Dr. Adams: We have looked through a number of possibilities other than calcium loss to explain a loss of density and have not been able to come up with anything other than a slight mineral loss. This can be equated to what has happened in astronauts, for example, where osteoporosis develops.

Dr. Querens: In New Orleans we have encountered quite a few nontoxic or noninfectious hip conditions, some which have had too much cortisone and some cases of sickle cell disease. Does sickle cell disease cause necrosis of the hip?

Dr. Walder: In the United Kingdom I have been involved in many legal cases with men who have bone necrosis and therefore I am in the habit of going very carefully into their background to make sure there is no excessive alcohol, no cortisone, no sickle cell anemia, and all the rest of the possible differential diagnoses. I think, as a general statement, we could say that these cases seem to be the result of the exposure to compressed air rather than anything else.

Part IV. DYSBARISM

PATHOPHYSIOLOGY OF COMPRESSION AND DECOMPRESSION*

H. V. Hempleman

Whenever men are subjected to breathing gases at pressure, there are three physical variables involved (pressure, time and temperature) and one chemical variable (gas composition). In addition to these physico-chemical variables and their interaction with the biological system, there are secondary factors, such as immersion—partial or complete—and psychological reactions.

Given this extremely large number of variables, it is not surprising to find that the literature abounds with uncertainties. The assessment of the present position in the pathophysiology of compression is rendered difficult by the fact that the evidence obtained has been analysed differently by the various investigators. For example, with the hand tremor which can occur following compression on oxyhelium, one investigator suggests the use of a detailed analysis to identify individual divers (2), whereas other investigators may give merely qualitative impressions of hand tremor. Thus, a reviewer must use his own judgment when comparing one set of results with another, and only rather gross statements are possible at present.

For several years experiments in the buoyant ascent escape technique have progressed using both animals and men (21). This technique involves rapid compression of experimental subjects to depths as great as 180 meters (600 feet) in times the order of 20 seconds, and it is clear from this work that such rapid compression does not of itself subsequently produce any untoward effects in healthy young males, except perhaps an occasional burst eardrum, which fortunately seems to heal readily. Equally clearly, it seems well-established, both from commercial work and from laboratory-type experiments, that compression to depths of the order of 125 meters (400 feet) can be accomplished using oxyhelium breathing gas, again without any detectable ill-effects, except perhaps some pain in the joints on movement. If, however, men are compressed at 30 meters (100 feet) per minute, to a depth of 246 meters (800 feet), there are very noticeable gross physiological reactions in most men—an effect which had been noted at this laboratory for some years. If men are compressed to 366 meters (1189 feet) in a 2-hour period, there is apparently less hand trembling than is seen in the much more rapid compression to 266 meters (800 feet) noted previously. Nevertheless, it is clear that the extra pressure has placed an

*Several excellent publications relating to problems of the pathophysiology of compression and decompression are available (7, 8, 24, 40) and these will form the background source for the present review.

additional and quite severe burden on the body. Such experiments lead to marked increases in theta activity in the EEG and periods of somnolence and wakefulness, with disorientation and difficulty in reading instruments (10). If 1 hour is taken to reach 300 meters (1000 feet), there appear to be no changes of note in the EEG, no periods of alternating somnolence and wakefulness and only slight hand trembling.

From these observations and recent small animal studies (29) it seems that two physical variables are of prime importance: rate of rise of pressure and pressure itself. The attempt by Chouteau et al. (16) to construct a hypothesis for predicting the onset of these signs and symptoms, often collectively referred to as the high pressure nervous syndrome (HPNS) is pertinent to this. The basic concept in the hypothesis is that the tissue responsible for HPNS cannot tolerate a pressure excess greater than 10 bar and has a response halftime of 2 hours. This concept would not seem to explain recently accumulated data. For example, it will be seen from experiments reported by Morrison (45) that, following a 1-day sojourn at a constant pressure of 1000 feet which would allow all tissues with half-times of less than 4 hours to be completely equilibrated, the subsequent change of pressure took the experimental subjects to 1300 feet, using quite conservative compression rates, and yet led to very obvious physiological responses. Thus, a 10 bar (300 feet) approximate change in pressure was, at this level of pressure, very effective in provoking marked physiological responses in the subjects, whereas only minor body reactions are noted on changing the pressure from atmospheric to even as great as 15 bar. It would seem then, for such a calculation to succeed, the ΔP value used must be made dependent upon the absolute pressure value and not, as at present, be independent of pressure.

An alternative hypothesis to explain the compression effects on men states that exposure to breathing mixtures containing helium or nitrogen can cause transient osmotic gradients in man during the period of uptake or output of these gases. Such gradients have been implicated in causing haemoconcentration, capillary stasis, impaired exchange of gases in joints, dry joints, aseptic bone necrosis and urticaria. However, there is experimental evidence showing that there is no haemoconcentration or haemodilution during inhalation of 0.7 atmospheres nitrous oxide (28). Furthermore, as is well-known from physicochemical theory, osmosis is a colligative property of matter and, therefore, the transient osmotic effect would be expected to provoke the same degree of physiological response for a given sudden change in breathing gas pressure, as was noted previously in the discussion of Chouteau's theory. In view of the fact that this does not seem to be so, and in view of the inability to demonstrate haemoconcentration, it would seem that gas-induced osmosis is perhaps of more secondary importance.

However, the work of Charmasson (13-15), who has demonstrated differences in the osmotic properties of solutions of macro-molecules (e.g. lysozymes) following pressure excursions, is of particular significance in connection with examining any theory of gas-induced osmosis. The effect of the pressure excursion is largely independent of the time of exposure at pressure, and there is a gradual return to the pre-exposure osmotic pressure value which occurs over a period of several hours, but there seems to be, nevertheless, a small residual change left after 3 days. The time scales involved in these physicochemical events and the relatively modest pressures necessary to effect them, from 30 to 150 bar, have obvious implications in the aetiology of the compression syndrome.

Little attention has been devoted to the considerably altered thermal responses of the body encountered during and following the compression phase. This problem was men-

tioned at the Fourth Underwater Physiology Symposium, and recent statements such as "subjects and attendants noticed sensation varying from being uncomfortably cool to uncomfortably warm. Nevertheless, there were no significant changes in either oral or rectal temperatures during the experimental exposures," (10) accord with the subjective statements given by some of our volunteers in deep helium dives. There is a need for proper quantitative work on this problem. In addition, there are numerous possibilities of secondary importance which could exert influence during compression: for example, continuous ear clearing—especially with a gas of markedly altered thermal properties—or compression of intestinal gases.

In summary then, the reversal of anaesthetic effects in newts by application of pressure, the demonstration that this pressure reversal effect can also be accomplished using mice, and the experiments with hydrostatic pressure on liquid-breathing animals, all confirm the view that the main precipitating factor when subjecting mammals to pressure is the pressure itself; the response to hydrostatic pressure, however, will be influenced by the nature of the gas (e.g. narcotic or nonnarcotic) and by the time course to reach a particular pressure level. For the helium molecule with apparently no narcotising effects, it appears that the time course used to reach pressure must be such that the rate of change of pressure decreases as the pressure increases. This allows time for adaptation to the effects of increased pressure, but there would seem to be a limit to this adaptation process which can only be extended by pharmacological means.

Pathophysiology of Decompression

A good deal of evidence has accumulated over the past few years (5, 6, 9, 12, 17, 18, 20, 48, 49) to show that manifestations of decompression sickness are markedly influenced by biochemical and biophysical processes, which are not apparently themselves directly concerned with the presence of gas emboli. An early and somewhat dramatic demonstration of this is the successful treatment of three cases of severe decompression sickness by medical management without resort to recompression procedure by Bühlmann et al. (11). Another case occurred, but this time a similar therapy was accompanied by heparin infusions. Once again resolution of a case of quite severe decompression sickness was accomplished without resort to recompression. Therefore it is possible to state with confidence that certain forms of acute decompression sickness can be successfully treated without recompression, but it would be a dangerous extrapolation to assume that all forms of decompression sickness are amenable to medical management of this sort. Except in rare instances, recompression is always successful in alleviating the signs and symptoms of acute decompression sickness, and in those instances where relief is not obtained or only partially obtained, it is most often traced to undue delay occurring before recompression is given. Thus it is possible to state that all manifestations of acute decompression sickness hitherto encountered are amenable to prompt recompression.

According to this fact, and bearing in mind the well-established observations that the quantity of gas absorbed by the tissues at pressure plays a significant part in determining the outcome of a decompression, it would seem that the most likely agent to blame for producing a pressure-sensitive disorder in the body is, of course, a bubble. Furthermore, there is good evidence (22, 26, 53) that bubbles can be detected ultrasonically in blood vessels and that these bubbles appear after only relatively mild provocation. To quote from

Evans and Walder: "using this technique we can see evidence of bubbles in the heart of a guinea pig which had received only a mild decompression, which would not normally be expected to lead to signs of decompression sickness" (22). Ultrasonic bubble detection work would lead one to suppose that gas is first released into the vascular system, and earlier work of Gersh and Catchpole (25) supports this view. It is worth noting, however, that Rubissow and Mackay (53) detected stationary bubbles around fatty tissues in guinea pigs' legs following decompression and found that guinea pigs could be safely decompressed by keeping the bubble size below a small threshold value. Nevertheless, despite some reservations, the evidence as noted by Behnke in his review is heavily in favour of intravascular bubbles as the initiating factor in decompression sickness.

In 1871, during the construction of the Illinois and St. Louis Bridge, the Medical Officer Jaminet (36) describes in some detail the onset and resolution of a serious post-decompression attack of paralysis to himself. The important point to note, common to many of these cases, is the time course of the condition. Jaminet notes that at "half past two o'clock pm, three quarters of an hour after leaving the chambers or caisson, the last effort brought me to my office, where, in a few minutes, I became paralysed." This condition persisted with paralysis of all limbs except the right arm until "at half past nine o'clock pm I commenced to move my legs a little as also my left arm." From then on he describes a continuing improvement in this condition. It is impossible to suppose that his condition would follow such a time course, with apparently almost complete recovery, if bubbles had formed within the nerve tissues, since this would almost certainly cause permanent tissue damage.

One must conclude therefore that the most likely explanation for such a clinical picture is a manifestation of partial occlusion of blood supply to some relevant part of the central nervous system. Again, more recently, and giving more observations on the pain of simple bends, Barnard (3) states that, although the pain is in the knee and muscular power is unaffected, sometimes the stretch reflex of the affected side is diminished as well as reflexes in the arm of the same side. These neurological signs are completely removed by recompression, and he concludes "this seems either to imply extremely slow damage, followed by more general reaction, or hypoxia of nerve cells, which is relieved by recompression." An objective examination of the literature from the time of Jaminet to the present suggests that the latter hypothesis is the correct one, but that there are varying degrees of hypoxia of nerve cells and that sufficiently severe hypoxia leads to permanent damage. This may have led to the confusion which has arisen.

From observations on very severe decompression sickness leading to chokes, which are seen as a presenting sign in decompression sickness work with small animals, the literature abounds with evidence that the death or disablement is due to the presence of gas emboli in the vascular system. At the other extreme of the manifestations of decompression sickness, the skin mottling which is sometimes seen has been described as closely resembling postmortem staining (Dartford Tunnel Report) (27) doubtless due to local occlusion of blood supply. Thus, the very severe forms of decompression sickness and some of the minor forms are undoubtedly caused by the lodging of bubbles generated intravascularly.

The site of origin of these intravascular bubbles is not known. There is evidence for the view that bubbles or bubble nuclei are already present in the vascular system and that any decompression is likely to cause expansion and growth of them. A case has been

established for regarding the heart as a source of gas cavitation bubbles. If indeed the heart is exhibiting gas cavitation at normal atmospheric pressure, then the evidence that bubbles appear first on the arterial side following decompression of small animals both in vivo and in vitro (31, 41) is not surprising. Some older ideas, prompted by the work of Newton Harvey (46) suggest that stable gas pockets are present in certain tissues, and that decompression sickness is caused merely by a combination of gas diffusion into these stable nuclei plus subsequent expansion as the pressure decreases. All such ideas imply that the body can withstand no stable supersaturation with gas at all.

A modification of this view is the concept advanced in a rather more mathematical form by Hills (32). Here, the assumption is made that, due to metabolic usage of oxygen, there is a degree of unsaturation in the tissues as a permanent feature and that it should be possible to decompress in such a manner as to prevent an excess tissue partial pressure of inert gas occurring.

Other observations (35) have shown that undisturbed supersaturated solutions of gas in water, glycerol and mixtures of water and olive oil do not form bubbles when care is exercised as to the cleanliness and wetability of the container. Instead the phenomenon of tribonucleation produces cavitation and leads to bubble formation under conditions requiring relatively moderate decreases in barometric pressure. Tribonucleation may occur biologically in knee joints where synovial fluid of high viscosity is present or perhaps in muscle tendon inserts to bone joints.

Evans and Walder (23) showed that exposure of shrimps to high hydrostatic pressure prevents the formation of decompression bubbles. From these and earlier studies, it is evident that hydrostatic pressure has a marked effect on bubble nucleation; numerous other physical factors are also very relevant, however, and it is impossible to decide which set of factors is uppermost with respect to the human body.

It would be pertinent here to enquire how the bubbles grow. The literature on the growth of bubbles in pure liquids in varying conditions of flow and with varying gas compositions describes a field still open to physicomathematical speculation. If one considers the same problem in relation to a non-Newtonian fluid subject to pulsatile flow, such as blood, which furthermore has a surface tension varying over a wide range due to the influx of surface-active molecules into an expanding surface, then one need hardly emphasise that an accurate account of bubble growth in biological fluids does not currently exist.

Nevertheless, examination of the complexities involved when dealing with simple pure liquids will yield some helpful gross statements. First, the rate of growth or decay, at a constant hydrostatic pressure, is dependent upon the gas partial pressure differences between the inside and the outside of the bubble (34). Second, surface tension is an important factor in the early growth of very small bubbles but ceases to influence the growth when bubbles exceed about 10 microns in radius (31). Third, the surface tension of bubbles formed in most biological fluids, e.g. blood and cerebrospinal fluid, will vary markedly due to the presence of proteins and surface-active materials in the interface. Fourth, bubble growth and dissolution are very dependent upon whether the bubble is surrounded by stationary or moving fluid (38). Fifth, when the bubble is resolved it will leave behind a bolus which can act as a source of a new bubble if decompression is re-started (42). Finally, bubble growth will depend upon gas composition of the bubble. For example, helium

diffuses more rapidly than nitrogen but possesses a lower solubility in the surrounding tissue and oxygen will be utilised in metabolic processes. Analysis of such unsteady state gas transfer is very complex, even when accompanied by simplifying assumptions (54).

To these considerations relating to single-bubble growth must be added two further important possibilities relating to multi-bubble situations. There is a possibility of coalescence of small bubbles leading to formation of a number of large bubbles (32), but there is also the more likely possibility that small bubbles with high internal pressures, due to surface tension, will lose their gas content by diffusion into larger nearby bubbles. Thus larger bubbles will tend to become larger and smaller bubbles will tend to become smaller in a mixed population of small and large bubbles trapped in a tissue. It is worth noting that when small bubbles with their greater internal pressure interdiffuse with or coalesce with large bubbles, the volume of the new larger bubble is greater than the sum of the volumes of the previous bubbles. These qualitative physical observations can be applied to understanding the decompression problem.

Clearly, the decompression time course from a saturation dive is not just a matter of knowing tissue inert gas clearance half-times. Ample evidence exists to show that one is normally dealing with some sort of tissue-bubble complex. An extreme example has been recorded following the Ocean Systems dive (47). It will be recalled that a diver suffered a mild but definite attack of the bends when flying at low altitude in an unpressurised aircraft 1 week after surfacing from this dive. This means, in general terms, that there is some process capable of provoking symptoms of decompression sickness extending over a period of several days. Such a process must possess a half-time in excess of a day, and the absurdity of attempting to use such half-times is apparent.

Two further sets of facts have been established since the last Symposium: 1) it is possible to accomplish quite large and rapid pressure reduction following a 100 meter saturation dive on oxyhelium, but once such a change has been made the period of time required at the new pressure level, in order to make a further similar large decrease in pressure, is measured in days and, therefore, is quite an impracticable procedure (Barnard (4)); and 2) continuous decompression seems reasonably successful over a wide range of decompression rates. For example, the very conservative type of continuous decompression used by the U.S. Navy has a small measure of failure, as does the much more rapid Bühlmann/COMEX form of time course. In view of the facts established on bubble growth and decay, a likely explanation of the above evidence may be found by examining the internal pressure of bubbles generated during decompression from saturation dives.

The internal pressure of bubbles is due to the surface tension and tissue mechanical factors. The excess pressure resulting from surface tension is inversely proportional to the radius, whereas, of course, the volume of a bubble is related to the cube of the radius. Thus, for example, doubling the volume of a bubble will affect the radius only by a factor of 1.26. This would mean that when dealing with small bubbles with their high internal pressure, due to surface tension forces, the rate of decompression may vary widely without noticeably affecting their internal pressures, whereas for large bubbles with small internal pressures, the rate of decompression will have to be extremely low. One must presume, then, that continuous decompression generates

small bubbles and allows comparatively rapid time courses, whereas stage decompression generates large, nearly symptomatic bubbles at the first stage, and the subsequent decompression is prohibitively lengthy.

Turning attention now to nonsaturation dives, it is first necessary to have some concept of how the tissues saturate with inert gas. It is clear from evidence available that, for the decompression problem, the tissue saturation times involve all tissue half-times from the fastest to the slowest. An examination of the submarine escape techniques will implicate some process with a half-time certainly not in excess of 5 minutes, whereas examination of prolonged air diving will reveal processes with half-times on the order of several hours. The only tissue of the body having all such half-times is the vascular network; since bubbles are apparently generated first in the vascular network, this would seem to be an attractive conclusion. It is difficult to explain with this hypothesis how one can suffer the same end result—namely, an attack of bends—following either a short deep dive involving short half-times, or a long shallow dive involving long half-times. One could avoid this difficulty by assuming that bubbles generated in the various tissues of the body are transferred to the bend-producing situation via the circulation. An alternative to this view is to suppose that a tissue type exists which, in itself, possesses all the necessary fast and slow half-times. Hills's heterogeneous tissue concept is an interesting example, challenging the accepted view that perfusion is the major factor in inert gas distribution in tissues (33). Certainly, the theoretical analysis of Jones (37), which is still quoted in favour of the all-perfusion concept, has been demolished by Hills. One further point should be added to Hills's criticism of the earlier work on whole-body inert gas exchange. Jones, for example, uses expressions involving five different time constants. A reputable text on applied mathematics will show that to justify the use of five exponentials requires data accurate to eight significant figures, which is patently absurd in the biological situation. Diffusion coefficients established by Hills for intracellular material are some 10^{-4} times smaller than those in the extracellular fluid. This tissue model has such wide ramifications in other areas of medicine and physiology that it is surprising to find that no one has attempted verification of this quite positive challenge to the accepted values. Whether considering a mainly perfusion situation or a mainly diffusion-dominated situation, if molecules of nitrogen take such considerable periods of time to saturate the tissue then such a tissue must be so poorly vascularised as to cause doubts about whether or not this represents any real body situation. Certainly, there is sufficient evidence from saturation diving to show measurable biochemical changes: for example, significant increases in creatinine phosphokinase, amylase and cholesterol at 19.2 and 23.7 ata (55). It is speculated, therefore, that all straightforward physical tissue models used to explain inert gas uptake in relation to the decompression problem will fail to account for the subsequent decompression requirements—mainly because the body state has changed in very prolonged dives, which in turn changes the decompression procedure required and, therefore, gives the appearance of having affected the degree of saturation of the relevant tissue or tissues. Past and present evidence (1) shows that at atmospheric pressure only 4 hours of oxygen breathing are necessary for 100% protection of men from altitude bends. This implies some tissue with a half-time certainly no greater than 1 hour.

There seems to be, therefore, a good case for examining the influence of pharmacologi-

cally active agents on the subsequent decompression. Such experiments have been conducted from time to time, mainly in an attempt to prevent the onset of decompression sickness, but there is a strong case for investigating the possibility of influencing the body prior to the decompression. In most cases, of course, it is difficult to decide which of these two processes has been influenced (43). The analysis by Reeves and Workman (52) which shows that heparin has no effect on bends sensitivity—whether the heparin is injected pre- or postexposure, is more helpful. The discovery of the smooth muscle acting factor (SMAF), possibly released as a result of tissue reaction to micro-bubbles or to their denatured protein sequalae, and its potentiation of decompression sickness is also important in this connection (19).

From the bubble facts mentioned earlier, it would seem that resolution of bubbles will be followed by generation of a bolus of denatured protein and surface-active material, which may well be expected to cause the persistence of foreign body responses after the effects of the original gas embolus have ceased.

Philp et al. (50) have shown that experimental air embolism is accompanied by thrombocytopenia and conclude "it would seem reasonable to postulate that, in some circumstances at least, the intravascular formation of bubbles could precipitate a syndrome similar to that seen in disseminated intravascular coagulation." This conclusion would entirely agree with the formation of the aggregations noted above, which will take only a few seconds to form following formation and resolution of very small bubbles, and which will break away from the surface of large, easily deformable bubbles, passing on into the circulation. It has also been demonstrated that arteriosclerosis of the lungs will occur from repeated intravenous injections of fine chopped blood clot or equally by repeated injections of oxygen, nitrogen and argon.

Martin and Nichols (44) have been investigating posdecompression changes in relation to a normal simulated dive devoid of any signs or symptoms of decompression sickness. Two main features have emerged from this work. First, and not altogether surprisingly, they have demonstrated evidence of measurable change in a variety of blood constituents immediately following a dive. More surprisingly, when studying platelets and enzymes, they have demonstrated a delayed effect of the dive which led, for example, to a maximum depression of the cell count 48 to 72 hours after the dive. This finding has been confirmed subsequently by Philp (51). These delayed effects, previously unrecognised, open up a new field for research in terms of patho-physiological responses. The implications for postdecompression delayed effects, such as bone necrosis or the "acclimatisation" acquired by tunnel workers, are obvious.

How successful is the present approach to the decompression problem, given all the complexities noted above, many of which cannot be quantified? The major problem facing most practical workers in this field is the avoidance of bone necrosis. It is impossible to know whether helium diving is capable of causing this problem because nearly all divers who subject themselves to helium also dive regularly on air and, since it is well-known that air diving can cause necrosis of the bone, there is as yet no satisfactory answer. Regarding air diving, there have been two attempts to produce decompression schedules to avoid the crippling forms of bone necrosis: namely, in the United States at Seattle, and in Great Britain by the Medical Research Council. Both these decompression routines have succeeded in their major objective, i.e. to avoid the crippling forms of bone necrosis, but, nevertheless, a small percentage of radiologically detectable

bone change still occurs. Since both these attempts make the decompression procedures more conservative, it follows that bone necrosis, like bends, is probably caused by gas emboli and not, as has been suggested, by gas osmotic changes during the compression phase.

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ALTERATIONS IN BLOOD VISCOSITY AND MICROCIRCULATORY PERFUSION IN EXPERIMENTAL DYSBARISM*

C. H. Wells, T. P. Bond and M. M. Guest

A pattern of reduced capillary flow, scattered stasis in microcirculatory channels, increased erythrocyte aggregate formation and increased flow through arteriovenous shunts has been observed in individuals subjected to any of a variety of physically traumatic events including crush injury (5, 27), tissue ischemia (5, 12), thermal injury (21, 22, 41), and infection (26, 28). Similar aberrations in microcirculatory perfusion have been observed in decompression sickness (18, 23, 44). These changes may occur without evidence of bubble embolism (23, 44). Hemoconcentration and substantial reductions in plasma volume also occur (2, 9, 16, 17) and undoubtedly contribute to the alterations in microcirculatory perfusion. Increase in blood viscosity has been found following any of several traumatic events (4, 25, 34, 41) and must be considered as a contributor to the aberrations in microcirculatory flow that occur after trauma.

This study was designed to investigate the possibility that similar changes in blood viscosity occur after decompression; to monitor hematocrit, some plasma components of the coagulation and fibrinolytic systems; and to observe blood flow in the mesenteric microcirculation.

Methods

Eighteen adult mongrel dogs, anesthetized with pentobarbital, were subjected to a mixture of 5% O_2 and 95% N_2 at a pressure equivalent to 200 feet of sea water (fsw) for 60 minutes. The animals were then decompressed at 1 atm pressure at a rate of 20 feet/minute.

The mesentery of each animal to be compressed was exposed by a midline abdominal incision, and the microcirculation within that tissue was recorded before and after compression-decompression by cinephotomicrography at magnifications from 10× to 90× and exposure rates from 24 to 300 frames per second (Bond and Guest (6)). Single-frame 35-mm photographs of the mesenteric microcirculation were also made.

Blood samples were collected from a catheter in a carotid artery before compression, immediately after and 60 minutes after decompression. Viscosity of heparinized blood was

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measured at each of six shear rates ranging from 230 to 5.75 sec^{-1} using a Wells-Brookfield LVT plate-cone viscometer. Coagulation time was estimated by the method of Lee and White (29); fibrinogen concentration by the method of Ware, Guest and Seegers (45); prothrombin time by Quick's one-stage assay (37); and partial thromboplastin time (PTT) by the method of Rodman et al. (38) except that soybean phosphatide was substituted for cephalin used in the original test (7). Reciprocals of the euglobulin lysis times measured by the technique of Celander and Guest (11) were multiplied by 100 to convert to units.

Date derived from samples collected immediately after decompression were compared with corresponding values from the same animal's precompression samples, using Student's *t* test for paired data. Data from samples collected 1 hour after decompression were similarly compared with corresponding precompression values. Of the 18 animals used in this study 10 lived through the decompression. Thus, there were 18 sets of data available for analyses from samples collected immediately after decompression but only 8 sets of data from samples collected 1 hour after decompression.

To determine the effect of a large gas-liquid interface, citrated blood was obtained from dogs which had not been compressed. The plasma was separated by centrifugation. Baseline assays for coagulation and fibrinolysis were performed by the same method as on blood from animals subjected to compression and decompression. The remaining plasma was then foamed by shaking for 5 minutes in contact with air. The continuous liquid phase was separated from the foam. Assays were performed on both the continuous liquid phase and on the foam phase after the foam had reverted to a continuous liquid, free of air bubbles.

Results

Cinephotomicrography of the mesenteric microcirculation immediately after decompression revealed little change in microcirculatory flow and minimal formation of erythrocyte aggregates. Bubbles of approximately 10 μ diameter frequently appeared but passed readily through the microvasculature. No bubble embolism was seen. The films taken 30-60 minutes after decompression showed severe impairment of microcirculatory flow with scattered stasis and widespread formation of erythrocyte aggregates. Bubbles of approximately 10 μ diameter were again evident in the cinephotomicrographs of the microcirculation but passed through the microvessels without apparent difficulty. No evidence of bubble embolism was seen. Figure 1 is a microphotograph taken 1 hour after decompression, illustrating aggregation of erythrocytes. At this magnification the 10 μ bubbles are usually not discernible.

Analyses of blood viscosity are summarized in Fig. 2. The viscosities of blood samples collected immediately after decompression were slightly greater than corresponding precompression (baseline) values at each of the six shear rates tested (5.75–230 sec^{-1}). These values differed significantly ($P < 0.05$) at two of the six shear rates tested. A more pronounced, shear rate-dependent rise in blood viscosity was found in samples collected 1 hour after decompression. The mean viscosities increased progressively from 124% of control values at a shear rate of 230 sec^{-1} to 163% of control at a shear rate of 5.75 sec^{-1}. These values were significantly greater ($P < 0.05$) than corresponding control values at each of the six shear rates studied.

FIG. 1. Erythrocyte aggregation with flow impedence in decompression sickness.

The mean hematocrit of samples collected prior to compression was 34; immediately following decompression it was 35, and 1 hour after decompression, 42. The difference between the latter value and its corresponding baseline value (precompression value) was significant ($P < 0.05$).

No significant changes from baseline values were found in plasma fibrinogen concentrations, Lee-White coagulation times, or one-stage prothrombin times either immediately after decompression or in samples collected 1 hour after decompression.

PTT in samples collected immediately after decompression was slightly but not significantly greater than in samples collected from the same animals prior to compression. The PTT of samples collected 1 hour after decompression was 114% of that of precompression samples. This difference is significant ($P < 0.05$). In the liquid fraction of foamed canine plasma the mean PTT was 20% longer than in unfoamed aliquots of the same plasma; this difference is significant ($P < 0.05$). Foaming plasma failed to cause significant changes in any other of the performed coagulation or fibrinolytic assays.

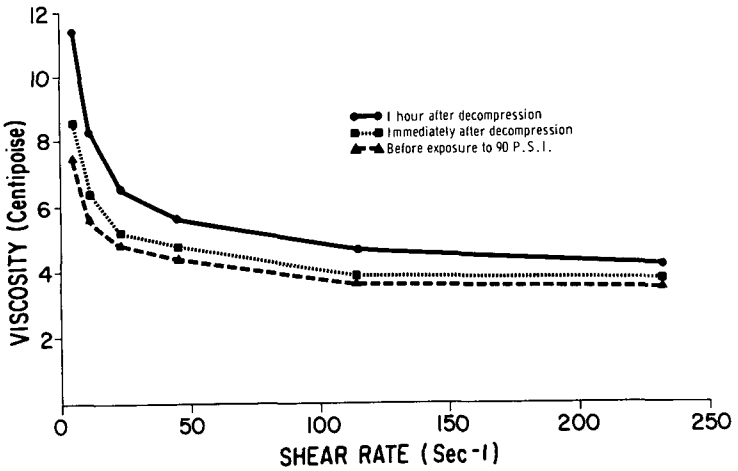

FIG. 2. Blood viscosity in dysbarism.

TABLE I

BLOOD COAGULATION STUDIES IN DOGS FOLLOWING DECOMPRESSION[a]

Measurement	Precompression	Immediately After Decompression	1 Hour After Decompression
Plasma fibrinogen concentration (mg%)	329 ± 14	318 ± 18	306 ± 33
Lee-White coagulation time (min)	5.8 ± 0.3	6.0 ± 0.8	4.8 ± 0.4
Prothrombin one-stage time (sec)	11.4 ± 0.3	11.7 ± 0.3	11.9 ± 0.4
Partial thromboplastin time (sec)	36.6 ± 2.1	39.2 ± 2.6	42.5 ± 5.3
Hematocrit	34.3 ± 1.4	34.9 ± 1.3	42.1 ± 3.0
Euglobulin units	82 ± 7[b]	85 ± 8	172 ± 30

[a]Values presented are means and standard errors of means.

[b]The mean precompression fibrinolytic activity of samples collected from those animals that subsequently survived less than 1 hour following decompression was 69 ± 8 euglobulin units. The corresponding precompression value from animals surviving 1 hour or more after decompression was 97 ± 11 euglobulin units.

TABLE II

BLOOD COAGULATION STUDIES OF FOAMED DOG PLASMA[a]

Measurement	Plasma Before Foaming	Foamed Plasma	
		Foamed Fraction	Fluid Fraction
Plasma fibrinogen concentration (mg%)	394 ± 25	399 ± 25	395 ± 25
Prothrombin one-stage time (sec)	11.2 ± 0.5	11.4 ± 0.5	11.1 ± 0.5
Partial thromboplastin times (sec)	46.2 ± 2.9	47.5 ± 3.0	55.4 ± 3.8
Euglobulin units	66.2 ± 4.0	68.0 ± 4.0	61.0 ± 3.6

[a]Values presented are means and standard errors of means.

Euglobulin lysis times (ELT) were less in samples collected 1 hour after decompression than in precompression samples. Converted to units, the precompression activity was 0.82 units immediately after decompression, and the 1 hour postdecompression activity was 1.72 units. This difference is significant ($P < 0.05$). Euglobulin lysis units in blood samples collected immediately after decompression (0.85) are not significantly different from the precompression values.

Discussion

From analyses of the cinephotomicrographic records, reduced velocity in larger vessels and stasis in many venules and capillaries occurred within an hour after decompression. Bubble embolism was never observed, although bubbles, which passed easily through the capillaries, did appear repeatedly (8). Swindle (43), Wagner (44), and Heimbecker et al. (23)

have also described impairment in microcirculatory flow during decompression sickness in the absence of demonstrable bubble microembolism.

It has been suggested that the alterations in flow during decompression sickness result from aggregates of erythrocytes which tend to block microcirculatory channels, as in other traumatic events including crush (5, 27), thermal injury (21, 22, 41), tissue ischemia (5, 12), and infections (26, 28). Photographic recordings of microcirculatory events during decompression sickness confirmed that aggregation of erythrocytes occurs (Fig. 1) and is especially marked 1 hour following decompression.

Viscosities of blood samples collected 1 hour after decompression were substantially greater than corresponding precompression values. The magnitude of the changes varied with shear rate, ranging from an increase of 24%, at a shear rate of 230 sec^{-1} to 63% at 5.75 sec^{-1}. Although the effects of an increase in viscosity of this magnitude on flow in the microcirculation are difficult to quantitate, it would appear that the resultant increase in resistance to flow could be a major cause of the circulatory impairment in decompression sickness.

Several factors have been shown to augment the viscosity of blood. The most important of these are an increase in hematocrit (19, 32, 36), erythrocyte aggregation (14, 30, 39), and a decrease in erythrocyte flexibility (13, 40, 42). Indirectly, through its effect on erythrocyte aggregation, an increase in the fibrinogen concentration also augments the viscosity of blood (15, 31, 33). In our decompressed animals no change in fibrinogen concentration was observed and there was no reason to suspect a change in red cell flexibility. On the other hand, the hematocrit increased and erythrocyte aggregation was observed in the microcirculatory photographic records. These factors appear to be responsible for the increase in blood viscosity and, pari passu, for an increase in resistance to flow in the microcirculatory bed.

The basic mechanisms underlying the increase in hematocrit and erythrocyte aggregation in decompression sickness are currently uncertain. A reasonable explanation for the rise in hematocrit is that plasma is lost because of an increase in capillary permeability. More obscure is the reason for the aggregation of erythrocytes in the absence of an increase in fibrinogen concentration. Heimbecker et al. (23) explored the possibility that hypercoagulability, presumably through enhancement of erythrocyte aggregation, is responsible for low capillary perfusion in decompression sickness. However, these investigators failed to demonstrate a change in blood coagulation by measuring polystyrene clotting times. On the other hand, Aggazzotti (1) has reported a shortened coagulation time in a majority of dogs and rabbits subjected to experimental dysbarism, and Barthélemy (3) has reported reduction in the severity of decompression sickness in rabbits and men treated with heparin. In this study, the only significant change in coagulation assays was the prolongation of PTT in blood samples collected 1 hour after decompression (Table I).

A significant prolongation of the PTT occurred in the decompressed animals when intravascular bubbles were present and in plasma foamed in vitro. Thus, the possibility exists that the alteration in activity of an intrinsic procoagulant factor or factors is related in some manner to the abnormal presence of gas-liquid interfaces. Prolongation of the PTT might be interpreted as an indication that conversion of prothrombin to thrombin occurred in the decompressed dogs. A prolongation of the PTT, due to a decrease in the activity of factor VIII, has been reported by Penick et al. (35) during experimental intravascular coagulation in dogs.

A highly significant increase in euglobulin lysis units was observed when the activity 1 hour after decompression was compared with the precompression level. The increase in fibrinolytic activator apparently was not due to interaction at a gas-liquid interface per se since no increase in activity was observed in the liquid or foam fraction of foamed plasma. The euglobulin assay is a measure of plasma activator of the fibrinolytic system and the activator appears to be derived from the endothelium of blood vessels (20). An increase in plasma activator occurs with vasodilation or the opening of previously stagnant segments of the circulation (24).

ACKNOWLEDGMENTS

This study was supported by Grants NIH HE 10893 and NIH 1501-FR 05427-08.

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ELEVATED BLOOD LIPIDS IN HUMAN VOLUNTEERS AFTER DECOMPRESSION

A. T. K. Cockett, S. M. Pauley, A. Pilmanis and A. P. Roberts

The important role of nitrogenous bubble formation in decompression sickness has been underscored for a number of decades. Nitrogen is released from lipid-laden tissues; these coalescing gaseous bubbles are thought to be the primary mechanism for symptomology ranging from mild bends to severe decompression sickness.

Approximately 10 years ago the finding of hemoconcentration in humans developing decompression sickness (2) was described. Subsequent studies (1, 3) clearly describe the magnitude of the plasma deficit developing in animals subjected to compression and rapid decompression after a 1-hour bottom time at 165 feet.

Bone marrow emboli were also reported in animals subjected to rapid decompression (4). Subsequent studies of visceral organs preserved in formalin from decompressed animals were performed several years later. Oil red O stains uniformly revealed numerous lipid emboli especially in the lungs, liver and kidneys of these animals. Control animals were carefully studied after fasting and sacrifice. Lipid emboli were seen in only a few tissue sections from the controls.

Studies focusing on a therapeutic regimen were also conducted in parallel. The benefits of heparin and dextran in reversing many of the pathophysiological alterations in animals subjected to lethal compression and decompression (5) were noted. Heparin has an antilipemic effect, a fact which suggested that lipid emboli play an equally important role in the genesis of decompression sickness.

Studies were then undertaken, initially in animals and later in human volunteer divers, to determine blood lipid profiles before and after decompression.

Materials and Methods

Animal Studies

Thirteen mongrel dogs were splenectomized and allowed a 3-week recovery period. The animals were anesthetized and compressed to 165 feet at the rate of 15 feet per minute. Bottom time extended for 60 minutes, after which the animals were brought to the surface at the rate of 15 feet per minute. Blood samples were obtained before and 3 hours following the dive.

TABLE I
LIPID PROFILE IN 13 DOGS OVERCOMPRESSED TO 165 FEET FOR 1 HOUR

Lipid	Predive	Postdive[a]	Significance of Difference
Cholesterol	144 gm%	160 mg%	$P < 0.005$
Phospholipids	298 mg%	325 mg%	$P < 0.02$
Triglycerides	26.7 mg%	37 mg%	$P < 0.02$
Total Lipids	518 mg%	561 mg%	$P < 0.03$

[a]Three hours after surfacing.

HUMAN STUDIES

The plasma samples in human volunteer divers were obtained in a series of dives in Hawaii and California over a 4-year period. Control blood samples were obtained at midday prior to a meal. Subjects ate low-fat breakfasts prior to diving.

Depths selected ranged from 100 feet to 200 feet. The single tank dives were in accord with the standard U.S. Navy diving tables. The postdive blood sample was usually obtained within 15 minutes after surfacing.

All plasma samples were analyzed by Bioscience for lipids which included cholesterol, triglycerides, phospholipids, free fatty acids and total lipids.

Results

Table I shows the results obtained in 13 dogs. Significant elevations of cholesterol are seen 3 hours after surfacing. The level of 160 mg% is significantly higher than the baseline control. Significant elevations are seen for phospholipids (325 mg%), triglycerides (37 mg%) and total lipids (561 mg%).

Table II illustrates the findings in human volunteer divers. Total cholesterol is significantly higher in the postdive samples (baseline, 192 mg%; postdive, 203 mg%). Unesterified fatty acids (0.515 to 0.686 mEq/L) and phospholipids (267 to 304 mg%) are significantly elevated in the postdive samples of the human divers. Symptoms of bends or decompression sickness were not encountered in any of the human divers.

Discussion

These studies clearly demonstrate a shift in an upward direction in the lipid profile following decompression.

In the human volunteers the changes in lipids could not be separated on the basis of diving depths. This is probably due to the small number of samples obtained at the deepest levels.

Hematocrit and white blood cell counts were also performed to rule out the effects of dehydration. On dives shallower than 100 feet, dehydration was noted as evidenced by an increase in WBC and Hct. This was due to water vapor loss after long bottom times. However, when subjects dove to 100 feet and 200 feet, bottom times were short and pulmonary

TABLE II
CHANGES IN BLOOD-LIPID PROFILE IN HUMAN VOLUNTEERS DIVING TO 100–200 FEET

Test (number of samples)	Predive	Postdive	Mean Difference	Probability of Difference
Total cholesterol (18)	192.2 mg%	202.9	10.7	$P < 0.02$[a]
Unesterified fatty acids (28)	0.515 mEq/L	0.686	0.172	$P < 0.005$
Phospholipids (30)	267 mg%	304	37	$P < 0.01$[b]

[a] Catalina dive.
[b] Hawaii dive.

water vapor loss was not significant enough to change Hct and WBC values. Therefore, the elevations of lipids were not due to dehydration in the 100-foot and 200-foot dives.

Previous emphasis on dextran replacement and treatment with heparin is believed to be valid. Heparin, by activating a lipoprotein lipase, may deter the coalescence of lipids and thereby minimize the formation of fatty emboli. LeQuire et al. (6) have demonstrated that pulmonary fat emboli produced by rapidly decompressing rabbits consist mainly of cholesterol. They point out that depot fat contains only small amounts of cholesterol, and that cholesterol aggregates probably are formed from plasma lipid or are extruded from the liver. It is interesting to note that both the dogs and humans in this study had significant elevations of cholesterol.

A second benefit of heparin—that of anticoagulation—may also be of value. This line of investigation is under evaluation. Medium molecular weight and low molecular weight dextran are well-known for their antisludging properties; this polysaccharide is also very important in expanding plasma volume to counter the loss of plasma seen in severe decompression sickness.

It should be emphasized that when severe decompression sickness is encountered, every effort should be made to recompress the individual. Since chambers are not readily accessible, dextran and heparin can be administered initially. Experience suggests that dextran (low molecular weight and medium molecular weight) is the agent of choice prior to and during recompression. When severe decompression sickness is seen, heparin and other modes of therapy should be used without hesitation, provided there is no history of a bleeding diathesis or trauma to the individual.

ACKNOWLEDGMENTS

This study was supported by the Office of Naval Research, Contract No. N00014-67-A-0398-0008.

The authors gratefully acknowledge active support of the Janss Foundation, Thousand Oaks, California; the Searcher vessel contributed significantly to the success of our dives: the Oceanic Institute, Makapu, Hawaii: Dive & Surf, Inc., Redondo Beach, California.

The authors acknowledge the editorial assistance of W. S. Cockett.

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PRESSURE STUDIES WITH MICE UP TO 270 ATA

M. J. Halsey, D. W. Kent and E. I. Eger II

There has been increasing interest in the effects of hydrostatic pressure on mammals (1, 7, 9). Recently there have been a number of important chamber dives during which men have been subjected to pressures around 50 ata (3, 4, 6, 11). The possibility of using drugs to enable man to dive to even greater depths was discussed at the Fourth Underwater Physiology Symposium (7). The experiments reported in this paper have been concerned with learning more about the detailed effects of deliberately adding an anaesthetic agent to helium–oxygen diving gas.

Nitrous oxide was used as the anaesthetic agent because its narcotic properties have been well-characterised. It has an intermediate narcotic potency (ED_{50} for mice = 1.5 ata) and thus it is easy to study the dose-response curve without having concomitantly large changes in the overall pressure. Equilibrium between inspired and alveolar gas concentrations is achieved relatively rapidly. It is not a profound respiratory depressant and is free from serious toxic side effects. The mice which were used have been extensively studied both in narcotic and pressure experiments. Their loss of righting reflex response is a reproducible end-point which provides an indication of their central nervous system function. In addition the morbidity and mortality of a group of mice can be studied in each experiment.

An initial attempt was made to determine the optimum conditions for survival at very high pressures, e.g., 3,000–8,000 feet of sea water. The effects of varying the gas mixture, oxygen and nitrous oxide partial pressures, and the rate of compression, total exposure time and environmental temperature were determined. As a result of these studies it became clear that at extreme pressures the mice were subjected to a severe stress before death. A study of the effect of prior acclimatization of the mice to increased exercise and respiratory work was therefore made. Finally it was determined if one of the limiting factors in survival at high pressures was the physical properties of the gas mixture rather than the effect of pressure per se. As a result of all these series of experiments it was possible to take mice up to 270 ata, which is equivalent to survival at depths of approximately 8900 feet of sea water.

All experiments with the helium gas mixtures were carried out in a high pressure chamber (maximum working pressure 4500 p.s.i.g.), having approximately a 20 liter internal volume with a 5″ diameter Plexiglas window at one end, and five 1-inch lighting and/or view ports along the side of the vessel. Pressures were measured on a 0–60 p.s.i.g. (calibrated against a mercury manometer), a 0–2000 p.s.i.g. and a 0–6000 p.s.i.g. (both calibrated against

a dead weight tester). The conventional flow-through system with premixed gases was not used but instead the gas inlet was via a manifold of valves, which allowed a variable quantity of any gas to be added to the chamber at any stage of the experiment. The concentrations of all the gases were measured with a gas chromatograph. Gases could be removed from the chamber by a flow-through system. Circulation of gases within the chamber was achieved by a powerful fan, incorporating a carbon dioxide-soda lime absorber. Ammonia was also removed with a scrubber arrangement. Temperature was controlled with an external heat source surrounding the main body of the chamber, and an internal heat exchange coil placed behind the gas circulating fan. Ambient temperatures were monitored via calibrated thermistors in different parts of the chamber. Humidity was controlled with a simple wick system. The concentrations of all the gases—including "metabolic gases" such as carbon dioxide, carbon monoxide, methane and ammonia—were monitored with two gas chromatographs.

Male Swiss Webster mice, 20–30 gm in weight, were put in a large rotating cage, which was divided into eight separate cylindrical compartments. The cage could be rotated in either direction at 4 rev/minute. In addition, three other mice had rectal thermistor probes inserted and were put in separate fixed cages. Some experiments were also carried out using electrical stimulation via electrodes on the tails of six mice.

Previous reports that, with helium-oxygen gas mixtures, mice have tremors and convulsions, display respiratory distress and die at pressures around 100 ata of helium (1, 7–9) were first confirmed. The nitrous oxide partial pressure which produced a loss of righting reflex in 50% of the mice (ED_{50}) at different pressures of helium was then determined. The ED_{50} was found to increase in proportion to the total pressure up to 150 ata (Fig. 1). At higher pressures a decrease in narcotic requirement was observed, and animals began to die above 200 ata.

The factors influencing the death of these mice were investigated. First, the necessity for the exposure time to achieve the higher pressures was tested to see if it were an important factor. The time dependence of the nitrous oxide potency at 100 ata was therefore investigated for a period of 3 days. The nitrous oxide potency was determined immediately after the initial compression. The animals were maintained at 100 ata, with continuous removal of carbon dioxide and ammonia from the chamber gases, and monitoring for car-

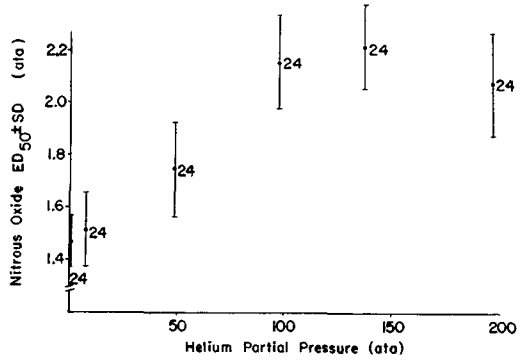

FIG. 1. Nitrous oxide anaesthetic ED_{50} in mice (± standard deviation) vs. helium partial pressure. The figures near each point refer to the number of mice used in each series of experiments.

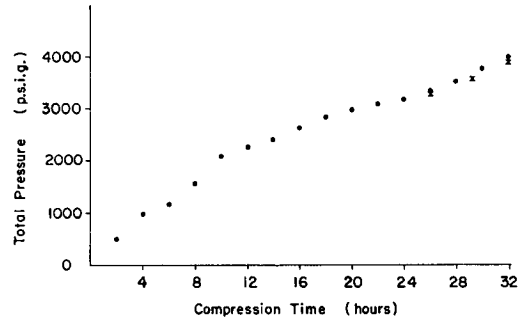

FIG. 2. Compression schedule for mice survival requirements up to 270 ata using optimum chamber conditions as described in the text.

bon monoxide and methane; no buildup of the concentrations of any of these four gases was detected. The potency measurements were repeated each day, and it was found that there was no significant change with time in the anaesthetic requirement of 24 mice at 100 ata. No diurnal variations in the requirement, as has been found in some other experiments (12), were detected. After 4–5 days, the mice generally deteriorated, but this was probably due to inadequate conditions inside the chamber.

Fifty mice were tested as to whether variations in nitrous oxide and oxygen partial pressures and compression rates could increase the number of survivors at higher pressures. The best survival rate was obtained when the nitrous oxide partial pressure was 0.5–0.8 ata; the oxygen partial pressure 0.3–0.5 ata; the compression rate less than 0.68 ata/min; and rectal temperature maintained between 36–37°C. It was postulated that, just as nitrous oxide partial pressure required for narcosis increased with total pressure, similarly the nitrous oxide partial pressure required to maintain "normal" function (i.e., to withstand the effects of pressure but not to be narcotized) would also increase in proportion to the total pressure. Results were consistent with this hypothesis at pressures up to 200 ata. However, when the conditions for maximum survival rate were used, although some mice died above 200 ata, others were pressurized up to 270 ata over a prolonged period (Fig. 2). It is interesting to note that with the most favourable compression conditions, no convulsions or respiratory distress was observed below 200 ata, and tremors were always very slight.

Two possible factors contributing to the morbidity of some of the mice above 200 ata were also investigated. It was recognized that they were unused to the stresses and respiratory efforts involved—therefore, the survival of mice acclimatized to increased exercise and respiratory work was examined. For these experiments, 3-week-old mice were intermittently exposed to an above normal carbon dioxide concentration and were forced to exercise in a large rotating drum. The duration and frequency of exposure, the inspired carbon dioxide concentration and the degree of forced exercise were all gradually increased over a period of 3 weeks. After the acclimatization period was over, these "trained" mice were then exposed in the helium chamber, and the results compared with those of the "normal" mice. However, the survival rates and righting reflex responses at high pressures of the two groups of mice were not significantly different.

Finally, the effects of increasing the viscosity and density of the gas mixture by substituting neon for helium were investigated. At sea level neon is five times as dense as helium,

and its viscosity is 1.6 times that of helium. For these experiments, a small 1-liter chamber with special gas handling facilities was used inside the large chamber, as has been described elsewhere (5). It was found that with the addition of a subanaesthetic partial pressure of nitrous oxide, all animals survived over 100 ata in the neon gas mixture for prolonged periods. They retained a normal righting reflex response, and there was no evidence of respiratory distress.

What conclusions can be drawn from all these animal experiments which are relevant to man's possible future diving limits? The neon experiments suggest that the physical properties of the gas mixture will not be the primary factor limiting exposure to very high pressures. The simple acclimatization experiments are consistent with the hypothesis that it is not diver training which will be the ultimate factor. The very high pressure studies agree with the predictions and experiments of other investigators (2, 10) in demonstrating that anaesthetics can prevent some of the central nervous system and lethal effects of high pressures. However, there appears to be a finite limit to the protective effect of nitrous oxide. It may be that, with other agents, greater pressures can be achieved. The theories behind such predictions are discussed elsewhere (5, 10).

While recognizing the many difficulties in interpreting whole animal data and extrapolating the results to man, it does appear that adding an inhaled general anaesthetic, such as nitrous oxide, to a helium-oxygen gas mixture would enable the so-called "pressure barrier" to be dramatically extended.

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VERY DEEP DIVING EXPERIMENTS ON MINIATURE PIGS

J. Parc, A. Michaud, J. Le Chuiton, A. Michel, L. Barthélèmy and G. Balouet

The Groupe d'Études et de Recherches Sous-marines (G.E.R.S.) of the French navy, in close collaboration with the physiological and histological laboratories of the Brest Faculty of Medicine, performed a series of very deep simulated dives using animals, over a 1½-year period in order to determine precisely the different parameters which play a part during the three phases of a dive: compression, stay at maximum pressure, and decompression. This paper relates the results of the first 17 experiments.

Pittman-Moore miniature pigs, weighing 40 to 60 kilograms, and 6 months to 1 year in age, were used. A comparative experiment was made on a goat the age and weight of which were equivalent to the pigs'. (See Fig. 1).

General Experimental Procedures

All of the dives were performed in a hyperbaric, spheric chamber, 1.6 cubic meters internal volume, allowing animal experiments up to a maximum 150 ata pressure. The chamber was fitted with feeding, watering, draining and lighting devices. A regenerating plant washed out carbon dioxide, organic pollutants and excessive moisture from the breathing mixture, by means of soda-lime, charcoal and silica gel canisters. Oxygen was replaced on demand through injections into the chamber. Required temperature was obtained by means of infrared heating of the walls or water cooling of gas tubing. These appliances are modeled after those of Chouteau et al. at C.E.M.A. (9, 34, 35, 37). (See Fig. 2).

The animals breathed a helium–oxygen mixture containing small amounts of nitrogen. Oxygen partial pressure and humidity were adjusted to different levels according to the different procedures, and carbon dioxide and other organic pollutants were removed as well as possible.

Prior to any experiment, the animals stayed for 24 hours at sea level in the chamber, and made a short 4 ata dive, in order to become accustomed to this new surrounding and to allow study of their surface bahavior.

Pressurization used pure gases and mixtures prepared in advance, stored in cylinders. During decompression, one part of the gases is purified, adjusted to the right level for oxygen, and stored again for a further experiment. Since compression, stay at the bottom, and decompression are studied, there are some differences in procedures. Nevertheless, it is

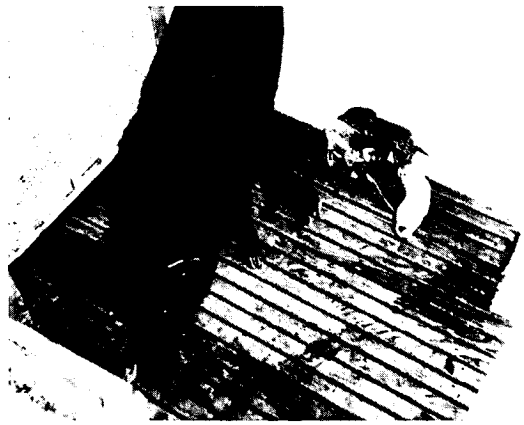

FIG. 1. A miniature pig used in the experiments. One pig, brought back alive from a 111 ata saturation dive, successfully impregnated a sow after the dive. This indicates that no major damage occurred during the dive.

possible to describe two different series: one included dives not exceeding 76 ata; the other, dives exceeding 101 ata.

DIVES NOT EXCEEDING 76 ATA

This first series included 10 experiments. Experiment number 1 did not exceed 41 ata, because of the difficulties met during compression (ear pain) and because of a defective control of the temperature in the chamber.

Experiments 2, 3, 4 and 5 reached 76 ata, and the duration of exposure at this depth was 1 to 7 days.

The five following experiments were made, for practical reasons, at 61 ata. The last was made on a goat.

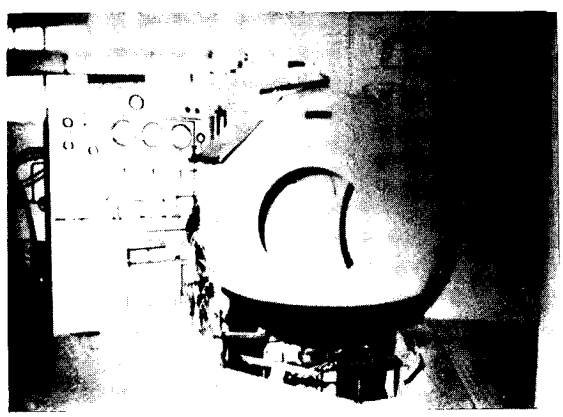

FIG. 2. The spheric hyperbaric chamber allowing animal experiments up to a maximum of 150 ata pressure.

Dives Exceeding 101 ata

The second series included seven experiments, all of which were made at pressures exceeding 101 ata (106–127 ata). As opposed to the first series, the results appeared to be aleatory: the behavior of animals was dissimilar, and only one animal could be brought back alive to the surface. Nevertheless, a number of established or assumed findings of the first series was corroborated. No physiological records were made. Only the behavior of animals was studied, and records were made with a magnetoscope or camera.

Results

Compression of Animals

Continuous and Staged Compression Produced Different Results. From the first experiment it was obvious that a staged compression was better than a continuous one. For pigs, the first compression was continuous, the animal reaching 41 ata with difficulty; it lasted 5½ hours. However, using the same animal, it was possible to reach 76 ata without difficulty, within 3½ hours, when a staged compression was used. It consisted of a succession of 10 ata jumps made within 5 minutes and followed by a 27-minute recovery period. The compression procedures which followed all derived from this one and showed constant effectiveness. (See Fig. 3).

The Importance of Homogenization of the Chamber's Atmosphere Was Clearly Revealed. It was observed, when oxygen was injected, that this gas flowed down along the wall to the bottom of the chamber. The phenomenon is very evident, because of the difference of refractive indexes of the gases. Several minutes were necessary before a steady $P_{I_{O_2}}$ was obtained. Some expedients (diffuser, deflectors) allowed us to improve the stirring

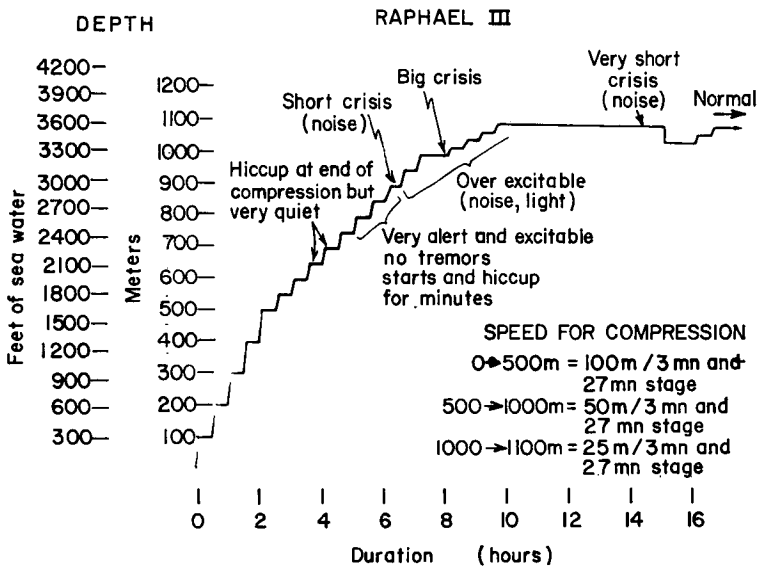

FIG. 3. An example of a deep dive procedure.

and hence the homogenization of gases in the chamber. At the same time, it was stated that the threshold for several disturbances, described later, was pushed back. For example, during the first experiments the disturbances occurred on reaching 61 ata. During the second series, even using the same compression procedure, they occurred deeper than 81 ata; the only difference was a better homogenization of the gases in the chamber.

The present compression procedure is a stage compression: to 51 ata, a succession of 10 ata pressure increases is made within 4 to 5 minutes, followed by 30-minute stops. Between 51 and 91 (or 101) ata, the magnitude of compressions is 5 ata, and the holding period, never shorter than 30 minutes, can be extended depending on the state of animal. Beyond 101 ata, the procedures are very variable, depending on reactions of the animal and on the capabilities of the appliances: compressions occurring within a few minutes or slow compression during 30 minutes, 2.5 or 5 ata at a time, and followed by holding period lasting 30 minutes to several hours. These empirical compression procedures are fundamentally flexible; for example, 2-hour stages at 51 and 76 ata were inserted during some of the last experiments. Those procedures are very fast also, for within 10 hours, a pig can be brought to a pressure higher than 100 ata. Of course, the animals show disturbances and fatigue; one must remember that limit procedures are studied to obtain information in an area in which it is lacking. (See Fig. 3).

Physical Parameters of the Dive

Gradually, some optimal values came out, applicable for miniature pigs, and also for goats. In addition, they confirm some results of Chouteau et al. (8-13, 17-23, 27-29):

1) Comfort temperature must average 31 °C ± 1.

2) Humidity (measured by a hair-hygrometer) must be low: 55%-60%. As was pointed out in 1970 (37, 39), and recently recalled in Marseille in 1971 (27), humidity is a very important environmental parameter. Chouteau mentioned this on the occasion of the Boucabloc and Boucafond experiments; several experiments performed at G.E.R.S. with Chouteau revealed the importance of this parameter—especially two comparative experiments lasting 1 week at a pressure of 76 ata. The latter experiments differed in humidity, which was high in one case, low in the other. With high humidity, the animals rapidly ex-

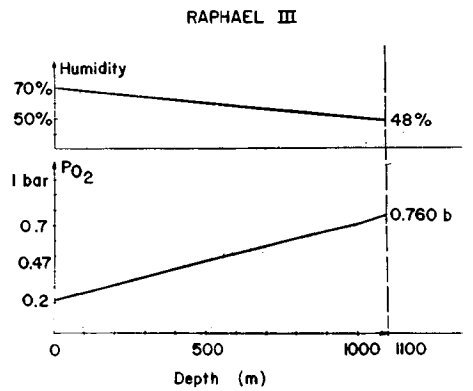

FIG. 4. During experiments 12 and 13, P_{IO_2} rose progressively during compression and humidity was lowered in the same manner.

perience an increasing dyspnea, with irritation and edema of upper airway. Postmortem examinations show lesions of the lungs, especially hemorrhages.

3) Atmospheric carbon dioxide must be removed as completely as possible. Twice, because of technical difficulties (regeneration of atmosphere was stopped because of a failure of electrical current), the carbon dioxide level was not controlled for a short period, and the animals were obviously impaired. As a general rule, carbon dioxide was adequately removed and became indetectable.*

Organic pollutants, and especially methane during the comparative experiment made on a goat, were detected as traces, even when using an original enriching method developed by the chromatography laboratory of C.E.R.T.S.M.

4) Oxygen partial pressure† was high in almost every experiment. It was maintained at 0.40, 0.45, or 0.50 bar, all the time during the first experiments, but was increased progressively and linearly, at the rate of 0.05 bar, every 10 bars total pressure, during experiments 12 and 13 (111 and 106 ata respectively).

The aim was to prevent hypoxia described at very high pressure by Chouteau, and the success of experiment 12 (Raphael 3) seemed to substantiate this. (See Fig. 4). Experiment 13, however, carried out in the same manner, induced death of the pig. This suggests that some other elements, currently unknown, play a part. Postmortem examinations showed evidence of only small Lorrain-Smith oxygen injuries in the lungs, in spite of relatively long exposures under high oxygen partial pressures.

The following two experiments: 14 (127 ata) and 15 (111 ata) were made in order to see to what extent high oxygen partial pressures would be better tolerated by the lungs under very high ambient pressures, than at surface level. Oxygen partial pressure was respectively 1.2 bar and 2.0 bars: the results were not very clear.

The last two experiments were made at 111 ata, and oxygen partial pressure was only 0.21 and 0.23 bar respectively, for the entire experiment. The behavior of animals was completely dissimilar: pig no. 17 showed tremors, clonic spasms and excitomotor crisis after 76 ata. At 111 ata, this animal showed evidence of hind leg paresis, then permanent excitomotor crisis, which did not stop with increasing oxygen partial pressure. The pig had to be decompressed after 1 hour and died during a stage at 84 ata.

However, pig no. 16 showed better behavior than the others during compression and the first 24 hours on the bottom with very few symptoms. Yet this animal remained awake, but became more and more tired. After 60 hours on the bottom a dyspnea occurred and, at the same time because of technical difficulties, carbon dioxide increased in the chamber. During decompression, begun in the 62nd hour, dyspnea increased, and the animal soon died. Notwithstanding the result of the following experiment, it seems that, if the stay on bottom had been shorter and if there had been no technical difficulties, it should have been possible to bring the animal back to surface alive.

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*Gas chromatograph Beckman model GC2A, and Carle. Results confirmed by a few analyses using the enriching method developed by the chromatography laboratory of C.E.R.T.S.M., 83800 Toulon Naval, France.

†Measured "in situ" with a Beckman polarographic electrode and, after expansion, with Servomex paramagnetic system, Beckman polarographic electrode, and gas chromatography.

Decompression (41)

The calculation procedures used by G.E.R.S. originate in the Haldane method (4, 5, 36) and use its perfusion equation; but instead of using (as most investigators do) a pressure difference (more or less constant for one tissue), G.E.R.S. uses a constant oversaturation ratio in order to compare dissolved gas tension to ambient pressure. Of course, there is a family relationship between the method used by Workman, Schreiner and C.O.M.E.X. (30, 44, 45) on the one hand, and the method used by G.E.R.S. on the other hand, but a fundamental difference still exists: constant ratio for G.E.R.S., a ratio varying with pressure (as the gradient remains constant) for the others.

During the first series of experiments oversaturation coefficients 1.10, 1.15, and 1.20 were tried, as well as the 120-minute tissue for continuous decompression. Then, with the same tissue, the 1.25 and 1.30 coefficients were used. Despite the fact that alive and comparatively healthy animals were brought back to the surface—after 24 hours to 1 week spent at 76 or 61 ata, those tissue-oversaturation coefficient combinations were not entirely satisfactory. As a matter of fact, the animals showed polypnea, which was controlled by means of a few 10-minute stages. Afterwards, the predicted decompression curve was overtaken, and the delay abolished through a temporary acceleration of the decompression speed. It was believed that a proper decompression would have to be close to a total 42 hours' duration, but that it would be necessary to use another longer tissue and consequently, another oversaturation coefficient. Therefore, first the 240-minute tissue and the 1.82 oversaturation coefficient were tried. Then, since the result was bad, the 180-minute tissue and 1.53 coefficient were used, which seemed to be satisfactory after a saturation dive at 61 ata was tried.

As mentioned before, only one animal could be brought back alive to the surface from a 111 ata pressure. After a 16½-hour stay under that pressure, the 180-minute tissue and the oversaturation coefficient 1.30 were used successfully.

Perturbations Shown by Animals

Perturbations in the animals' behavior were observed, some of them recalling those described by Fructus and Naquet as "high pressure nervous syndrome" (3, 6, 31, 43).

This begins in animals with a certain degree of irritability: very active exploratory behavior, startling reaction on hearing external noises, and piloerection all occurring at intervals interrupted by absolutely quiet episodes. A next stage includes intermittent muscular fasciculation high on the legs, sometimes with clonic contractions. Beyond 81 ata the animals stand almost continually, lying down only a few moments and then getting up soon, sometimes with a jump. It seems that this behavior is due in part to "irritability," but also to respiratory difficulties since standing aids effective ventilation. Of course this continuous standing leads to increasing fatigue.

Sometimes, epileptic excitomotor crisis occurs: a succession of starts on extended legs which causes a backward motion of the animal. Sometimes the pig circles the chamber's floor backwards two or three times. The crisis is accompanied by squeals, the animal breathes with open mouth, and sometimes a small quantity of sputum appears, that the pig chews. Sometimes the crises follow one another continuously, leading to the death of the animal. Often, however, their duration does not exceed a few seconds. After the crisis, a

small decrease of oxygen partial pressure can be seen, probably due to a temporary increase of metabolic oxygen consumption.

Examination of the nature and the causes of those symptoms suggested that a number of factors seemed to intervene:

1) The homogenization of the chamber's atmosphere is a very important factor, as it is possible to increase the pressure at which disturbances occur (33, 37–40, 42), by means of a good gas stirring. It seems possible to make disturbances occur under higher pressures, if the appropriate mixture was given to the pigs through a mask.

2) Compression is another important factor because, from the threshold-pressure, disturbances occur during pressure elevations and generally become weaker and less frequent as the duration of the overall period of consecutive stages continues. For example, when reaching the 111 ata pressure, the pig Raphael 3 showed less and less frequent and important excitative disturbances; after 6 hours, its behavior, except for standing on its legs and for not eating or drinking, was absolutely similar to the pig observed at 4 ata.

3) Apparently, it is impossible to exclude, at least as an associated factor, hyperoxia (Paul-Bert effect) as a cause for the crisis, since some alveolar hypercapnia (which should contribute to its occurrence) is very likely present. However, one must remember that the crisis was also observed under oxygen partial pressures averaging 0.45 bar, and during the last experiment, even under 0.23 bar.

As a rule, from the start of decompression, the animals (as if they were relieved) lay down. Decompression was a recovery period for the only animal brought back alive from a 111 ata maximum pressure: he slept much, ate and drank abundantly, contrary to his behavior under maximum pressure (only pigs number 16 and 17 ate beyond 81 ata). Excitomotor crisis was never noticed during decompression.

As for respiration, except that described before crisis and the polypneic episodes pointed out during inadequate decompression curves, the animals always breathed with the mouth closed, 12 to 14 movements in a minute. The increased respiratory dynamic resistances induced an inspiratory cock of the snout, a discrete presternal depression, and standing beyond 81 ata. The apparent small magnitude of respiratory disturbances was somewhat astonishing as was the fact that the pigs recovered normal breathing within a few minutes after the most animated crisis—this indicates very important adaptation possibilities. In fact, even when clinically discrete, dyspnea is important, as shown by the postmortem examinations (reported hereafter), and the frailty of the equilibrium; increased humidity and momentary augmentation of carbon dioxide partial pressure are sufficient to break it down.

Histological Examinations

The pigs died during the experiments, or were sacrificed thereafter and were the subjects of postmortem examinations.*

The site of the most obvious changes is the lungs, which show typical aspects of recent red infarcts, sometimes with very congestive thrombosed vessels. The parenchyma shows the

*G. Balouet, U.E.R. de Médecine, Brest, France, performed the autopsy.

aspect of a reticulated atrophic pneumonia. The vessels reveal a fibrous thickening of the arterial wall: this is somewhat similar to the lesions of endarteritis observed in pulmonary hypertension.

Those pulmonary anatomic alterations are aggravated by high humidity. The few cases submitted for 10 hours to high oxygen partial pressures (1.2 bar, 2.0 bars) suggested a discrete Lorrain-Smith pulmonary oxygen poisoning effect.

The remaining viscera, expecially the liver, show congestion and hemorrhagic lesions.

In the central nervous system, one can see as a rule a moderate edema of white substance of the brain and of the cerebral stem, whereas the gray substance looks normal.

Discussion

Satisfactory Dive Procedures

Often during the first experimental series and only once during the second one, the procedures were considered satisfactory. The animals tolerated compression with minimum disturbances. Sojourn under maximum pressure—even studded with some short excitomotor crises at first (which then became less and less frequent) and despite lack of sleeping, eating and drinking—allowed the pigs to begin decompression in a state sufficiently good to bear it without difficulties (i.e., without polypneic episodes, without paresis, and without articular pain inducing visible lameness).

It is clearly recognized that these procedures were barely tolerable by the animals, and anatomical examinations showed that visceral damage always existed. But it is not absolutely correct to call this "dysbarism," for the animals can overcome the disturbances within a few days. For example, the pig which was brought back alive to surface could, a month after, successfully impregnate a sow. This research is to determine limits, and effects must be expected (1,2).

Limits and Thresholds

At the present time, it is possible to pressurize a miniature pig to a pressure of about 81 ata, without visible behavioral disturbances; to maintain it under that pressure for several days; and to bring it back to surface without major damage.

Beyond this threshold-pressure, disturbances appear, varying greatly from one animal to another, and survival becomes an apparently aleatory phenomenon. It seems that, if duration on the bottom allows progressive disappearance of some abnormal phenomena, after a certain point, continuous fatigue suffices to impair the survival.

These results confirm and add to the results of others (especially Chouteau) using animals whose dimensions are comparable to ours (8-16, 18-24, 28, 29, 32).

Since many limits and thresholds have been exceeded shortly after their proclamation, likewise these limits will probably be extended. In particular, it will probably be possible, using a more conservative compression curve, to bring animals to decompression in a better state.

Nature of Abnormal Symptoms

Undoubtedly, part of the symptoms observed during the compression and the stay beyond 81 ata have some relationship with what has been described as the "high pressure

nervous syndrome" (6, 31). It is difficult, nevertheless, to assign only one cause to this pathological phenomenon, when several parameters vary at the same time and seem to have some effect on its precocity and importance (pressure, partial pressures of gases, speed of compression, etc.). Numerous etiological hypotheses have been propounded, and several probably play a part for the constitution of the syndrome. These include thermal stress, osmotic dysbarism (Kylstra, Longmuir and Grace), vagotonic disturbances of an hypoxic origin (Chouteau 8, 14, 15, 16, 24, 32), circulatory perturbations, and inert gases' pharmacological effects (6). It must be pointed out that a discrete edema of the white encephalic substance was noticed, in contrast with the normal aspect of the gray matter. The different saturation rates of the different parts of the nervous system, the perfusions of which are dissimilar, may affect the character of the syndrome (25, 26).

Respiratory disturbances observed beyond 81 ata are probably due to increased dynamic inspiratory resistances of the airways (edema of mucous membranes, in addition to an increased humidity and increased density of breathing mixtures). It is only logical to assume that a part of the origin of the disturbances is a possible change of surfactant (7).

Polypnea occurring during inadequate decompressions seems to be due, in part at least, to an excess of gas released in lung capillaries.

Applications to Manned Dives

Miniature pigs have a metabolism, a tissue composition and irrigation, and a weight not very dissimilar to humans. Hopefully, some of these results can be applied with great care to manned dives. This is easily true for some environmental parameters such as temperature or humidity.

However, the results obtained so far (and those we hope to obtain) concerned with compression or decompression, cannot be applied—just as they are—to manned dives. In the best case, it is hoped that some general information on the evolution of calculation parameters as a function of pressure and duration of the saturation dive can be derived. Human experiments, under less extreme pressures, cannot be avoided.

Acknowledgments

This study was supported by a grant from the Direction des Recherches et Moyens d'Essai (DRME), 5 bis avenue de la Porte de Sèvres 75015 Paris, France.

The authors would like to thank Prof. J. Chouteau from C.E.M.A. who provided the goat used in the comparative experiment, and whose friendship and advice were unfailing.

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FUNDAMENTAL STUDIES IN DECOMPRESSION FROM STEADY-STATE EXPOSURES

E. E. P. Barnard

The series of experiments described here was designed to investigate the time-course of decompression following steady-state exposures during which the divers breathed oxyhelium mixtures. In such exposures it is by no means certain when equilibrium can be said, for practical purposes, to be attained, but Hempleman and Trotter (5) found no significant difference between 4- and 8-hour exposures breathing 20/80 oxyhelium mixture upon subsequent decompression. Unpublished experiments by Young extended the exposure time to 24 hours and were essentially in agreement with the work of Hempleman and Trotter. The threshold exposure breathing 20/80 oxyhelium was found to be about 2.4 atmospheres (equivalent to about 10 meters when breathing P_{IO_2} equal to 0.22 bars). This figure was accepted without further test as a basis for the present experiments. For convenience also an initial exposure time of 24 hours was taken to be sufficient for the achievement of a steady state with respect to the inert gas dissolved in the body.

Method

Divers were compressed, three at a time, in the pressure chamber of the Deep Trials Unit (1). Throughout all but the first two dives in the series divers breathed 0.22 ± 0.02 bars oxygen except when diving in the wet chamber. The first day was spent diving and on subsequent days, during the decompression, the wet portion was decked in and the diving chamber used to carry out dry exercises for two sessions daily. Most dives were carried out in water at 15 °C and the chamber temperature was controlled for comfort in the region of 26°–29 °C. Humidity was uncontrolled and, with the presence of a free water-surface, was always in the region of 100%.

Carbon dioxide levels were maintained below 0.01 bar by internal scrubbers using soda-lime absorbent. Nitrogen was removed from the atmosphere by evacuating the pressure chamber before the dive and then compressing with oxygen and helium to the required pressure. Divers entered via the lock which was cleared of air by layering in helium gas while the divers breathed oxygen from the Built-in-Breathing-System (B.I.B.S.). With this method the nitrogen contamination could be kept below 2% of 1 atmosphere at the start of an experiment.

Compression and decompression rates did not remain constant throughout the experiments. Initially they were at the rate of 12 meters/minute. Technical problems of compres-

sion caused a change to 5 meters/minute and for some of the deeper exposures the rates were reduced to 1 meter/minute.

During the shallower dives the Surface Demand Diving Equipment (S.D.D.E.) was used with appropriate mixture for depth, the P_{IO_2} depending upon the oxygen consumption of the diver. At deeper depths a Windak apparatus, modified by Mr. Kettle of the Admiralty Experimental Diving Unit, was used. Inspired oxygen levels were uncontrolled and always higher during diving at maximum depth than in the chamber above the water. This entailed some adjustment of chamber levels to keep the P_{O_2} constant.

Instrumentation

The normal instrumentation of the Deep Trials Unit includes a Servomex OA 137 paramagnetic oxygen analyser and a Hilger SC/F Mark II infrared CO_2 analyser. Gas samples for checking these instruments and for the estimation of contaminants in the atmosphere were removed for analysis on an Associated Electrical Industries M.S.10 mass spectrometer. The gas used in this experiment was recovered and purified by a British Oxygen Corp., liquid nitrogen cryogenic unit which gave 90–95% recovery of helium.

Experimental Design

The production of a schedule for a dive to 1500 feet (2) suggested that currently existing mathematical models were inadequate when it was necessary to extrapolate to greater depths. The present series of experiments is intended to study depths only down to 250 meters, nevertheless it is important to be able to extrapolate information gained from 0–250 meters, should a need arise to dive deeper.

The first consideration, therefore, was to discover a satisfactory empirical equation which could be used to describe the shape of the decompression curve. The possibility exists that if assumptions are made at the outset as to the form of the decompression curve, then some solutions may be excluded since it is by no means certain that there is only one unique solution to each decompression.

The approach selected therefore was to search for the dive which would fit the previously determined stops. The initial stop was the 10-meter exposure calculated from the data of Hempleman and Trotter (5).

The method of searching for each new dive is presented in flow-chart form in Fig. 1 from which it can be seen that the experiment was designed to be variable in both depth and in time. The direction in which the search for a new dive was followed was determined by the previous result, for if bends resulted then the modification was first away from and then toward trouble, while if no bends occurred, modification was toward trouble and then away. This method of defining an edge may be expected on average to give rise to decompression sickness 50% of the time, providing that the alterations are large enough to go from the bends to the no-bends situation.

Steps of about 2 meters are usually large enough to show this characteristic in men, but the effects of acclimatisation may complicate the issue. A ratio method of calculation was therefore adopted to define the increments.

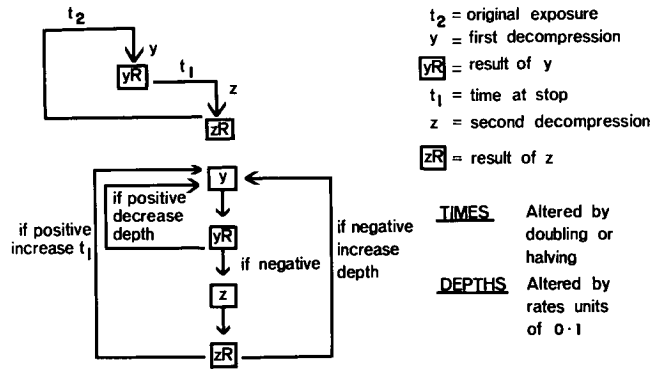

FIG. 1. Experimental design.

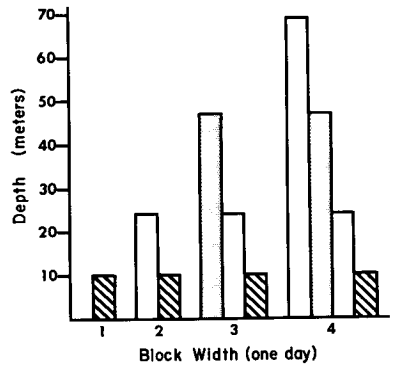

FIG. 2. Progressive stages in the development of schedules for steady-state exposures.

Results

Figure 2 shows that it was possible to define a series of 24-hour stops at 10, 24, 47 and 69 meters. This was achieved in 19 dives representing 57 individual exposures.

Although a duration of 1 day had been selected at the outset as being convenient both to the experimenters and in practice to the divers, it was not assumed that this was in any sense the correct time interval. A number of experiments was carried out in which one of the 24-hour intervals was shortened in an attempt to define more closely the shape of the decompression curve (Fig. 3). From this it can be seen that the shortened stops all led to the development of decompression sickness at the subsequent stop.

100 METERS

The progression of experiments up to and including 69 meters was relatively straightforward; however, an attempt to find the dive which would be followed by stops at 69, 47, 24 and 10 meters was not. In Dive 20, which was for 24 hours at 100 meters, one man com-

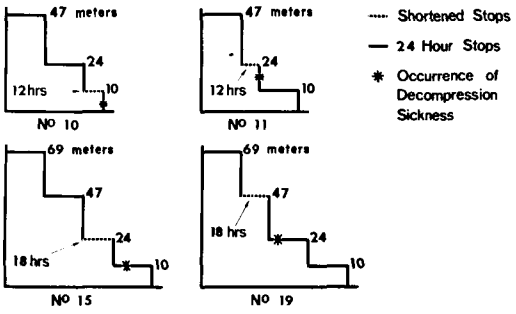

FIG. 3. The effect of shortening decompression.

plained of pain in both knees while exercising at 69 meters but was symptom-free when his knees were not under load; he did not complain on any subsequent drop until leaving 24 meters for 10 meters. Another of the divers, however, developed a bend in the right knee on dropping from 24 to 10 meters. Although no divers had developed a clear case of decompression sickness at the first stop (69 meters) it was assumed that the drop from 100 to 69 meters was too great and the following dive was therefore altered to 92 meters.

Dive 21 produced no symptoms on dropping from 92 to 69 meters but one diver developed acute symptoms on leaving 69 for 47 meters. These took the form of a Menière-like syndrome which appeared to be completely relieved by recompression to 69 meters. Subsequent examination by Dr. R. R. A. Coles of the Institute of Sound and Vibration, University of Southampton, led to a diagnosis of decompression sickness affecting the vestibular system, possibly due to a lesion in the brainstem.

The next dive was an attempt to assess whether troubles at a stop later than the first, without any preceding trouble, might be due not to a pressure difference (ΔP) which was too large, but instead to one which was too small. Dive 22 was therefore for 24 hours at 108 meters. One diver had some aching in the knees following exercise at 69 meters and all three developed severe knee pain on dropping from 69 meters; the decompression was halted at 51 meters. Recompression to 60 meters was largely successful, although one diver who did not experience complete relief still had some residual aching 7 days after the dive. These and two subsequent dives are shown in Fig. 4. This shows the attempt to avoid trouble on Dive 23 by extending the stop at 69 meters. Following ascent to 47 meters one diver complained of deafness in the right ear. Recompression to 75 meters and treatment of a preexisting otitis externa with Ampicillin 250 mg q.i.d. to a total of 7 gm gave no immediate relief but a subsequent drop from 62 to 54 meters seemed to produce a marked improvement. Postdive examination confirmed the clinical impression that the deafness was at least partly perceptive in nature.

Dive 29 illustrates the end of the second phase of schedule development since it shows a major departure from the original plan. The pressure drops found to be satisfactory in earlier experiments were halved and carried out in half the time; the overall pressure course thus remained the same except for the insertion of a 5-meter stop. Following this one diver had symptoms in both knees during the last three drops and another had slight discomfort on surfacing.

This phase therefore began with the failure to carry out a dive to 100 meters (no. 21) and

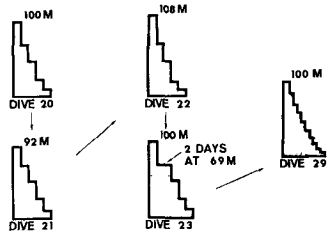

FIG. 4. Schedules for steady-state exposures: second phase of development.

ended with Dive 29 which was unsuccessful but seemed at last to show a way of exceeding 100 meters.

THE THIRD PHASE

The third phase, Dives 31–39 inclusive, involved attempts to use the information of the earlier dives to predict dives at greater depths. The first method used was to regard the time-course of decompression as representing a series of estimates of the decompression rate at various depths. Any values which departed markedly from the regression line were then adjusted. The greatest deviation was for the 47-meter stop which was therefore adjusted downwards to 42 meters. The resulting schedule gave rise to brief and transient aches in two out of three men.

In the following dive the time at maximum depth was extended to 60 hours to see if any gross difference from the previous dive could be detected. All divers complained of mild symptoms beginning as deep as 66 meters. For subsequent dives, therefore, two modifications were made: first, an adjustment of the mean rate of ascent at greater depths and second, the restriction of pressure drops to a maximum of 11–12 meters. This approach allowed extension to 137 meters with only slight occasional and transient symptoms related to each pressure drop.

EXPONENTIAL SOLUTION

Dive 34 was based upon a fresh approach. A comparison of the schedules for 33 and 34 in Table I shows that the form of the decompression has been altered to the familiar Haldanian pattern of equally spaced drops for varying and gradually increasing times. The initial depths and the total times are the same, but Dive 34 was calculated according to the following exponential equation, Eq. 1:

$$(L_\infty - L_t) = (L_\infty - L_o) \cdot e^{-kP} \tag{1}$$

Where

L_∞ = decompression time for dive of infinite depth

L_t = decompression time for dive of depth P

P = depth of dive for which decompression is required

A semi-logarithmic plot of $(L_\infty - L_t)$ against P, of the data from Dive 32 gava a best estimate for L_∞ = 200 hours (Fig. 5). From the duration of Dive 33, the value of k = 0.00788 was derived.

TABLE I

Dive 33		Dive 34	
Depth 137 m	Time 24 hours	Depth 137 m	Time 24 hours
125	8	130	4
113	8	125	6
101	8	115	6
89	8	105	7
77	8	95	7
66	8	85	8
54	12	75	9
42	12	65	10
33	12	55	10
24	12	45	11
17	12	35	12
10	12	25	13
5	12	15	14
		5	15
	Total = 132 hrs		Total = 132 hrs

Dive 34 produced the familiar complaint of minor aches and pains, some alleviated and others exacerbated by exercise, but all resolving quickly after each ascent. Extrapolation was therefore extended to 150 and then 180 meters. In this latter dive, decompression from 105 to 95 meters gave rise to minor aches and pains in two divers. Empirical adjustment of the deep stops was made in Dives 37, 38 and 39, all of which gave rise to mild bends at depth.

Parabolic Function

The schedule for Dive 40 has been calculated, but not tested yet, on still another basis. The deep portion of Dive 39 could be described by a decompression rate of about 1.67 meters/hour. This function was subtracted from the decompression curve to give a differ-

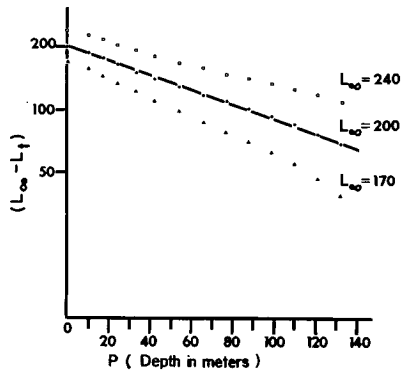

FIG. 5. Fitting of decompression curve—Dive 32.

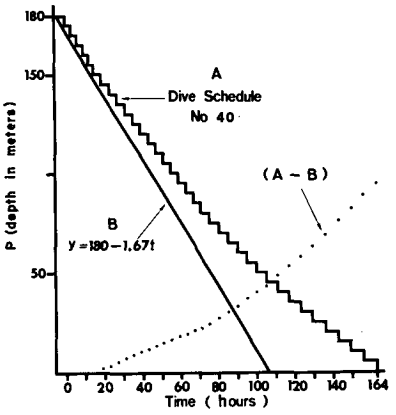

FIG. 6. Departure of decompression schedule from linear equation. (Difference plot A — B)

ence function. The latter function gave a reasonably straight line on a log-log plot. The technique is illustrated for Dive 40 in Fig. 6.

The empirical equation derived by this method is shown in Eq. 2:

$$P_t = P_o - 1.67t + 0.0104t^{1.67} \tag{2}$$

Where:

P_t = depth after time t
P_0 = original depth in meters
t = time in hours

DECOMPRESSION SICKNESS

Brief mention must be made of the endpoint on which all these theories are based. As previously mentioned, the theoretically expected incidence of decompression sickness was 50%. So far 44 cases have occurred (39% of exposures) 19 of which required recompression treatment (17%). Most cases have been simple limb-bends but two atypical cases have already been described.

Discussion

This necessarily condensed account of some 14 months' work involving 113 men-exposures naturally excludes much of interest but is intended principally to illustrate the methods used.

The initial attempt to be purely empirical failed, party because curiosity led to continuous attempts to interpret the data, but mainly because it was not found possible to extend the coarse steplike approach to decompression beyond about 100 meters. The second phase described was therefore a series of attempts to find a way out of the maze. Conjointly with Walder and Evans we had been carrying out routine monitoring with an ultrasonic transducer throughout these dives in an attempt to detect bubbles in vivo and to correlate these findings with the amount of decompression sickness. The impression gained from this work led directly to the hypothesis that the decompression steps then used were too large, and a reduction in these steps seemed to allow further progress.

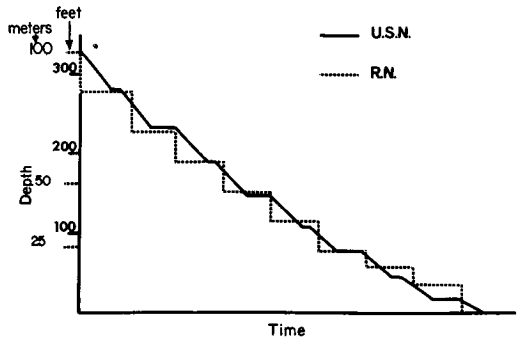

FIG. 7. Comparison of U.S. Navy schedule with Dive 28.

The third phase began when sufficient points had been defined to attempt an estimate of the shape of the decompression curve.

Previous attempts by the author to use a single exponential had been unsuccessful (6). It was clear that the solution was not linear. The attempt to use the exponential Eq. 1 was abandoned since it was found that decompression at depth required more time than predicted, indicating that the curve was not asymptotic to a line parallel to the y axis.

The model upon which the current solution is based is as follows: Equal masses of gas are considered to be liberated by equal decrements of pressure, hence a linear term should be involved. Secondly, equal masses of gas occupy volumes which are inversely proportional to the pressure. The physical dimensions of such volumes of gas will be inversely proportional to some power of the pressure; for spherical bubbles, indeed, the radius would be inversely proportional to the cube root of the pressure. The underlying assumption which we believe to be supported by experiment (4) is that this type of decompression always leads to the formation of intravascular gas emboli.

The general method described—that of extrapolation followed by empirical adjustment and refitting of the curve with further extrapolation—represents a method of progressive refinement by which it should be possible to exclude unsatisfactory mathematical relationships, but which does not in itself suggest any particular solution. The decompression curve so far developed with its deep linear portion and its shallow curved section—shaped like a hockey stick—presents a striking similarity to the U.S. Navy schedules for these depths (3) shown in Fig. 7, but it remains to be seen whether future experiments will confirm this convergence.

ACKNOWLEDGMENTS

Grateful acknowledgment is made to the divers of the Royal Navy and especially the Admiralty Experimental Diving Unit under Lieutenant Commander J. Naquin, USN, who carried out the dives described.

To the Officer in Charge of the Deep Trials Unit, Lieutenant Commander W. B. Filer, RN (Retd.) and his team of experts for their willing cooperation. To the Superintendent of

Diving, whose zeal in maintaining general interest in these experiments and in seeking volunteers for them was unremitting.

To my colleagues, Captain R. C. Bornmann, MC USN; Surgeon Commander D. E. Mackay, RN; Surgeon Lieutenant Commander R. de G. Hanson, RN; Surgeon Lieutenant D. R. Leitch, RN; and to two helpful visitors, Surgeon Lieutenant B. Reid, RN and Surgeon Commander J. Smith-Sivertsen of the Royal Norwegian Navy, who shared the medical responsibilities over a long period.

To the skillful assistance of Mr. J. Williams and Petty Officer M. S. Martin, and finally to the Superintendent of RNPL, Dr. H. V. Hempleman, for much helpful discussion during the past 12 years.

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DECOMPRESSION SICKNESS STUDIES

J. M. Hallenbeck, A. A. Bove and D. H. Elliott

The treatment of decompression sickness by immediate recompression is usually fully effective. Such treatment becomes difficult only when there is no chamber readily available or when the recompression is, for some reason, ineffective.

In 38% of a group of civilian divers there was a delay of more than 6 hours before treatment (40). Initial recompression was not fully effective in 24% of the cases of decompression sickness treated at the U.S. Navy EDU during 1963 and 1964 (47). Recurrences after recompression have occurred in some 20% of cases treated with air tables (7, 30). Although initial recompression breathing oxygen brought complete relief to 84% of all patients treated, 17% of civilian cases (in whom severity is often associated with delay) were left with substantial residual injury (47).

There is, therefore, a need for alternative or supplementary treatments to be given under these circumstances. The occasional use of drugs such as heparin has been reported (2, 33) but no rational approach to drug treatment has been defined. The ability of heparin and other drugs to reduce the incidence and severity of decompression sickness (5, 14, 35) is not necessarily relevant to their possible application in the treatment of the acute illness. Therefore, a study to evaluate selected drugs in the treatment of decompression sickness, with particular emphasis on the neurological complications, was begun. First, however, it was felt necessary to try to clarify certain aspects of the pathophysiology of decompression sickness and, in particular, to investigate the time-course of events leading to decompression paraplegia in order to provide a better basis for drug studies.

There are several problems which any hypothesis of decompression sickness must explain. One is that of the silent bubble, the presence of which has been suspected for some time. Several laboratories using ultrasound have shown that silent bubbles exist in the circulation of apparently healthy individuals after decompressions which cause no overt manifestations of decompression sickness (43, 46). Evidence has also been provided from double dive experiments (17) that silent bubbles from a first dive may precipitate decompression sickness after a second dive under conditions that a priori might be thought to be relatively safe. A remaining problem is that of the role of the silent bubbles during the latent period between the decompression and the onset of manifestations. This may be considered in terms of the quantity of silent bubbles that might be considered safe. From this arises the concept of some "dose" of intravascular bubbles that causes a particular pathological re-

sponse, a dose measured at the lungs in terms of not merely the size and number of bubbles but also as a function of time.

A second problem is that of the relative importance of the various pathological mechanisms which the bubble can initiate. In addition to any embolic effect such as vascular obstruction with ischemia or infarction and release of local humoral agents, the bubbles can produce a number of indirect effects in the circulating blood. There is growing evidence that a number of aspects of decompression sickness can be considered as secondary consequences of circulating bubbles. For instance, a significant fall of platelets after safe and unsafe dives (34, 37, 38, 41, 42) has been related to disseminated intravascular coagulation as a possible feature of decompression sickness (25, 26, 32, 36).

Another problem is that of the distribution of neurological lesions in decompression sickness. Several considerations favor the brain to be that portion of the central nervous system predominantly affected by arterial emboli. In clinical disorders such as subacute bacterial endocarditis, fat embolism or the presence of a mural thrombus in the left atrium, the brain is the principal target organ, whereas arterial embolism of the spinal cord is extremely rare. For instance, in a series of 3737 autopsies at a hospital for neurological diseases there were only 11 cases of vascular disease of the cord and none with arterial emboli (6). In fact, a brief study of current textbooks of neuropathology shows that most authors when describing arterial emboli refer to the cord only because it is the site of the lesions of decompression sickness.

Furthermore, it has been demonstrated experimentally that bubble emboli are not only distributed with arterial flow, but also according to their buoyancy (45), a finding supported by the dominance of brain involvement in air embolism complicating the pulmonary barotrauma of submarine escape training and related rapid ascent accidents. Finally, with gross proportions of gray to white matter roughly the same in brain and cord, the brain constitutes some 98% of the mass of the human CNS. Having some 60 to 70 times more total blood flow than the cord (27), the brain should accordingly receive proportionately more systemically distributed emboli.

Standing in stark contrast to the cerebral distribution of arterial emboli is the dominance of spinal cord lesions in the neurological manifestations of decompression sickness. Besides a possible greater lipid content (11), the explanations for this can be related to the blood vessels of the cord. The arterial supply is said to be less in the cord than in the brain and to be relatively sparse in the deeper regions of the white matter (10, 22). The rate of blood flow through the white matter of the cord is only some 60% that of the white matter of the brain (27). The venous drainage feeds into Batson's plexus (3), the longitudinal vertebral venous plexus of veins and sinuses in which flow is closely related to intrathoracic pressures. Thus a study of the pathology of decompression sickness also requires a reexamination of the role played by these factors.

Hypothesis

Previous reviews have considered the site of origin of the bubble (12, 16, 31): de novo in the capillaries and the veins; extravascularly and perhaps via the lymphatics to the general circulation; or as a result of what might be termed micro-barotrauma, a release of very small bubbles from a few over-distended alveoli during the course of decompression, bub-

bles which rapidly pass through the arterial circulation to capillaries where excess gas tensions may make them grow. Whatever their origin, intravascular bubbles may cause purely obstructive effects in both the pulmonary and systemic capillaries and also, as a consequence of their surface activity, nonobstructive indirect effects throughout the circulation.

A 40–100 Å layer of electrostatic forces at the gas–blood interface tends to orient exposed globular proteins such that their hydrophilic groups are in the blood while their nonpolar groups protrude into the gaseous phase with a resultant disruption of the native secondary and tertiary configuration of the proteins (28, 29). Thus surface activity of the bubble can lead to platelet aggregation, clumping of red cells, unmasking of active sites of enzymes, denaturation of other enzymes, formation of lipid emboli from lipoproteins and activation of the Hageman factor. This will begin a series of events leading to activation of the complement system, intravascular coagulation and the release of kinins and other smooth muscle activating compounds (39) (Fig. 1). By incorporating these fundamental reactions of the blood into a current hypothesis of decompression sickness some new possible patterns of dynamic pathology emerge.

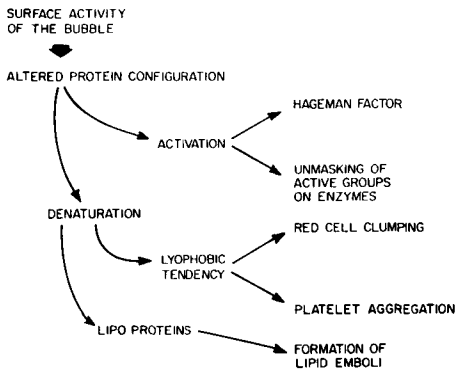


FIG. 1. Possible consequences of the surface activity of intravascular bubbles.

Thus silent bubbles, along with some platelet and red cell aggregates, lipid emboli, vasoactive compounds and altered proteins are carried to the lungs. The release of local humoral agents (13) may be a consequence of pulmonary microvasculature obstruction by these various emboli. It seems probable that the response of the lungs to emboli will depend on the rate of arrival of the emboli. A few bubbles may lose their gas by excretion through the lungs and the normal dynamic equilibrium within the blood will be restored. A few more bubbles may, by their obstructive effects and by their continued surface activity, have some small effect. It is significant that fatigue and anorexia which are symptoms associated with subclinical disorders of the pulmonary circulation are often present in the presymptomatic phase of acute decompression sickness. A greater rate of arrival of bubbles may cause an asymptomatic tachypnea associated with interstitial pulmonary edema. Yet more bubbles may lead to a rise of pulmonary artery pressure, central venous pressure, bronchospasm and, secondarily, a marked increase in the cyclic swings of respiratory inthrathoracic pressures.

In a particular individual this may account for the onset of the acute respiratory crisis known as "the chokes." The interstitial pulmonary edema together with any edema elsewhere will lead to hemoconcentration, impaired flow in the microcirculation, and eventually hypovolemic shock. Alternatively, or sometimes additionally, this chain of events may lead to spinal decompression sickness.

Associated with the increased swings of intrathoracic pressure is a change in the flow patterns of venous return. This is particularly so in the longitudinal vertebral venous plexus. Congestion will occur and may lead to local obstruction of venous outflow. Thus a situation is produced which favors the formation of local bubbles and hemorrhagic infarcts in the brainstem and spinal cord.

Spinal decompression sickness is frequently considered to be associated with a period of respiratory embarrassment (4). In a series of 24 neurological cases of decompression sickness (15), chest symptoms were recorded in 12. Of the persons in whom the records describe separate times of onset, six had chest symptoms which preceded the spinal symptoms.

The roles of congestion in Batson's plexus and of arterial embolism were considered by Haymaker and Johnston (23). It is hypothesized here, however, that there is not merely a generalized passive congestion of the plexus as a result of pulmonary vascular obstruction, but a localized area or areas of complete obstruction to venous flow arising from the relationship between venous drainage of the epidural vertebral venous plexus and from the swings of intrathoracic pressure. To these may be added the indirect result of the specific surface activity of intravascular bubbles. It is believed that the sum of such factors without arterial bubbles, is sufficient to account for local bubble formation and spinal cord infarction.

Experimental Procedures and Results

Surface Activity in Vitro

Studies of the acceleration of clotting of whole blood and of cell-free plasma by bubbling in vitro were undertaken (20) to evaluate the possible significance of bubble surface activity. If the Hageman factor is converted into the active form by bubbles, not only should the clotting sequence be initiated but also complex interactions will begin leading to the production of kinins, plasmin and C'1 esterase.

Samples of whole blood and cell-free plasma from human volunteers were each divided into three aliquots, and these were given distinct but simultaneous treatment for 1½ minutes. One aliquot was bubbled and agitated in a partially evacuated siliconized syringe, another was only agitated in a siliconized syringe, and the third was exposed to glass. Lee-White clotting times were then run concurrently on each of these aliquots in siliconized tubes.

Platelin was added to the cell-free plasma as a source of phospholipid. Omission of this step prolonged clotting for several hours, thus confirming the observation by phase microscopy that platelets were indeed absent in the cell-free plasma.

The results (20) show that bubbles in whole blood accelerate clotting even more than exposure to glass, but that in platelet-free plasma the acceleration, though present, is less dramatic. Samples of blood were also taken from persons who had ingested 1000 mg of

aspirin 3 hours previously in order to inhibit platelet aggregation. These also showed acceleration by bubbling but less than that in the normal whole blood.

Our interpretation is that bubbles accelerate clotting not only by promoting platelet aggregation, but also by accelerating it in the absence of platelets by the activation of a plasma factor, probably Hageman.

Decompression Sickness in The Dog

Animals

Conditioned male mongrel dogs, 16 to 20 kg, were used. At least 3 weeks prior to studies of plasma volume and, in some animals, hematological indices, a splenectomy was performed. In some dogs splenectomy will cause a hemolytic anemia due to previously dormant *Hemobartonella canis* infection; therefore blood smears to exclude active hemobartonellosis were made from all dogs before participation in the dive program.

Dive profiles

All dives were at 75 feet/minute to 220 feet on air with a decompression rate of 60 feet/minute. In addition to a safe dive, 220 feet for 5 minutes, an unsafe dive profile was designed in order to provoke "limb bends." The concept was that, by recompression during the latent period while still sign-free after an unsafe dive, the limb-bend would occur under pressure where it is easily controlled during the subsequent slower decompression (Fig. 2). Another profile was used to provoke the neurological manifestations of decompression sickness. After decompression from 30–40 minutes exposure to 220 feet the dog was recompressed as necessary to minimize respiratory distress and the first neurological signs were usually observed in the course of that "therapeutic" recompression.

Venous samples were taken before the dive, 1 hour and 4 hours after surfacing, and again the next morning, some 23 hours after surfacing. No attempt was made to pursue any deviations from normal after the time-course of the acute phase of the illness. In addition to samples related to the unsafe dive profiles which produced limb-bends or paresis, samples were taken from dogs subjected to safe dives and to baseline control periods. Series were run on two groups of dogs, intact and splenectomized.

Results are shown for hematocrit (Fig. 3) and platelets (Fig. 4) expressed in terms of percent change from predive value. Other results (44) include platelet adhesiveness, blood viscosity and fibrinogen. A significant feature, present in both the intact and the splenectomized series, was that the dives in which limb-bends occurred showed very little difference from the safe dives.

Neuropathology

Shortly after a dive which produced paresis, dogs were examined neurologically and then given intravenous Evans Blue 30–60 minutes before anesthesia and sacrifice by perfusion with 10% formalin. Evans Blue, a dye that becomes bound to albumin in vivo, is excluded from all areas of the CNS that have an intact blood–brain barrier. When the blood–brain barrier is damaged the lesions are revealed macroscopically by blue staining.

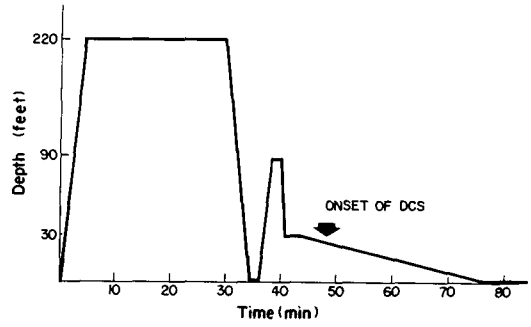
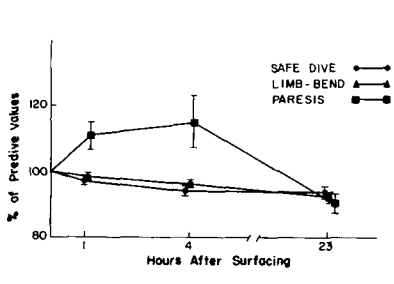


FIG. 2. An example of the dive profile which provoked a limb-bend in a conditioned mongrel. (Dog 609, 13 January 1972.)

The locations of the lesions as predicted by the preceding neurological examination were in close agreement with those demonstrated at autopsy by the Evans Blue. In 17 cases of paralytic decompression sickness, all had involvement of the spinal cord and in two there were also brainstem lesions. There was a surprising absence of cerebral involvement. The infarcts were grossly hemorrhagic and the white matter was principally affected. Together with the relative sparing of the gray matter these form a pathological picture typical of venous infarction of the CNS (1, 24). Venous congestion and bluing was also observed in the paravertebral muscles corresponding to the region of spinal cord damage. Epidural and subarachnoid hemorrhages were also noted. The dorsal root ganglia, which are in close anatomical relation to large tortuous veins (47), stain deep blue after paretic dives.

Air injected through a catheter at the root of the aorta, with the dog anesthetized in the prone position, produced bluing that was confined to the uppermost parts of the CNS, namely in the regions of the occipital lobe and the cerebellum. When air was injected through a catheter in the descending aorta, there was bluing in a portion of the lumbar cord. In contrast to the lesions of decompression sickness these lesions were of the gray matter with little associated hemorrhage.

Thus the nature and distribution of the lesions in these studies of paretic decompression sickness (18) appear to favor venous rather than arterial obstruction as the cause of spinal cord damage.



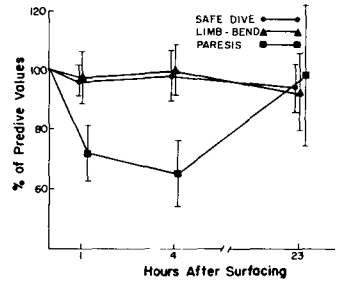


FIG. 3. Hematocrit as a percentage of predive values (mean ± standard errors) after safe dives, limb-bends and paresis in splenectomized conditioned mongrels.

FIG. 4. Platelet index as a percentage of predive concentrations (mean ± standard errors) after safe dives, limb-bends and paresis in splenectomized conditioned mongrels.

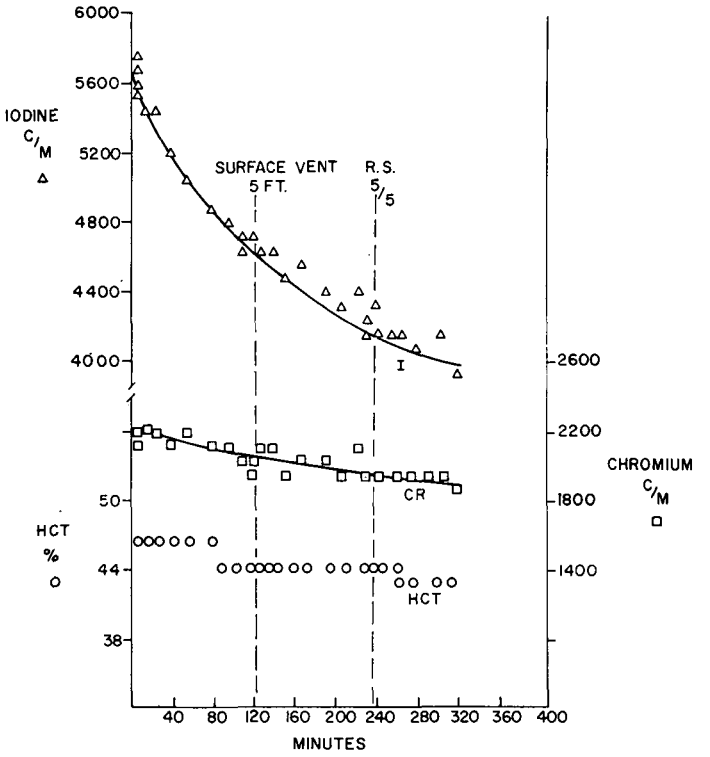


FIG. 5. ^{125}I albumin and ^{51}Cr RBC kinetics after a sham dive and a shallow dive (5 ft for 5 min). (Dog 614, 27 January 1972.)

Labeled albumin may give a misleading picture of blood volume when there is also an increased permeability that permits albumin loss into the tissues. Besides ^{125}I-labeled albumin, ^{51}Cr-tagged red cells were used in this study of decompression sickness (8). Red cells will not leak from the capillaries as readily as albumin and represent an intravascular indicator not affected by water or protein shifts from the intravascular space. After injection of the isotopes, samples were taken for at least 100 minutes to establish a baseline washout curve prior to any experiment. Then a dive was performed on the awake dog after which samples were taken to measure changes in the isotope concentration curve from the predicted curve established during the initial period. As a measure of the reproducibility of this technique, 200 ml of blood were withdrawn from one animal and later replaced. Total blood volume increase calculated from ^{51}Cr was 207 ml and from ^{125}I was 265 ml. Some examples are shown from the series (8): the first dog had, after the baseline period, a 12-minute period at atmospheric pressure to simulate a dive interval and, after a further 100 minutes of sampling, a dive of 5 feet for 5 minutes. The samples were then continued for a final 100 minutes (Fig. 5).

Another dog after the baseline period was given a safe dive, 220 feet for 5 minutes; 120 minutes later the dog was given a dive, 220 feet for 27 minutes sufficient to cause a limb bend and mild "chokes" from which the dog made a complete recovery spontaneously (Fig. 6). Contrast this with the next example, a dog which after the baseline period was

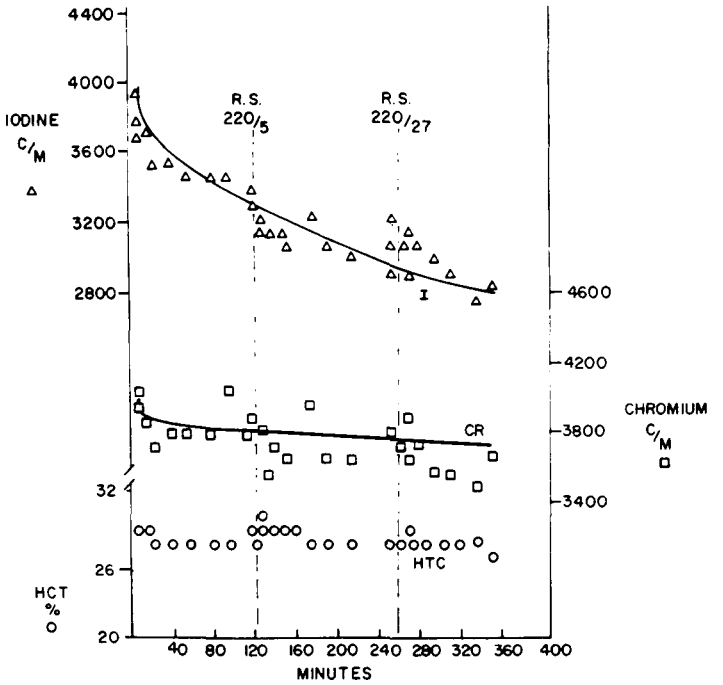


FIG. 6. ^{125}I albumin and ^{51}Cr RBC kinetics after a safe dive (220 ft for 5 min) and a limb-bend dive (220 ft for 27 min). Mild chokes, limb-bend noted upon surfacing; dog recovered completely. (Dog 695, 2 February 1972.)

given a dive to 220 feet for 60 minutes. Severe "chokes" and a paraparesis were noted on surfacing and during recompression the dog's condition deteriorated rapidly until death some 70 minutes later (Fig. 7). A marked increase in ^{51}Cr concentration and hematocrit indicate that blood volume has diminished. ^{125}I concentration remains fairly constant, however, suggesting that plasma loss occurs isotonically.

ANGIOGRAPHY

Infusion of a volume of air into a peripheral vein caused bronchospasm, tachypnea, tachycardia, and large respiratory swings of central venous pressure. There was also an early rise of systolic right ventricular pressure. During this phase, blood flow from the vertebral veins into the thorax was either unchanged or increased. As more air was injected, a greater rise of systolic right ventricular pressure occurred and central venous pressure and end-diastolic right ventricular pressure also rose. A tachypnea of 70 to 85 per minute was reached and at this time azygos cinevenography (Fig. 8) showed that the flow from the vertebral system into the thorax was slowed. Contrast medium in the thoracic vertebral veins flowed cephalad and caudad and, in one instance, particulate contrast medium in the azygos vein was seen to flow into the vertebral venous system (9).

Similar studies in decompression sickness were undertaken using a dive profile similar to that described previously but, since the animals were anesthetized with morphine and chloralose, a slightly longer bottom time was required. Cord lesions were manifested by extensor rigidity, loss of panniculus reflex and, occasionally, paralysis of the diaphragm requiring respiratory assistance.

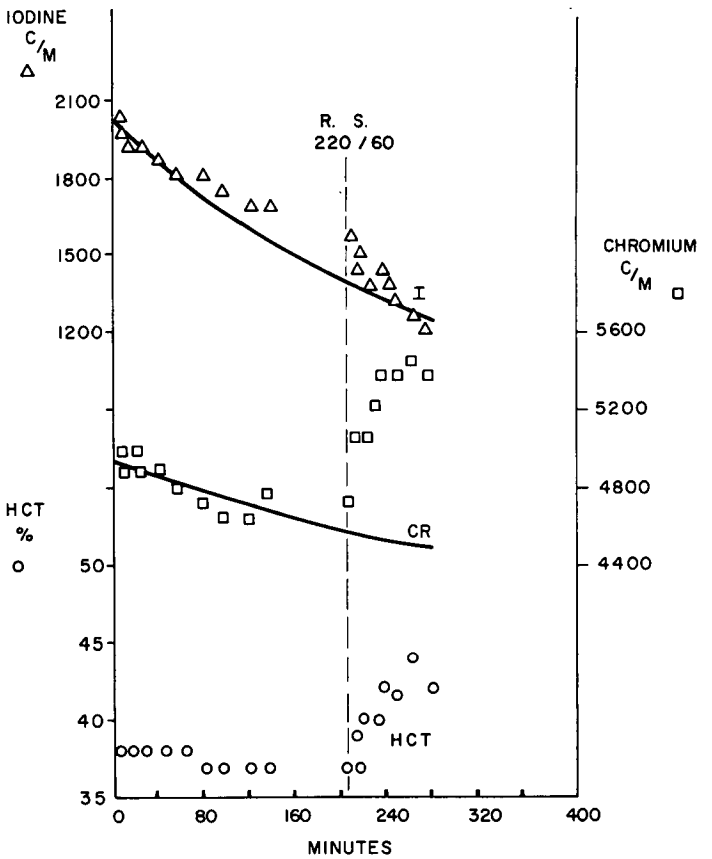


FIG. 7. ^{125}I albumin and ^{51}Cr RBC kinetics after a safe dive (220 ft for 5 min) and a paretic dive (220 ft for 32 min). Severe chokes, paresis noted upon surfacing, then death. At R.S. + 70. (Dog A23, 1 June 1972.)

Azygos cinevenography was supplemented by intraosseous venography in which injections of radiopaque medium, given through a needle inserted into the spinous process of a vertebra, quickly pass into the vertebral venous system. Both azygos and intraosseous venography (Fig. 9) demonstrated changes from predive flow patterns after the onset of neurological decompression sickness, suggesting obstruction of parts of the epidural vertebral venous plexus in the paretic dog (19).

CSF MANOMETRY

Under similar dive conditions, with and without angiography, tracings were made of cerebrospinal fluid pressures in addition to the intravascular pressures. Manometric responses of cisternal spinal fluid pressure to abdominal compression and to lung inflation were normal before the dive (Fig. 10). Cisternal pressure rose shortly before the onset of a cord lesion and returned to normal during the next 30 to 45 minutes. However, after the signs of spinal cord damage had appeared, the lack of manometric response in the cisterna magna to lung inflation and abdominal compression (Fig. 11) suggested an obstruction of the vertebral venous system.

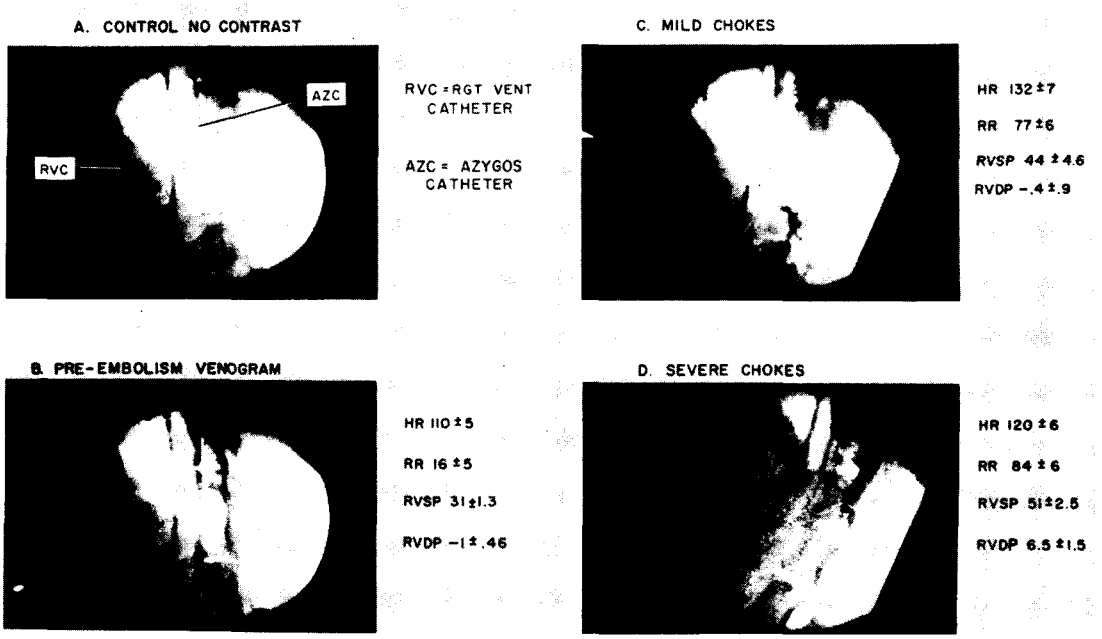


FIG. 8. Still photographs from azygos cinevenography before and after venous air embolism, from mongrel dog under chloralose anesthesia.

Conclusion

While the evidence in support of the hypothesis described is incomplete, this summary of work, detailed elsewhere, does serve to emphasize the complexity of the pathological processes that occur during decompression sickness. This work and the evidence available from other laboratories fit to form a logical and cohesive pattern (21). The focal point is now upon the surface effects of the bubble and much has yet to be learned of the ramifications and implications of this upon the course and treatment of acute decompression sickness. However, it is important to remember that while the many different pathological pathways that may be followed are known, it is not yet possible to evaluate their relative importance or to assess fully the importance of their cumulated effects vis-a-vis the direct obstructive effects of the bubble. The importance of this approach toward a clarification of the pathophysiology of neurological decompression sickness is in suggesting new possibilities, particularly in terms of alternative and supplementary treatments when recompression is unavailable or ineffective.

ACKNOWLEDGMENTS

The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

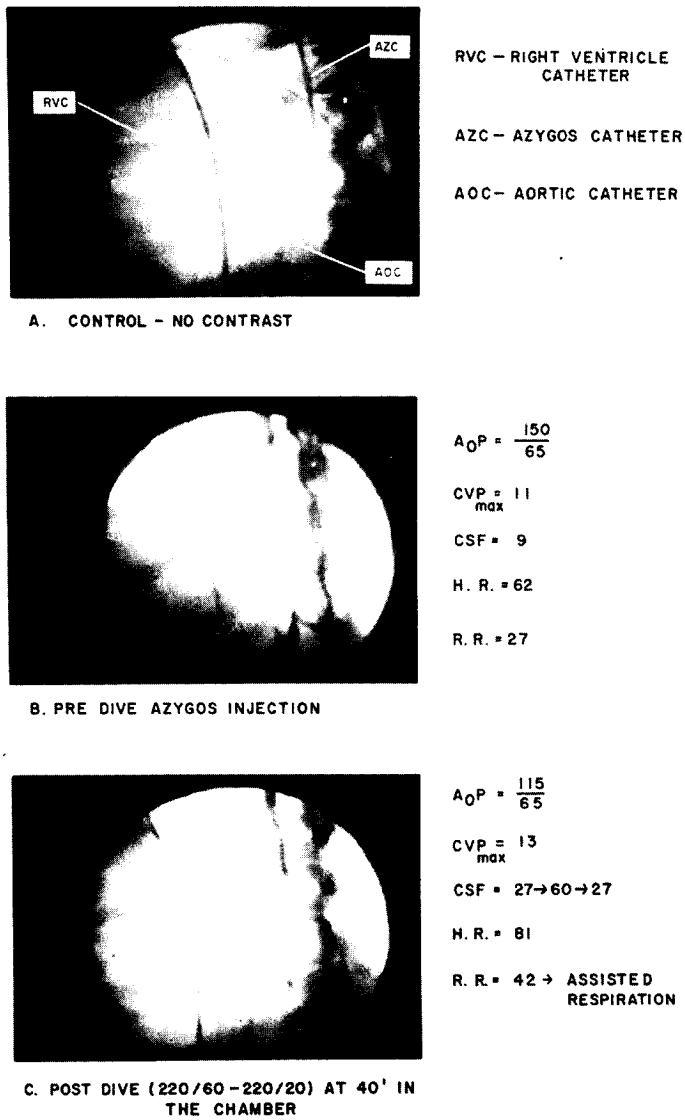


FIG. 9. Still photographs from azygos cinevenography in acute decompression sickness. Changes in azygos-vertebral filling pattern after development of chokes in chloralose anesthetized dog.

The major portion of this work was supported by the Bureau of Medicine and Surgery, Navy Department (Work Unit M4306.01.1150AAK9 and Work Unit M4306.01.1060BGK2). Other portions were supported by the Temple University Medical School, Philadelphia (USPHS NIH Grant No. HE08886) and the F. G. Hall Laboratory for Environmental Research, Duke University Medical Center (USPHS NIH Grant No. HL07896) to whom the authors extend grateful acknowledgments and thanks.

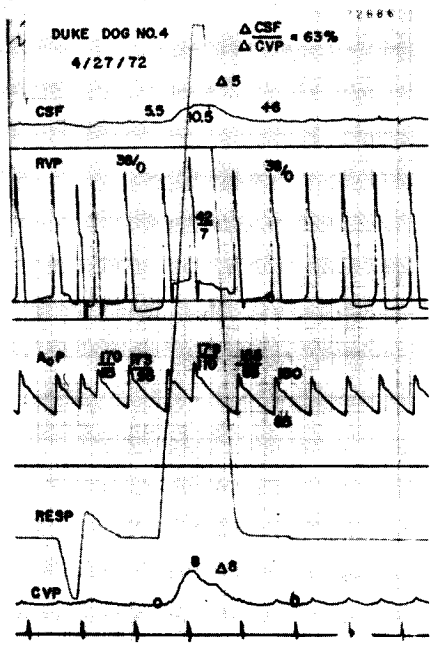


FIG. 10. Manometric responses during a control period.

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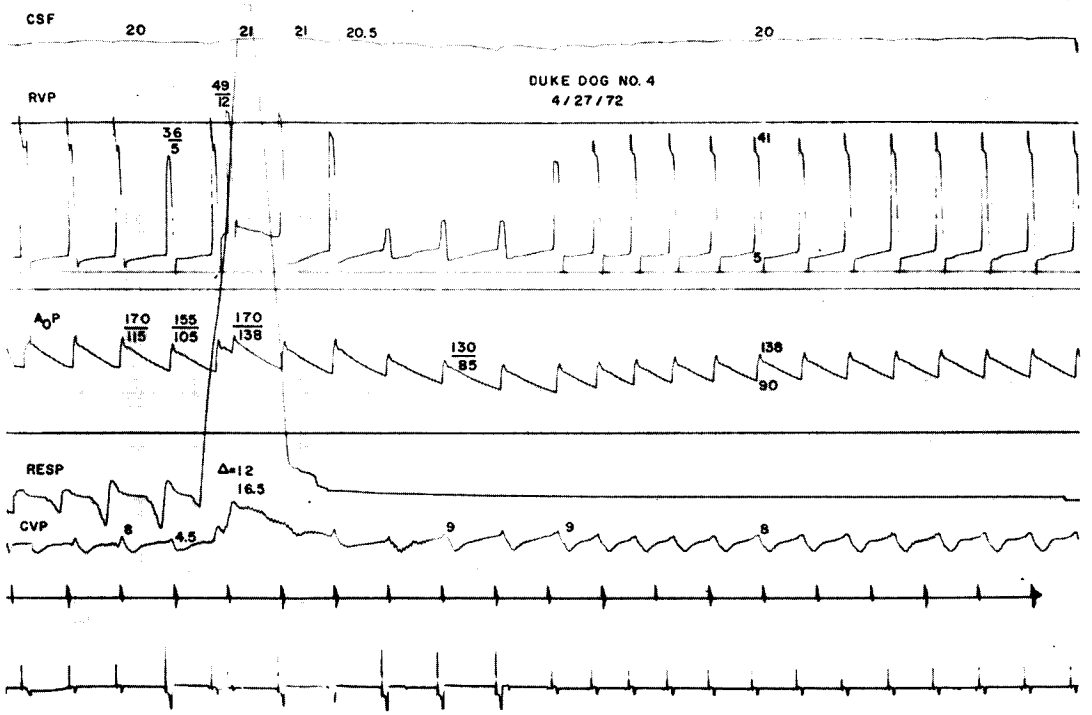

FIG. 11. Manometric responses during acute decompression sickness.

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PART IV. DYSBARISM*

DISCUSSION

C. M. Hesser, Chairman

Dr. Spencer: I wonder if Drs. Elliott, Hallenbeck or Bove could tell us more definitively something about the obstruction in the vein. Can you find, for example, thrombus formation in the vertebral veins at autopsy or is it possible that it is a change in direction of blood flow that produced the angiographic results?

Dr. Hallenbeck: That is a good question: the point that the angiographic studies show just the dynamics of flow and do not actually show obstruction is a very good point. We did some very recent experiments not reported here in which we drilled a hole into the confluence of sinuses of the dog, which is conveniently encased in bone, and inserted a polyethylene catheter. We have visualized vessels in the dog by occluding the external jugular veins and then injected contrast material into the confluence of sinuses. Normally exit via collateral paths permits filling of the sinus system. We have done the vascular visualization also in dogs when spinal cord damage was evident. We again occluded the external jugular vein, but instead of collateral channels being open, there were actual cutoff points in veins. There was a cutoff point in the internal jugular and one in the vertebral. They are the collateral channels of flow and all that was seen was blood stagnating above our ligatures. This was a reasonable demonstration of obstruction in the venous circulation. We do not want to appear to be challenging the existence of arterial emboli. I think they have definitely been shown to exist. What we question is the dominance of arterial embolism as a cause of spinal cord lesions. In no other known condition where arterial emboli occur (examples might be subacute bacterial endocarditis, fat embolism, bland embolism from such things as a thrombus in the left atrium or left ventricular thrombus, or what is common in diving—air embolism) does the spinal cord figure prominently as the site of damage; this really stands in stark contrast with the situation in decompression sickness, where the spinal cord is a predominant site of lesion. So we have looked to other explanations.

The other thing is that in our air embolism experiments there was an early phase when flow appeared to actually increase in the vertebral venous system. One can visualize this possibly as occurring because of a collapse of the inferior vena cava at the diaphragm during periods of pressure differential across that diaphragm. When there are large swings of intrathoracic pressure early and the negative pressure goes way down, you might expect with the higher abdominal pressure the inferior vena cava could collapse. The vertebral venous system is a great low pressure valveless system which is exquisitely sensitive to changes in pressure in the cavities, and during this period we have observed increased flow through the vertebral system.

One could then postulate that during this time, early, when bubble-laden blood is going to the lungs, there is some favoring of the longitudinal vertebral venous system and bubbles actually would get into it at that period of time.

Dr. Bove: The reason we decided to label two intravascular markers was that hemoconcentration was occurring as measured by hematocrit; we felt that if this were due to a plasma shift because of the leak of plasma into some space—either pulmonary or systemic interstitial—as an isotonic shift relative to protein, we would not see albumin changes because the concentrations of albumin, including the isotope label, would not change. Simply, more labeled albumin would go out into the interstitial space.

This we feel has occurred. We see marked hemoconcentration, marked concentration of the red cell label, while the albumin shows little change. There are changes in albumin; in some other experiments it went either up or down. But basically the albumin is not easily predictable.

There is some evidence from Haymaker's review of pathology indicating that severe pulmonary edema is one of the terminal aspects of decompression sickness. I believe Dr. Wells has also found in some animals (pigs, I think) that pulmonary edema does occur during the time of hemoconcentration. So we feel this type of information

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**Panelists*: C. Agarate, E. E. P. Barnard, A. A. Bove, D. H. Elliott, J. M. Hallenbeck, M. J. Halsey, H. V. Hempleman, C. M. Hesser, A. Michaud, S. M. Pauley, F. Sicardi, and C. H. Wells.

indicates there is plasma leakage. It probably leaks into the lung more than any place else. After all, the lung is the end organ receiving most of the embolic insult. And this deranges lung function to cause oxygen unsaturation and eventually CO_2 retention and loss of pulmonary function—probably because of this massive plasma leak into the lung which results in hemoconcentration and possibly shock.

Dr. Freed: Is the composition of lipid emboli in decompression sickness known? I ask because most circulating lipid is bound in the form of lipoproteins. This is the substrate for lipoprotein lipase and has a characteristic composition. What lipoprotein lipase does is release, from triglyceride largely, free fatty acid.

I wonder, then, what the composition of a lipid embolus is and what the activity of lipoprotein lipase in this respect can be?

Dr. Pauley: I believe Dr. Hillman actually produced bends in rabbits and is trying to determine the composition of the lipid emboli. I believe they found that they were mostly cholesterol, which they feel did not come from fatty stores. The lipid emboli they felt were being generated in the plasma rather than from fatty tissue breakdown.

Dr. Freed: Then I would suggest that probably the largest store of cholesterol available to the circulation is the cholesterol in the red cell membrane, and there are enzymes that can transfer it to circulating materials.

I would also suggest that somebody do some lipoprotein studies. These are very easy to do. You can do them with paper electrophoresis and find out whether there are shifts in the quantities of the different classes.

Dr. Orris: Heparin has been mentioned several times today among the factors affecting the coagulation of blood. I have two questions I would like to ask about heparin, one clinical and the other scientific.

Is it the consensus of the panel that heparin should be used in the treatment of decompression sickness?

The scientific question is this: Heparin is a normal and present constituent of all human blood, but I have seen no evidence that this has been taken into consideration. If it is a constituent of human blood, then it would seem advisable to study the degree to which it is present and perhaps do some further research as to the effects—when it is present in larger than usual amounts (or Vitamin K)—on these compression studies.

Has anything been done on the amount of heparin present in various individuals, to a greater or lesser degree?

Dr. Pauley: As far as I know, in decompression studies nobody has measured heparin levels. But it is feasible that with massive emboli to the lungs, where I believe heparin is in high concentration, with capillary damage you could have an extrusion of heparin. I would tend to doubt that the amount would have any detectable effect, or would change the clotting mechanism to the extent of reversing the symptoms of bends.

Our results with heparin are different from Dr. Reeves' and Dr. Workman's studies, but our models were different as well. We produced severe bends in our dogs. We had an LD-100 model, whereas the Reeves-Workman study used a bends threshold which was considerably less stress on the dog. They were unable to show any protective effect or therapeutic effect of heparin. However, in our dogs if we gave heparin immediately following a rapid, severe decompression in a dose of 2 mg per kg, we could completely reverse the symptoms of decompression sickness—without any recompression at all. It was a very dramatic effect.

We have subsequently used heparin in human cases along with dextran and have been pleased with the result. As far as recommending heparin in routine therapy, I believe it should be used on a routine basis. I think there is enough evidence now, particularly with our papers today and yesterday and the things coming into the literature which are suggesting that heparin would definitely be advantageous to use in decompression sickness. Whether you want to use it in just severe cases and not do it in "pain only" bends would be up to the individual, but so far as our work with it is concerned we have had no untoward effects. There has been no bleeding, no problems with using heparin. All that we have seen have been good results.

Dr. Elliott: Remember that heparin has a multitude of effects, and probably many we have never heard of, including, for instance, reduction of vaso-activity in the lungs. So I think heparin may be a many-edged sword in our therapeutic arsenal.

Dr. Hempleman: There have been four documented cases only of treatment of severe decompression sickness without using recompression, using purely medical management. Three of those were done by Dr. Bühlmann without the use of heparin quite successfully and one recently with plasma expanders and heparin.

It is also worth mentioning that where heparin seems to have been successful experimentally the type of decompression is very severe decompression sickness really, and this I think is the difference between findings of Reeves and Workman and the Cockett group. It is one further example of the enormous number of variables there are in this situation. I think it is true to say that wherever heparin has been used regularly there has been recompression with it as well and there are, as I say from a survey I did, only four people who have actually not resorted to recompression—those with and those without heparin—and all gave successful conclusion.

Dr. Bühlmann: Ten years ago we treated with plasma and without recompression, the cases with only skin

decompression sickness. If we had involvement of the spinal cord we used recompression and plasma, but never heparin, not routinely.

Dr. Hempleman: Dr. Bühlmann, you did say, though, that you treated with recompression and plasma. There were three, were there not, that were not treated with recompression?

Dr. Bühlmann: There were three cases from 1962 without recompression, but in the last 10 years we have had other cases.

Dr. Walder: The study by Hallenbeck, Bove and Elliott seems to have established that the intrathoracic pressure can rise high enough to prevent dye from getting into the intrathoracic veins. However, this plexus of veins about the vertebral columns is very extensive and it is therefore unlikely that the venous drainage from the cord is completely stopped. It seems to me possible that some of the venous drainage may go by other routes—that is, out into the paravertebral muscles. Perhaps one way in which this could be determined would be to measure clearance rates of some marked isotope from the cord tissue.

Dr. Hallenbeck: It is true that the venous network around the cord is very rich. There are anastamoses between the internal vertebral venous plexus, the external vertebral venous plexus, and the parallel veins within the cavities. The cord, though, has a venous drainage which is analogous to its arterial supply. What began embryologically as a segmental drainage through ventral and dorsal routes ends up in the adult as a very incomplete segmental drainage with perhaps six to ten ventral reticular veins draining into the cord, with perhaps six to eleven dorsal reticular veins, and they drain into the longitudinal vertebral venous sinus.

There is no collateral flow to be drained by way of the paravertebral muscles. If at the level of the longitudinal vertebral venous sinus there was an obstruction, it would compromise the reticular veins.

Dr. Miller: Everybody who has worked with mice at high pressures reports that the partial pressure of oxygen is immaterial to performance at high pressure. In the work done by Chouteau on larger animals such as pigs and goats, it was thought that oxygen is important.

It seems to me that it is established that in larger animals oxygen is important. Is there a scaling factor in respiratory function between mice and larger animals? In other words, are small mice useless models for respiratory problems of men? Larger animals should have more trouble getting enough oxygen at 100 atmospheres than mice do.

Dr. Lundgren: As far as I know there is a sizeable difference in alveolar size between, say, dogs and mice. I think it is about the order of three—the mouse being at an advantage provided that interalveolar diffusion is of any importance in this connection.

Dr. Lambertsen: Relative to the question about oxygenation in man, at 400 feet absolute pressure breathing nitrogen even in severe exercise, there is no important change in arterial oxygenation from the level of arterial oxygenation at sea level. That is one bit of information. This situation is comparable to the respiratory gas density, at any rate, that one would have at approximately 3000 feet.

During neon breathing at 700 feet of sea water measurements of arterial P_{O_2} could not be done, but heating the hand to speed the flow of blood through the hand allowed the measurement of P_{O_2} in arterialized venous blood. It was close to normal for arterial blood. So there seems under these circumstances not to be important interference with oxygenation in man.

Dr. Behnke: I would like to ask Dr. Halsey a question that emanates largely from some experience with toxic substances in closed spaces, particularly submarines. He did mention ammonia and hydrogen sulfide. On compression what is the possible role of the gases in the intestinal tract, such as ammonia (which certainly affects the nervous system), hydrogen sulfide and at times the toxic amines (which certainly on rapid compression would be increased in concentration in whatever the pressure multiple is—up to 30 or 54)?

Dr. Halsey: We in fact did not measure the hydrogen sulfide concentration; we were concerned simply with determining whether these gases might interfere with the results and assessment of the narcotic potencies and the righting reflex. We just measured for them. I agree with you that all of these gases might indeed have an effect, but we have got no detailed results or studies that would tell us what effect that might have.

Dr. Behnke: I would say that slow compression would allow for the diffusion of these gases without an increase possibly in their tension—which might explain the beneficial effects of slow compression.

Part V. INERT GAS EXCHANGE AND BUBBLE FORMATION

PHYSICAL ASPECTS OF BUBBLE FORMATION IN TISSUES

G. Karreman

This review of physical factors in bubble formation will depart from the familiar history of investigations of decompression sickness, as well as general concepts of inert gas exchange between blood and tissues. These have been the subject of papers in each of the preceding symposia. Further, in examining bubble formation the primary attempt will be to describe factors in terms employed in application of mathematics to biophysical phenomena.

Physical Factors Involved in Bubble Formation

Cavitation or conditions generating bubble nuclei facilitate development of actual gas bubbles. Dean (4) has advanced a theory for the formation of bubbles by mechanical action which avoids the necessity of the existence of extraneous bubble nuclei. He has shown that free vortices in liquids produce sufficient tension to rupture the liquid, and he has suggested that in such a way bubbles are formed when liquids are subjected to mechanical disturbance.

A second, well-known physical factor to be considered in detail is the presence of excess gas in solution near a nucleus or cavity. A convenient measure of the tendency of a gas to come out of solution was introduced by Harvey et al. (10). According to Henry's law the concentration of gas dissolved in a liquid is at equilibrium proportional to the partial pressure (p) of the gas in contact with the liquid. This pressure determines the gas tension (t) in the liquid. If the hydrostatic pressure (P) in the liquid is P atmospheres, the difference $t - P = \Delta P$ is the primary driving force for bubble formation in compression and altitude experiments. Different gases, even at the same tension, will behave differently when rates of diffusion are important, in which case diffusion as well as solubility constants must be taken into account. Of course ΔP varies considerably in different regions of an animal as a whole immediately after decompression. The fundamental problem is to determine the ΔP at which bubbles can appear under different conditions. Harvey et al. (10) made a detailed study of liquid models. For cavitation (de novo formation of bubbles) in a homogeneous fluid at rest without contact with a gas phase but containing dissolved gas, it is necessary (at least in water-glass systems) that ΔP be of the order of 100–1000 atmospheres (10). This also applies to de novo formation at smooth surfaces, whether hydrophilic or hydrophobic in character. It is confirmed experimentally by the fact that liquids at rest may be highly supersaturated with gas and remain so under proper conditions. Another case in which de

novo formation of a gas nucleus may occur even when $\Delta P = 0$ is with the presence of a surface crack or an acute angled cavity.

Introduction of a gas mass, however small, changes conditions completely. In this case the gas mass moves immediately by diffusion from the supersaturated solution, and a bubble may be rapidly formed, similar (although there are differences) to seeded crystallization from a supercooled or supersaturated solution.

Since a ΔP of 100–1000 atmospheres caused exclusively by a gas tension increase cannot exist in the resting animal—either in the usual pressure chamber or after exposure to a high altitude—Harvey and co-workers believe that most bubbles formed in a resting animal come from minute gas nuclei sticking to surfaces on the outsides of cells.

The bubble grows by movement of the gas into the nucleus. This growth of a gas bubble in a liquid supersaturated with that gas is of primary interest in the study of decompression sickness. Harvey et al. (10) present a differential equation for the growth rate of a gas bubble in terms of the rate of change of its radius without giving its derivation and stating the assumptions on which it is based. It is assumed that 1) the bubble is spherical with radius (r); 2) it contains molecules of a single ideal gas; 3) the gas is distributed up to the gas-liquid interface; 4) gravity is neglected so that the external pressure (P) on the bubble is everywhere uniform; 5) the bubble grows in a state of mechanical quasi-equilibrium; 6) the concentration of dissolved gas in the fluid is uniform except in a shell surrounding the bubble; 7) the diffusion gradient is uniform through the shell; 8) no gas is produced or consumed within the bubble.

On the basis of these assumptions the following differential equation was obtained for the time rate of increase of the radius, shown in Eq. (1):

$$\frac{dr}{dt} = \frac{RTaD}{\Delta r} \frac{\Delta P}{P} \frac{r - (2\gamma/P)}{r + (4\gamma/3P)} \tag{1}$$

where:

r = radius in cm
R = gas constant, 8.3136×10^7 erg/°C/mol
T = absolute temperature
a = solubility in mol/dyne-cm, defined by $c = a\tau$, c being the concentration in mol/cm^3 and τ the gas tension in dynes/cm^2
D = diffusion coefficient in cm^2/sec
Δr = thickness of diffusion shell (3×10^{-3} cm); for $r < 3 \times 10^{-3}$ cm, $\Delta r = r$
P = hydrostatic pressure in dynes/cm^2
τ = gas tension in water measured by gas pressure with which dissolved gas is in equilibrium
$\Delta P = \tau - P$, in dynes/cm^2
γ = surface tension in dynes/cm
t = time in seconds.

Without integrating Eq. (1) it is useful to plot the time rate of change of radius $\dot{r} = dr/dt$ vs. r, as is done in Fig. 1. From Fig. 1 it is seen that the critical value $r^* = 2\gamma/P$ of r is unstable: for if $r > r^*$, it is seen that $dr/dt > 0$ and dr/dt increases to its asymptotic value

$$\frac{dr}{dt} = \frac{RTaD}{\Delta r}\,\frac{\Delta P}{P}$$

After dr/dt has approached this value, r increases approximately linearly with t, and the bubble would grow indefinitely if there were no obstacles in its way.

Harvey et al. (12) also observed bubble formation in *Nitella* cells by mechanical manipulation like gentle pinching or twisting when the cells were supersaturated with high nitrogen pressures.

The solution of stationary bubbles was studied by Lieberman on a theoretical basis (21). He also analyzed the effect of surface contamination on diffusion and found the bubble solution in the presence of contaminants not greatly affected. Experimentally, he found that air bubbles of dimension less than 1 μ attached to hydrophobic particles are not soluble, existing indefinitely, and that hydrophobic particle residues frequently remain after bubble formation as such, functioning as bubble nuclei for cavitation.

Buckles (2) observed bubbles in the cheek pouch of the hamster. He studied 1) the microcirculatory effects of safe decompression; 2) the onset of symptoms; and 3) the resolution of bubbles. He observed that rapid decompression (4 ata/min) produces considerable arterial vasoconstriction, extending throughout arterioles and often resulting in a temporary arrest of capillary flow. In anesthetized hamsters bubbles are formed between 5 and 6 ata with a 4 ata/min ascent rate, whereas no bends are formed in hamsters saturated with nitrogen at 7 ata with an ascent rate of 2 ata/min. While bubbles may occur elsewhere in the hamster, nucleation does not occur in the pouch (although bubbles do appear in the pouch after certain decompressions). They appear first in an artery and later in a vein, entering always from the proximal end, moving in a distal direction toward the capillary. During recompression arterial bubbles are pushed distally and often break into smaller bubbles at bifurcations. Venous bubbles move in a proximal direction, breaking into many small bubbles. Many bubbles in both arteries and veins remain affixed to the endothelial wall.

Most of Buckles' analysis of the principles of formation and resorption of bubbles was actually based on original contributions of others (2). He referred to the several investigations of the creation of a gaseous phase within a supersaturated liquid phase as best summarized by Harvey's original mechanical stress theory (11). Harvey's mechanical stress theory proposed that the bubbles form de novo within blood, extracellular fluid, or tissues due to a highly localized negative pressure induced by some kind of mechanical stress. As noted by Harvey (12), single cells could be supersaturated to 80 atmospheres without nucleation, but

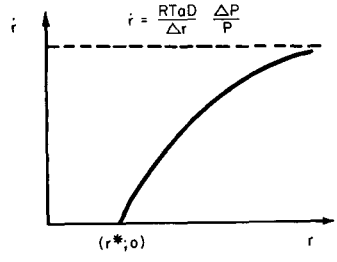

FIG. 1. Time rate of change of radius, $\dot{r} = dr/dt$, versus r. For r greater than the critical value $r^* = 2\gamma/P$ the ordinate $\dot{r}$ is positive and approaches the asymptote $\dot{r} = (RTaD/\Delta r)\,(\Delta P/P)$.

intracellular nucleation occurred often at locations distal to the point of contact when these cells were merely touched by microprobes. Furthermore, kidney crushing seldom results in nucleation, but when muscle is crushed against bone, nucleation always occurs.

Buckles (2) uses the following expression:

$$A\{\exp(-\Delta F/kT)\} \tag{2}$$

for the rate of bubble formation as already used by Becker and Döring (1) in their theory about the rate of gas nuclei formation. Buckles (2) then uses the following equation derived by Frenkel (7) for the free energy ΔF required for the de novo nucleation leading to the creation of a bubble of radius r shown in Eq. (3):

$$\Delta F = 4\pi r \frac{\gamma - rP_B \ln P_T/P_B}{3} \tag{3}$$

By substitution of Eq. (3) into expression (2), the rate of bubble formation is found as a function of the radius (r), the surface tension (γ), and the tension (P_T) of the inert gas dissolved in the surrounding tissue; P_B is the sum of the hydrostatic pressure (P_H), the distending excess pressure ($P\gamma$) due to the surface tension (given by $P\gamma = 2\gamma/r$) and the sum of the constraining pressures.

Buckles (2) also studied the growth-decay kinetics of bubbles: in the process of bubble growth, the transfer of any one of the three quantities of inertia, heat and mass may limit the growth rate of the bubble. This gives the essential difference between bubbles formed during boiling, cavitated bubbles and bubbles formed during decompression. Decompression bubbles are primarily limited by mass transfer because of their slow growth, their existence in a thermal environment, and because their major constituent is the inert gas in the surrounding tissue. By using the basic equation for the flux (J) of gas across the boundary between the bubble and surrounding tissue:

$$J = KA_B(P_B - P_T) \tag{4}$$

in which A_B is the surface area of the bubble, Buckles obtains Eq. (5) for the time rate of growth (dV_B/dt) in which V_B is the total volume of the gas bubble:

$$dV_B/dt = M(P_T/P_B) - 1 \tag{5}$$

with

$$M = KmT/273 \tag{6}$$

in which m is a function of A_B, V_B and the length-to-diameter ratio of the bubble. The proportionality constant K is the product of the diffusion coefficient and the solubility of the gas within the surrounding tissue. K will depend on the model assumed to describe the gas flow as shown by Scriven (25). K may be inversely proportional to the bubble size and depends on the major mode of transfer: for instance, in a vein the convective transfer due to the venous blood flowing past the bubble or, if the bubble is located within a tissue gas, its loss by diffusion. Buckles points out that the several assumptions that have gone into

Eq. (5) may not be right, such as where bubbles grow and where they are resolved. The total resolution of small bubbles during decompression is very sensitive to the local blood flow surrounding that bubble. Buckles points out rightly that in the in vivo studies the observed formation may not directly relate to the development of decompression sickness produced by evolution of bubbles in tissue fluids. He concluded that the observations of bubble resolution in the in vivo pouch are probably highly pertinent for therapy.

Döring (5) applies the theoretical method developed by Becker and Döring (1) to the probability of formation of nuclei in superheated vapors in liquid phases. A limit is found for both the superheating and the maximum tension which can be achieved in agreement with experiments.

The tensile strength of liquids has been treated by Plesset (23) who derived Eq. (7) for the probability (w) of appearance of a vapor bubble of radius R:

$$w = \exp\ (-\ 4\pi R^2\gamma/3kT) \tag{7}$$

where:

R = the radius of the bubble
γ = the surface tension
k = Boltzman's constant
T = absolute temperature

Another equation for the probability (w) of creation of a nucleus derived by Landau and Lifshitz (20) is shown in Eq. (8):

$$w = \exp\ \{-\ 16\pi\gamma^3/3kT(P_i - P)^2\} \tag{8}$$

in which P_i and P are the pressures inside and outside the bubble, respectively.

From Eq. (7) it can be seen that for water at 27 °C, for which $\gamma = 72$ dynes/cm, the probability is a sharply decreasing function of R for 10^{-8} (cm) $< R < 10^{-7}$ (cm), given in Fig. 1 by Plesset, quoted above. For $R = 10^{-7}$ cm, ln $w = -72.8$, and the corresponding tension $2\gamma/R = 1440$ atmospheres, which is also sharply decreasing with R between 10^{-8} (cm) $< R < 10^{-6}$ (cm), given in Fig. 2 by the same author.

Isobaric gas counterdiffusion has been studied experimentally and theoretically by Graves et al. (8). Experimentally a steady-state supersaturation with bubble formation was found at the interface of the two layers of a properly arranged composite through which two species counterdiffuse when the two layers differ in their relative permeabilities to the two species. This steady-state investigation has been extended recently by this author (16) who has given a kinetic analysis of the counterdiffusion of gases through such a composite. In addition to the variables used by Graves et al.—namely the pressure of each gas in each of the two layers and the distance along the normal of the interface as well as the diffusion and solubility constants of each gas in each layer—the distance over which each gas diffused in each layer as a function of time was introduced as a new variable. For long times the kinetic pressure obtained in this way at the interface approaches the steady value obtained by Graves et al. (8) as, of course, it should. These studies may be important in understanding the cause for the disturbances subjects have experienced recently in simulated diving experiments such as intense itching, gross maculopapular skin lesions, and severe vestibular derangement with vertigo and nystagmus.

These isobaric gas counterdiffusion studies should be extended, in the opinion of the present author, to 1) other geometries than the linear one studied so far; 2) more species and other pressures; and 3) study of other biophysical mechanisms which may be involved also in bubble formation and resolution. Such mechanisms are probably concerned with nucleation, growth of gas pockets at surfaces, release of gas nuclei from the surface, and growth and resolution of streaming gas bubbles.

Nucleation

For this process the original concept of Harvey et al. (10) (supported by Knapp [19]) of a nucleus consisting of gas in a crevice in a surface hydrophobic to the liquid might be investigated. For the explanation of the specific effects of different gases the accumulation of gas molecules at a surface due to cooperative specific adsorption, as introduced by Ling (22), will be discussed here. According to this mechanism, gas molecules can be specifically adsorbed at chains of interconnected sites of lipoprotein molecules in the surface leading to cooperative phenomena based on reinforcement of adsorption due to nearest neighbor interactions. This reinforcement is due to the mechanism of chemical induction as studied by Ingold (13). Basically, this is a quantum mechanical mechanism which has been applied to biologically active molecules (24) e.g., to drugs (18), including tranquilizers (17). The wave mechanical computations for large molecules have recently been reduced a hundredfold by the new method of Clementi (3), making the quantum mechanical treatment of large biologically active molecules practically feasible. The gases in the pockets may be adsorbed multilayers above the surface(s) due to the interaction of permanent and induced dipoles in the gas molecules.

Growth of Gas Pockets at Surfaces

The growth of the gas pockets attached to the surfaces should be studied by investigating the mechanical factors involved. These factors are determined by the pressure of the gas in the pocket at the surface and the pressure in the liquid, as investigated by van der Walle (27). The increase of the specificity of surface sites for gas molecules compared to that for liquid molecules will clearly lead to the accelerated (due to the cooperative mechanism) adsorption of the gas at the surface and hence to the growth of the bubble. The present author thinks it will be worthwhile to study the variables—specifically the (mean) radii of curvatures—which determine the form of the interface between the gas bubble and the liquid as function of time, not only in a deterministic way as done by van der Walle, but also by a stochastic treatment. A stochastic treatment of cooperative adsorption has been given recently by this author (14, 15) as well as a kinetic treatment of cooperative specific adsorption in an attempt to obtain a new physical explanation of physiologic excitation.

Release of Gas Nuclei from the Surface

As the gas bubble attached to the surface grows, the stability of the attachment will change and eventually become unstable. It would be useful to study the stability of the pressure and radii of curvature of the gas bubble still attached at the surface as a function of time in order to find the critical pressure at which the gas bubble will be released from the

surface. From a deterministic treatment, Strassberg (26) already obtained a threshold pressure for vaporous cavitation of such nuclei. Because of the fluctuations which play an important role in the behavior of such small gas nuclei it will be useful to treat the time behavior of the variables stochastically.

Growth and Resolution of Streaming Gas Bubbles

Transport of a bubble in a flow field will lead to a change in the static pressure in the fluid surrounding the bubble. Consequently, the pressure, temperature and density in the bubble, as well as the bubble radius, will change, yielding a diffusion of gas between the liquid and the bubble and a flow of heat across the bubble-liquid interface. Furthermore, evaporation and condensation can occur at this interface and inside the bubble. The relationship between the bubble radius and the static pressure is determined by several equations, namely:

1) *A dynamic equation*, which takes into account the effects of surface tension and dynamic viscosity.
2) *The gas diffusion* equation if gases other than the liquid vapor are involved. The solution of this equation gives the equilibrium pressure of dissolved gas in the liquid as function of space and time.
3) *The heat conduction equation* which describes the conduction (without convection) of heat. The solution of this equation yields the temperature as function of time and space and is important for the understanding of the formation of bubbles when the environmental temperature changes, as occurs during the ascent of divers.

Equations for 1-3 have been given by van der Walle (27).

Important aspects of the growth of bubbles are concerned with:

Static Stability of Small Bubbles. This problem has been studied by Harvey (9), who took into account only the diffusion and solubility of dissolved gases in bubbles and the surface tension, and by van der Walle (27), who neglected frictional effects and found that the bubble grows continuously even without gas diffusion, its growth speed being determined by either dynamic limitations or heat conduction effects.

Dependence of Growth Speed of Small Bubbles on Gas Diffusion Effects. In normal flow problems van der Walle (27) found that this is only important for very small bubbles with radii of the order of 10^{-4} cm, whereas it is less important for larger bubbles.

Dependence of Dynamic and Heat Conduction Effects on Ultimate Growth Speed of Larger Bubbles. The dynamic and heat conduction effects exert, according to van der Walle (27), opposing effects on the growth speed, which is determined essentially by the rate of evaporation.

The combined effects of these factors—dynamics, gas diffusion and heat conduction—determine the growth and resolution of bubbles, as determined by the dependence of their radii on time. The above effects were already discussed, corresponding equations given, and simplified cases of them solved—sometimes only in limiting cases—by van der Walle (27).

It is important to include the neglected biophysical mechanism of viscosity and extend it with other neglected biophysical mechanisms. All the differential equations involved, of which several are partial ones, can be solved with the aid of electronic computers, simul-

taneously in general, as was not done by van der Walle (27). In addition, it would be useful to develop similar equations stochastically (or probabilistically).

Detailed consideration should be given to aspects of stability, and especially instability, of cavitation inception and bubble formation and resolution. By means of the method of state variables (for which especially the radii of curvature of the bubble and gas pressure may be taken), as applied recently (15), special investigation of instability aspects will lead to a threshold of pressure for cavitation, for detachment of gas nuclei from the surface.

The above-mentioned suggested studies will apply also to the dynamics of bubble formation with physical and biologic membranes in vitro. In fact, the higher purity of synthetic physical membranes will allow testing of many of the theoretical predictions: 1) the influence of the composition of the membranes on threshold presures; 2) the influence of the interface on the rate of bubble formation and the growth rate of the bubbles; 3) the effect of mixtures of different gases at different pressures on the bubble formation rate. The relevance of these studies for biologic membranes has been indicated in the experiments in which different substances (olive oil and water) were used for the layers of the composite membrane (8). In these experiments the influence of changes described under 1) through 3) can be studied and compared with the theoretical predictions. The studies of the inception and mechanisms of bubble formation are not only useful for a better understanding of the cause of decompression sickness after diving and in aerospace medicine, but also for a better understanding of the process of embolization, with applications to open-heart surgery.

From the stochastic studies planned for the investigation of the mechanism of bubble formation and resolution the following results may be expected to be obtained. The stochastic treatment of bubble formation will probably yield the distribution of the number of bubbles of a certain radius (R) as a function of the pressure (p) above a critical pressure (p^*) of resolution as well as the time rate of the change of that number due to the processes of gas diffusion, evaporation and condensation, and heat conduction outlined above.

It would be worthwhile to derive decompression optimization procedures in multi-gas exposures at increased pressure by the methods of optimal control theory. By these methods an objective function is extremized (minimized or maximized) subject to some constraints. At the present time the aim is to derive optimal rates of ascent for decompression in diving or simulated high pressure experiments.

These methods of optimal control theory can be applied in the following way. At each pressure and temperature the number (N) and size of gas bubbles are given from the results already mentioned above as well as the formerly suggested studies on bubble formation and resolution. Denoting the minimum number (N_m) of bubbles at the highest obtained pressure, this number will increase due to bubble resolution upon ascent: it will be denoted $N(h, t)$ ($\geqslant N_m$) at depth (h) under the water surface as it depends indirectly on h through its dependence on p, which is a function of h, and on the time (t) during which the processes of gas diffusion and condensation take place, either during ascent or at a (temporarily) stationary level. It is suggested to take as the objective function to be minimized with appropriate constraints concerned with the oxygen uptake and waste (e.g., carbon dioxide) removal:

$$\int_0^t \{N(h, t) - N^*(h)\}^2 dt \tag{9}$$

where:

$N^*(h)$ = equilibrium number of gas bubbles at the pressure and time (t) at the level (h)
$N(h, t)$ = number of gas bubbles left over from the higher pressure at the lower level.

The velocity of ascent is, of course, obtained by Eq. 10:

$$v = \mathrm{d}h/\mathrm{d}t \tag{10}$$

from which it follows that:

$$\mathrm{d}t = \mathrm{d}h/v \tag{11}$$

in which v is the function of h to be determined. From Eqs. (9)-(11) an objective function as used in modern control theory with a positive definite integrand to be minimized is obtained. The feasibility of this approach for the problem of minimization of the power of the heart in the cardiovascular system containing 24 pressures and flows has recently been demonstrated by Doubek (6) by solving the nonlinear Riccati equation used in modern control theory.

ACKNOWLEDGMENTS

The author thanks Dr. C. J. Lambertsen for encouragement and advice during the preparation of this paper.

This work has been in part supported by Public Health Grant HL 07762.

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THE ORIGIN OF INTRAVASCULAR BUBBLES PRODUCED BY DECOMPRESSION OF RATS KILLED PRIOR TO HYPERBARIC EXPOSURE

J. Smith-Sivertsen

The various manifestations of decompression sickness are believed to be caused by intra- and extravascular bubble formation during and after decompression. It is generally accepted that the supersaturation of gas in blood and tissue that makes such bubble formation possible is brought about by gas uptake through respiration and circulation during exposures to higher pressures. Diffusion of gas through the skin of man and animals is regarded as negligible, although the itching commonly occurring during decompression in a pressure chamber has been explained as a result of such diffusion (3).

This implies that intravascular bubble formation is dependent on ventilation and circulation during exposure to pressure. Such bubble formation does, however, occur in animals killed prior to exposure to high pressure. The appearance of bubbles in these animals is still dependent on the time of exposure and therefore not likely to be caused simply by mechanical air trapping (2).

The following experiment attempted to trace the origin of intravascular bubbles found in dead animals exposed to pressure. The experiment can be divided into two parts. The first part was intended to verify that the occurrence of bubbles is dependent on the time and the depth of exposure, and to prove that these bubbles are not artefacts introduced by the surgical technique used for opening the animals but are true products of decompression. Furthermore, an attempt was made to determine the location of bubbles and where in the circulation the bubbles are most likely to be formed.

In addition, experiments were done to determine the origin of the bubbles by trying in different ways to eliminate gas uptake from the lungs and through the skin.

Methods

One hundred fifty-six Wistar rats, 6–10 weeks of age, were killed by chloroform inhalation 10–15 minutes before exposure in air to pressures ranging from 6 to 18 ata. The exposure times varied from 5 minutes to 8 hours. The rates of descent and ascent were kept at 100 feet per minute. After decompression the animals were opened and examined for macroscopic intravascular bubbles in the following four main locations:

1) *The peripheral vessels.* Bubbles were looked for in the superficial vessels of the extremities and in the subcutaneous tissue of the trunk. No distinction was made between arteries and veins.
2) *The pulmonary vessels.* Again no distinction was made between arteries and veins.
3) *The central veins.* Bubbles were looked for in superior and inferior vena cavae.
4) *Central arteries including the left ventricle of the heart.* The latter was examined by extracting blood through a transparent polyethylene catheter introduced through the myocardium.

A control group of animals, not exposed to pressure but otherwise treated in the same way, was examined 1–8 hours after they had been killed.

To eliminate a possible influence of chloroform on the bubble formation, another group of animals was killed by intraperitoneal injection of barbiturate.

From four of the animals samples of gas were drawn from the aorta and analyzed by gas chromatography for nitrogen, oxygen and carbon dioxide contents.

In the second part of the experiment the killed rats were exposed to 12 ata for 2 hours. Gas uptake from the lungs and through the skin was tentatively prevented by the following measures:

1) In an attempt to empty the lungs, 25–30 cc of pure water was injected into the pleural cavities making the lungs collapse. The rats were then exposed in air.
2) A second group of rats was exposed submerged in water to prevent gas diffusion through the skin.
3) The two measures mentioned were then combined. A third group was exposed submerged after the lungs had been collapsed by intrapleural water injection.
4) Air trapped in the fur of the submerged animals was then considered responsible for the bubble formation. This possibility was ruled out for the next group, by shaving the rats before submerged exposure.
5) In the fifth group shaving was combined with intrapleural injection of water before exposing the rats submerged.
6) The final group was exposed submerged, shaved and pulmonectomized. The removal of the lungs was performed underwater in order to prevent suction of air into the pulmonary vessels during the surgical procedure.

After the exposures the animals were examined in the same way as in the first part of the experiment. The 17 rats previously exposed to 12 ata for 2 hours in air served as a control group.

Results

The results of the first part of the experiment are presented in Tables I, II, III and IV. Table I shows that the occurrence of bubbles is definitely time- and depth-dependent. More than 6 hours seem to be required at 6 ata for bubbles to be formed, while at 9 ata bubbles are seen after only ½ hour of exposure. Nearly all animals show bubbles after 4 hours or more at 9 ata, 2 hours or more at 12 ata, 1 hour or more at 15 ata and ½ hour or more at 18 ata.

TABLE I

NUMBER OF RATS IN WHICH INTRAVASCULAR BUBBLES WERE FOUND, RELATED TO THE TIME AND DEPTH OF EXPOSURE[a]

Pressure (ata)	Time							
	5	30	60	90	120	240	360	480
6						0 (5)	0 (5)	3 (5)
9	0 (5)	1 (5)	13 (20)		12 (20)	18 (20)		
12		7 (12)	10 (15)		17 (17)			
15	0 (2)	4 (10)	6 (6)	4 (4)				
18		5 (5)						

[a] Numbers in parentheses indicate number of rats exposed.

Between the dives that produce no bubbles and those that produce bubbles in all the animals, there is a range of time-depth relationships in which bubble formation seems to be somewhat random. In two animals of equal weight, age and sex, lying side by side in the chamber and exposed to identical conditions, the vessels of one might be filled with gas while in the other bubbles could hardly be found at all. Doubling the time of exposure from 1 to 2 hours at 9 ata seems to reduce the number of animals showing bubbles, as does an increment of 3 ata from 12 to 15 ata for the animals exposed for ½ hour. This might be an expression of the same randomness.

There were no visible intravascular bubbles in the control group of animals not exposed to pressure.

Table II shows the location of the bubbles in the positive cases. The variations in pressure and time did not change the distribution significantly. Bubbles were found most frequently in the left ventricle and in the aorta where they were present in 95% of the positive cases. The next most frequent location was the peripheral vessels (58%). In 41% bubbles were found in the central veins, and in only 10% of the positive cases bubbles could be seen in the pulmonary vessels.

In the cases where the distribution of bubbles was limited to only one of the four main locations considered, the aorta-left ventricle was the most likely, with only three exceptions (Table III). In only 5% of the positive cases were bubbles found elsewhere when not present in the aorta or the left ventricle.

Killing the animals by barbiturate injections instead of by chloroform inhalation neither reduced the number of positive cases nor altered the distribution of bubbles significantly.

The gas analysis showed nitrogen concentrations close to those of air, but low oxygen and high carbon dioxide levels (Table IV).

The results of the second part of the experiment are presented in Table V. Water injection in the pleural cavities did not prevent bubbles from being formed in any of the main locations, neither did submergence in water nor the combination of the two measures. The shaving of the rats did, however, prevent bubble formation in the peripheral vessels and in

TABLE II

NUMBER OF RATS PRESENTING INTRAVASCULAR BUBBLES AT THE DIFFERENT LOCATIONS

			Location of Bubbles									
			Peripheral Circulation			Pulmonary Vessels	Central Veins			Arterial Side of Systemic Circulation		
Pressure	Time (hr)	Total Number Animals Showing Bubbles	Skin	Extremities	Total	Total	V. cava super.	V. cava infer.	Total	Left ventricle	Aorta	Total
6	8	3	2	0	2	0	1	0	1	1	2	3
9	4	18	13	8	13	4	7	5	7	16	15	17
9	2	12	8	7	9	1	8	5	8	7	10	11
9	1	13	7	1	7	0	2	2	3	12	8	13
9	½	1	0	0	0	0	0	0	0	1	0	1
12	2	17	9	7	9	2	11	5	11	16	12	16
12	1	10	6	3	6	2	4	1	4	9	8	10
12	½	7	5	4	5	1	2	0	2	4	4	5
15	1½	4	2	2	2	0	1	0	1	4	4	4
15	1	6	2	2	2	0	2	1	2	4	6	6
15	½	4	0	0	0	0	1	0	1	3	1	4
18	½	5	3	1	3	0	1	0	1	3	4	5
Totals		100	57	35	58	10	40	19	41	80	74	95

TABLE III

NUMBER OF RATS PRESENTING INTRAVASCULAR BUBBLES IN ONLY ONE OF THE FOUR MAIN LOCATIONS

			Bubbles Found *Only* in:				
Pressure (ata)	Time (hr)	Total Number Animals Showing Bubbles	Peripheral Vessels	Pulmonary Vessels	Central Veins	Aorta or Left Ventricle	Bubbles Found But *Not* in the Aorta or Left Ventricle
6	8	3				1	1
9	4	18	1			5	
9	2	12				3	1
9	1	13				4	
9	½	1				1	
12	2	17			1	4	1
12	1	10				3	
12	½	7	1			2	2
15	1½	4				2	
15	1	6				4	
15	½	4				3	
18	½	5				1	
Totals		100	2	0	1	33	5

TABLE IV

PERCENTAGE OF NITROGEN, OXYGEN AND CARBON DIOXIDE IN SAMPLES OF GAS DRAWN FROM THE AORTA OF FOUR DIFFERENT RATS

Sample No.	N_2	O_2	CO_2
1	79.3	2.60	
2			22.6
3	81.3	4.2	
4			16.2

the central veins of the submerged rats, whether or not the lungs had been collapsed, but bubble formation still occurred in the aorta and the left ventricle. The latter could be prevented only by taking out the lungs before exposure.

Discussion

It has not yet been established with certainty where in the circulation the intravascular bubbles of decompression sickness are formed and first appear (1). In live animals bubbles can move quickly, may pass the capillaries or anastomosis of the lungs or the systemic circulation and then coalesce to become visible far from the place where they originated. In a dead animal exposed to pressure one would *not* expect such migration of bubbles, especially not through the capillary bed since this would require perfusion induced by intracirculatory pressure differences. It seems therefore likely that the bubbles in this kind of experiment are found in or close to their site of origin. This would imply that bubbles are most easily formed in the aorta or left ventricle. However, the possibility of retrograde migration from the periphery due to buoyancy or to the wider diameter of the central arteries cannot be ignored.

It is still not clear how the blood of a dead animal can become saturated with gas when there is no ventilation or circulation during exposure. The time of exposure required for bubbles to be formed in a dead rat is certainly longer than in a live rat, but the difference is surprisingly small. The gas could either diffuse from the surface or from gas cavities inside the animal, most likely from the lungs. Gas uptake mainly through the skin would imply a gas tension higher in the peripheral than in the central arteries and one would expect bubble formation to start in the periphery. Alveolar gas as the source of supersaturation is consistent with the central appearance of bubbles, but then one would expect bubbles to be found more frequently in the pulmonary vessels. In any case, the outcome of removal of the lungs prior to exposure indicates that the lungs, in some way, are important for the central arterial bubbles to be formed, while bubbles in the central veins and peripheral vessels might be explained by gas uptake through the skin alone.

The relevance of these results to the decompression syndrome in man is not obvious, but there may be some indications that the lungs are the possible site of origin of bubbles, even in live organisms. It is known that parts of the lungs are normally poorly ventilated and

TABLE V

NUMBER OF RATS PRESENTING BUBBLES TOTALLY AND AT THE DIFFERENT LOCATIONS[a]

Condition	Number Exposed	Number Showing Bubbles	Location of Bubbles			
			Peripheral Vessels	Pulmonary Vessels	Central Veins	Aorta or Left Ventricle
Exposed in air	17	17	9	2	11	16
Lungs collapsed, exposed in air	10	10	5	2	5	10
Exposed submerged	5	5	4	1	3	5
Lungs collapsed, exposed submerged	5	5	5	0	4	5
Shaved, exposed submerged	5	4	0	1	0	4
Shaved, lungs collapsed, exposed submerged	5	5	0	0	0	5
Shaved, pulmonectomized, exposed submerged	10	1	0	—	0	1

[a] Various measures have been employed to prevent or reduce uptake of gas from the lungs and through the skin during exposure.

perfused and that surface tensions in the lungs are low. Furthermore a general underestimate of the skin surface as a recipient of gas during hyperbaric exposure may be suggested. Finally the discovery of intravascular gas in postmortem examination of victims of diving accidents should not automatically be associated with air embolism or decompression sickness as the cause of death. The uptake of inert gas seems to continue in the deceased when he is still under pressure, and formation of free gas can occur as well in the vessels of a dead body.

Conclusions

1) The intravascular bubbles found in rats killed prior to hyperbaric exposure are true products of compression-decompression.

2) The bubbles are found most frequently in the left ventricle of the heart and in the aorta; they are seldom seen in the pulmonary vessels.

3) The results may indicate that two different sources of gas uptake and subsequent bubble formation exist in this type of experiment: while bubbles in the left ventricle and the aorta seem to be dependent on the presence of lungs, whether collapsed or not, the bubbles in the peripheral vessels and the central veins appear to be caused by gas diffusion through the skin.

ACKNOWLEDGMENTS

The experiments were performed at the Royal Naval Physiological Laboratory in Alverstoke, England. I am greatly indebted to the Superintendent H. V. Hempleman for his guidance and interest and for giving me the opportunity to use the facilities of the Laboratory. Thanks are given also to Mr. J. Florio who did the gas analysis.

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SAFE DECOMPRESSION WITH THE DOPPLER ULTRASONIC BLOOD BUBBLE DETECTOR

M. P. Spencer, D. C. Johanson and S. D. Campbell

The etiologic agent of decompression sickness has been held to be the formation of supersaturation bubbles in the tissues and blood. Many consequences result from the presence of these bubbles which alter circulation, respiration, biochemistry and blood constituents. Devices are greatly needed which will detect the presence, size and number of both static and moving decompression bubbles. The Doppler ultrasonic flowmeter represents a sensitive device to detect the presence of moving intravascular gas emboli. Its usefulness rests primarily on its objectivity, simplicity of operation, adaptability for transcutaneous and non-invasive detection and—as will be shown here—its reliability in detecting venous gas emboli before development of decompression sickness. As a research tool, the flowmeter can be used to elucidate the events related to bubble nucleation, circulation and dissipation. Special modifications that make it a much needed tool for developing safe, efficient decompression procedures include the diagnosis and treatment of bends and aeroembolism. It also possesses a potential, through wise usage, in the prevention of bends and bone necrosis in divers and caisson workers.

The use of Doppler ultrasonics for bubble detection is an application of the Doppler ultrasonic blood flowmeter first proposed and demonstrated to be useful for the cardiovascular system by Satomura (13,14) and introduced in the United States by Franklin, Schlegel and Rushmer (4). In brief, it involves irradiating the body with high frequency sound, nominally between 5 and 10 megahertz (MHz), and receiving the reflected signals scattered from moving acoustical interfaces. As a blood flowmeter the interfaces are primarily blood cells and the received Doppler-shifted frequency spectrum is interpretable as the velocity distribution of flow streams under the ultrasonic beam. In the case of motions of the heart and vascular walls the interfaces are represented by the walls of the heart chambers and blood vessels. When compared with the transmitted frequency, the backscattered frequencies provide the difference frequencies which are in the audible range.

Spencer and Campbell first used the Doppler flowmeter for objective detection of circulating decompression gas emboli moving in the flow streams of the larger arteries and veins (17,18). Gillis et al. (6), following our progress, published simultaneously. Gas emboli circulating were also found at the time of open-heart surgery in the cardiopulmonary bypass circuit and in the heart and carotid arteries after closure (20). The first decompression investigations performed on sheep disclosed that decompression gas emboli are heard with

the Doppler flowmeter as chirps, whistles and snaps on the audio output and occur early in the peripheral veins. They occur in the systemic arteries only upon gross violation of accepted decompression schedules. Venous and arterial gas emboli are obliterated by hyperbaric recompression, but arterial bubbles without adequate treatment cause death following convulsions and unconsciousness.

After exceeding some tissue and blood critical supersaturation state the venous gas emboli collect in the small peripheral blood vessels and are extruded by blood flow, local tissue compression or muscular contraction into the systemic veins where they embolize to the lungs. Venous gas emboli may pass through the pulmonary vasculature of sheep when experimental nitrogen gas injections exceed a rate of 0.015 ml/kg/min and when the pulmonary systolic arterial pressure rises above 35 mm Hg (10,22). Venous gas emboli in sheep have been heard as long as 72 hours after decompression when signs of illness are not apparent (16) and were shown to form before signs of decompression sickness (19,15), in swine (8). Decompression venous gas emboli are easily produced on U.S. Navy tables of exceptional exposures over 150 fsw (5,15,19,23) and are also a frequent occurrence on certain U.S. Navy tables for normal exposure (15).

Early attempts at demonstration of venous gas emboli in human subjects, on exposures and tables producing positive results in animals, were disappointing (7,19). None was unequivocally detected in the peripheral veins in the extremities and neck using transcutaneous detectors, even though signs and symptoms of decompression sickness occurred. The first human decompression venous gas emboli are now known to have been heard in 1968 on a diving instructor following decompression on the U.S. Navy tables of exceptional exposures after 200 feet for 30 minutes (19). They were not clear-cut chirps and whistles and therefore were questioned at the time. Later results showed that loud clicks are produced by large bubbles in animal veins or by nonoptimal positioning of the transducer at a 90-degree angle with the blood vessel rather than at a more acute angle which allows the development of more recognizable whistles and chirps. The second recognized human signals occurred in the right brachial vein downstream from an upper arm site of redness and pain, produced by a 15-minute excursion to 300 feet with decompression according to the U.S. Navy Manual.

Because of the early difficulties in human investigation, it was recognized that a precordial blood bubble detector should be developed to facilitate the recognition of venous gas emboli arising from any point in the body as they flow through the right atrium, right ventricle and pulmonary artery on the way to the lungs. Following the development of the precordial detector (21,23) reproducible venous gas emboli signals were heard transcutaneously without discomfort, danger or inconvenience to the subject. Results of human experimentation have confirmed and extended the animal findings. Venous gas emboli are now reliably detected by the precordial sensor and the early failure to find them in the periphery resulted from the fact that they develop first in specific localized areas without general distribution. They are undetected with peripheral monitoring unless the sensor happens to be localized over the veins draining an area of bubble formation.

This study describes the use of the Doppler precordial blood bubble detector in developing decompression tables by statistically defining the limits of no-decompression air exposures by the incidence of venous gas emboli to the incidence of bends; experience in the use of the precordial blood bubble detector in the treatment of bubbles and bends was also developed. In addition, the general location of origin of early venous gas emboli, their

time of onset, reproducibility and propensity to formation in "bubbles prone" individuals are also described.

Methods

Twelve expert SCUBA divers, including three fulltime instructors, ranging from 20 to 35 years old, volunteered for the study (see Table I). The hyperbaric exposures, carried out in dry hyperbaric chambers, were selected on the basis of guidance from nitrogen elimination data of Behnke (3) (see Fig. 1) and the *U.S. Navy Diving Manual* no-decompression exposures. The principal exposures were: 30 feet (1.91 ata) for 720 minutes (12 hours); 70 feet (3.12 ata) for 50 minutes; and 150 feet (5.55 ata) for 15 minutes. All compressions were at 100 feet/minute and decompressions were consistently at 60 feet/minute. The 12-hour "dives" were conducted in a double chamber complex where the divers slept overnight, entering the chamber at 8 PM and emerging at 8 AM the following day. The 150- and 70-foot compressions were carried out at the Institute of Applied Physiology and Medicine in a 60-cubic-foot chamber where the subjects were either sitting or reclining. Compressed air was used throughout all exposures and the chambers were ventilated every 20 minutes for 5 minutes when in the larger complex and for ½ minute of every 5 minutes (250 cubic feet flush) when in the smaller chamber.

Immediately before and for 1–5 hours after decompression, the divers were monitored with the precordial blood bubble detector for the presence of venous gas emboli. The predive recording gave a baseline with which to compare the postdive recordings and also made certain that all the divers were bubble-free prior to compression. None of the subjects had been diving within 24 hours of the experiments. Following decompression the divers were given an initial monitoring immediately upon exit from the chambers. Postexposure precordial recordings were made at 5-minute intervals during the first half-hour and at

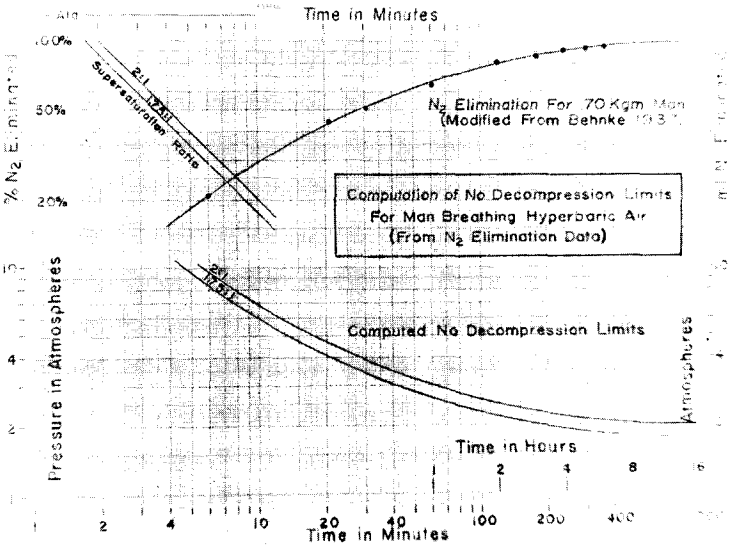

FIG. 1. Computation of no-decompression limits for man breathing hyperbaric air from nitrogen elimination data.

TABLE I

DOPPLER FLOWMETER INDICATIONS OF BUBBLE OCCURRENCE[a]

Diver			No-Decompression Profile (Depth/Time)[b]	Precordial Bubbles	Peripheral Bubbles[c]				Time of First Bubbles	Duration of Bubbles	Symptoms	Rx	Duration of Pain or Other Symptoms	Location of Pain or Other Symptoms
Age (Years)	Weight (kg)	Height (cm)			B_r	B_l	F_r	F_l						
	S.C.		30/720	+2	+	–	–	–	6	84	A, C		75	R. arm
35	82	175	70/50	+2	+	+	–	–	10	110	A, B		120	L. arm
			150/15	+1	+	–	–	–	2	33	A, C		30	R. shoulder
	A.G.		30/720	+3	–	–	+	+	30	150	B	O_2P[d]	20	R. knee
20	75	183	70/50	+1	–	–	+	+	30	25	A			
			150/15	+3	–	–	+	–	13	55	A	O_2P		
	M.P.		30/720	+1	–	–	–	–	<500	530	A			
21	75	178	70/50	+3	–	–	+	–	10	95	A, C	O_2[e]	20	R. knee
			150/15	+3	–	–	–	+	<15	95	A, C	O_2	130	R. shoulder
	P.M.		30/720	+3	–	–	–	+	>90	115	D	O_2P	145	L. thigh
23	75	173	70/50	–	–	–	–	–						
			150/15	+1	–	–	–	–	<15	170	A	O_2		
	B.A.		30/720	+3	–	–	–	+	<15	200	A, C	O_2	32	L. knee
19	91	185	70/50	–	–	–	–	–			A			
			150/15	+1	–	–	–	–	<15	170	A, C	O_2	20	L. knee
	M.R.		30/720	+3	–	–	+	–	30	740	A	O_2P		
21	70	173	70/50	–	–	–	–	–			A			
			150/15	+2	–	–	–	+	5	80	A	O_2		
	A.F.		30/720	+1	–	–	–	–						
23	74	178	70/50	+1	+	–	–	–	30	30	A			
			150/15	–	–	–	–	–			A			
	D.J.		30/720	–	–	–	–	–			A			
28	80	178	30/720	+2	–	–	+	–	30	200	A, C	O_2	250	R. knee,
			70/50	–	–	–	–	–			A			both arms
			70/50	–	–	–	–	–			A			and hands
			150/15	–	–	–	–	–						
			150/15	–	–	–	–	–						

	G.A.		30/720	–	–	–	–	–		
21	89	188	70/150	–	–	–	–	–	A	
			150/15	–	–	–	–	–		
	J.E.		30/720	–	–	–	–	–	B	
22	79	178	70/50	–	–	–	–	–	A	
			150/15	–	–	–	–	–		
	G.G.									
27	79	178	30/720	–	–	–	–	–		
	M.S.									
50	84	188	70/50	–	–	–	–	–		
	L.B.									
29	67	173	150/15	–	–	–	–	–	A	

Average All Divers				Precordial %	Peripheral %					30/720	70/50	150/15
26	74.5	179	30/720	67	8	0	17	25	Average time 1st bubbles	88	20	11
			70/50	33	17	8	17	8	% Venous gas emboli (bubbles)	67	33	50
			150/15	50	8	0	8	17	% Bends pain	25	8	25

+ = Bubbles
– = No bubbles
1 = Minimal detectable bubbles
2 = Moderate bubbles < blood sounds
3 = Copious bubbles = to blood sounds
4 = Extreme bubbles > blood sounds

A = Skin itching
B = Vague, uneasy feeling
C = Mild pain
D = Moderate pain
E = Severe pain

B_r = Right brachial vein
B_l = Left brachial vein
F_r = Right femoral vein
F_l = Left femoral vein

[a] All divers are experienced, healthy, normal males.
[b] All times are in minutes, depths in feet.
[c] No bubbles have ever been heard in either jugular vein.
[d] O_2P means relieved by oxygen under pressure.
[e] O_2 means relieved by oxygen.

15-minute intervals during the 30 to 60 minute postdive period on the two deeper dives. On the shallower 30-foot dive, recordings were made immediately upon exit from the chamber, and then at 15-minute intervals during the first hour and at 30-minute intervals during the 60–300 minute period. This sequence was followed as long as Doppler ultrasonic signals of venous gas emboli were not detected over the precordium.

The precordial transducer was constructed with one-half inch square crystals spaced 2 cm apart and tilted so that they focus around 5 cm below the skin surface, on the right side of the heart. If positioned as illustrated in Fig. 2 along the left midsternal border, the transducer focus includes the right atrial appendage, the right ventricular outflow tract and the pulmonary artery. Correct positioning is confirmed by finding the closure sound of the pulmonary valve which has a chirping quality similar to some venous gas embolism signals but, of course, occurring regularly at end systole. All venous gas emboli signals were recorded on a cassette stereo tape recorder along with voice notations. The tapes were later replayed for confirmation and documentation of the signals. If precordial venous gas emboli were detected, the divers were then asked to flex the limbs—one at a time—while monitoring was continued. Bursts of the venous gas embolism signals from bubbles thus dislodged more specifically indicated the regional source of the venous gas embolism. A 5-MHz pencil-type 2 cm lens-focusing Doppler probe was then substituted for the precordial probe and a search was made over left and right femoral, brachial and jugular veins. When monitoring the brachial or femoral veins, manual compression of the upper and lower sections of each limb usually confirmed the source of venous gas emboli. In addition, the arteries were monitored for possible arterial bubble signals.

The time of first occurrence of the signals following decompression was recorded as well as an estimate of their duration and a graded indication of the quantity along with the absence or presence and severity of any symptoms of decompression sickness.

The grading system for the venous return bubbling rate heard over the right heart consisted of a five-point scale, judged at the time of maximum frequency and amplitude.

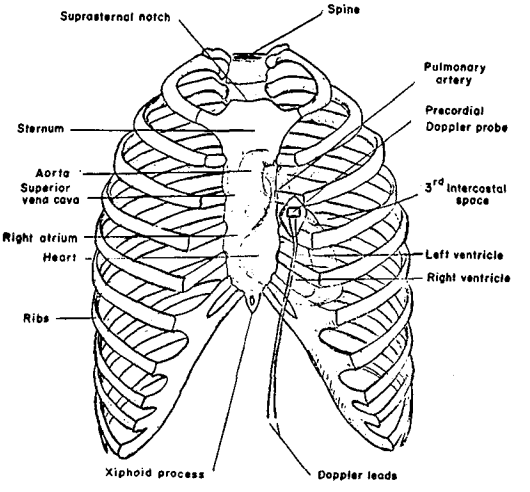

FIG. 2. Positioning of precordial blood bubble detector along the left sternal border.

Zero indicated a complete lack of bubble signals. Grade one indicated an occasional bubble barely discernible within the cardiac motion signal and with the majority of all the cardiac periods free of bubbles. Grade two designated that many, but not all, of the cardiac periods contained several bubbles clearly distinguishable within the cardiac and blood flow signals. Grade three designated when most of the cardiac periods contained bubble signals which overrode the blood flow signal. Grade four was the maximum detectable bubble signal, continuous throughout systole and diastole of every cardiac period and completely overriding the amplitude of the cardiac signal.

Subjects were recompressed for therapy if bubbles occurred in more than grade 2 quantities, or if pain developed in the extremities. Recompression treatment in the 30-foot dives was to 60 feet on the U.S. Navy treatment table number 5 (26). In the treatment of two cases following the 150-foot exposure recompression, treatment was only to 30 feet for 1 hour on 100% oxygen. In most cases where bubbles were heard in grade 1 or grade 2 quantities, the subject breathed 100% oxygen at 1 atmosphere until the bubble signals diminished or disappeared.

The hyperbaric exposures made in this study (30 feet/12 hours, 70 feet/50 minutes and 150 feet/15 minutes) were selected on the theoretical prediction to lie somewhere on the gradient of no-decompression limits ranging between no bubbles on any diver and 100% bubbles on all divers; this was also true on a gradient ranging between no bends and 100% bends. These gradients for air exposures may be visualized as a series of parallel lines representing isoembolic contours and isobends contours extending over a practical range from approximately 30 feet for several hours to some practical depth and time around 10 atmospheres. The prediction of the location of this gradient, on a time-pressure plot, was based on Behnke's nitrogen elimination data (3). His averaged cumulative quantities of nitrogen eliminated by three subjects, whose weight averaged 64 kg, was normalized to 70 kg body weight and plotted as a function of time on log scales (see Fig. 1).

The curved nature of the nitrogen elimination data on a log-log plot is interpreted to mean that nitrogen elimination is not a single power function of time but represents the action of a series of simultaneous exponentials. The exact shape of the no-decompression isoembolic and isobends lines along the no-decompression limits gradient is of prime concern in this study. For the purpose of guidance in finding the no-decompression gradient, a circle fit to the N_2 elimination data on a log-log plot was considered adequate. The lower section of Fig. 1 gives the theoretical limits based on the N_2 elimination data, for supersaturation ratios of 2:1 and 1.75:1. The no-decompression calculations were based on Behnke's method which assumes that: 1) nitrogen uptake and elimination follow the same course with any step-change in respired P_{N_2}; 2) a uniform tissue supersaturation ratio may be tolerated where no-decompression dives occur; 3) the percentage uptake for all body tissues is constant at any point in time; and 4) if a subject initially equilibrated at one atmosphere breathes hyperbaric air, the time (t_x) at which he is y% saturated is assumed to be equivalent to being 100% saturated at y% of the actual respired atmospheric pressure. On the assumption that a subject can tolerate a 2:1 supersaturation ratio for all tissues, he then can be safely returned to 1 atmosphere at t_x. For example, using Fig. 1 it can be seen that if a subject makes a step excursion from 1 to 4 atmospheres, in 29 minutes he will have taken up 50% of the nitrogen that he will accumulate when saturated at 4 ata. He may then safely return to 1 ata because his equivalent 100% saturation is at 2 ata. Likewise, the subject is safe to decompress from air respired at 8 ata after 8 minutes. The

upper left hand corner of Fig. 1 discloses a simple graphic method of finding the no-decompression limits using any assumed supersaturation ratio. At all supersaturation ratios the shape of the no-decompression compression limit lines are an inverse image of the nitrogen elimination curve. The computed no-decompression line on the basis of a 2:1 and 1.75:1 supersaturation ratios were used as references for the additional results of this investigation.

Figure 3 contains a plot of the bends threshold data for goats after Hempleman (9) and swine bends percentage versus exposure from Gillis (7). Hempleman's data tend to follow an isobends contour parallel to the nitrogen elimination predicted curve. The swine data range is not sufficient to determine its shape but suggests a gradient of isobends lines over the range studied. The swine bends gradient appears to be very broad ranging at 5.5 ata from 0% for 15 minutes to 80% for 50 minutes. The sheep bubble data (15) indicate 100% venous gas emboli on all exposures except those at 7.5 ata, where the gradient appears to be broad (as in the 5.5 ata bends gradient for swine).

The 2:1 N_2 predicted curve falls here between bubble ratios of 1:3 and 3:5. Thus the predicted no-decompression curve, at a ratio of 2:1 approximates a 50% bubbling rate. The sheep and swine data (7,15) both indicate a bubbles gradient far in advance of the bends gradient.

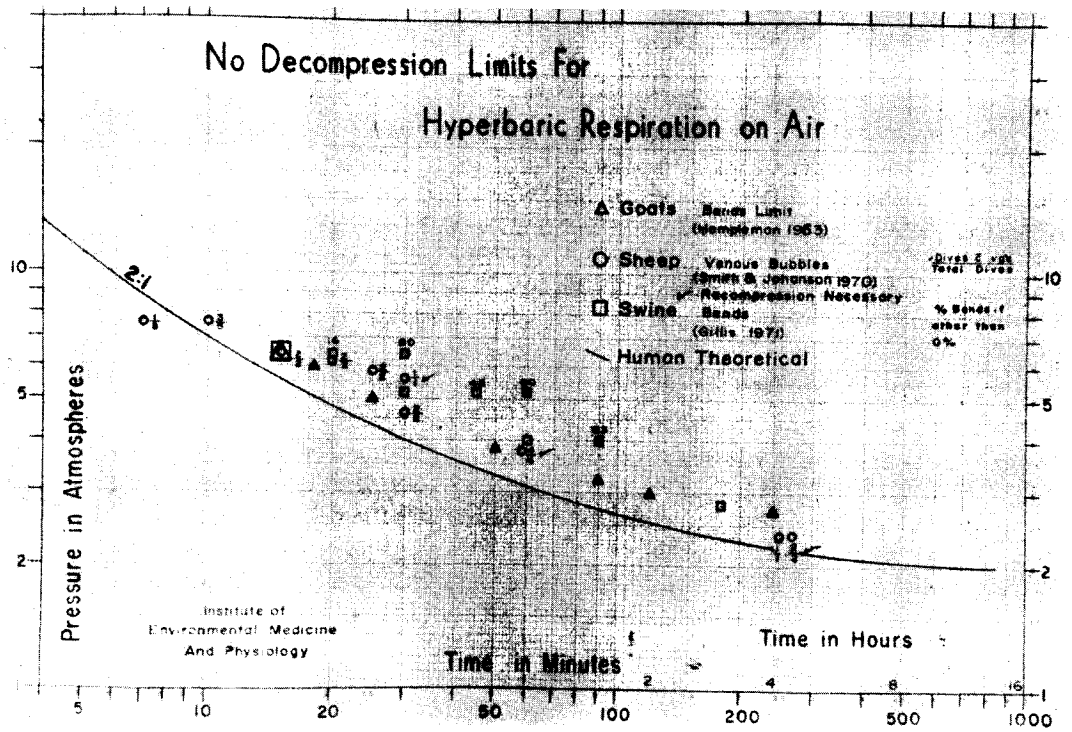

FIG. 3. Animal no-decompression data from goats, sheep and swine bends and sheep venous gas emboli. No-decompression limits for hyperbaric respiration on air.

Human No-Decompression (Direct Decompression) Limits Defined by Bends

The present U.S. Navy limits for hyperbaric air dives are plotted in Fig. 4 and follow approximately a predicted 1.9:1 limits curve within the range of 35 feet (2.1 ata) to 110 feet (4.3 ata). It deviates in a conservative direction at the greater depths. Figure 5 which gives experimental data of Hawkins et al. (8) and of Albano (1) indicates, as do the N_2 predicted lines, that greater depths and times may be allowable in the upper range than provided for on the U.S. Navy tables (26). It is clear that the Navy limits, which are based on bends *symptoms*, are far to the left and below the animal limits which are based on bends *signs*.

Human Limits Based on Venous Gas Emboli

The three principal human experimental hyperbaric chamber exposures are plotted in Fig. 4. The attendant percentage of venous gas embolism occurrence as well as the percentage of bends occurrence among the experimental divers is given beside each of the exposures. Table I gives further details on the results. At 5.6 ata (150 feet) a 50% bubbles rate and a 25% bends rate corresponded to the predicted 2:1 limit. The results at the 1.9 ata (30-feet) exposure, however, indicate that selection of a lower supersaturation ratio would be necessary to produce 50% bubbles. This finding is at variance with the presently accepted concept that 30 feet (13.3 p.s.i.g., 1.9 ata) is a safe no-decompression exposure for any period of time because a coincident 25% bends rate was found after 12 hours. The USN limit of 70 feet/50 minutes was the middle exposure to determine more precisely the curvature of the isoembolic and isobends lines. The percentage range and the percentage bends were the lowest of the three selected exposures. These three data points indicated that the

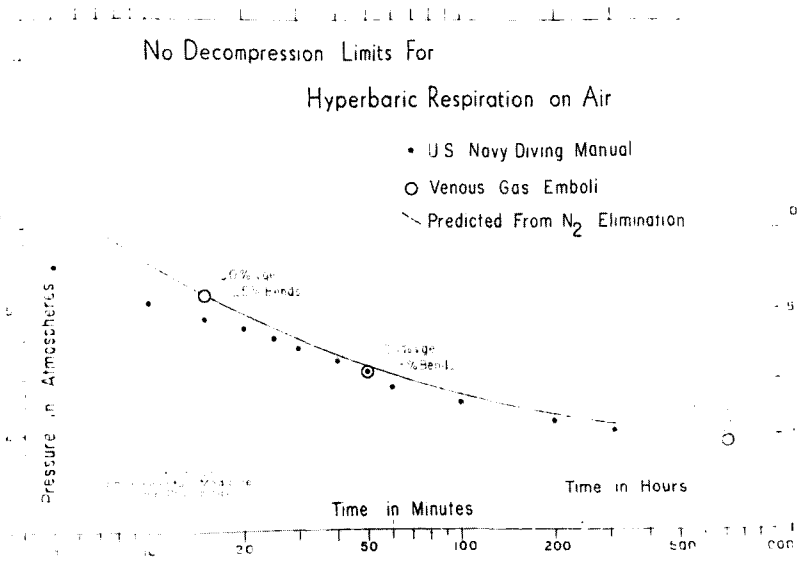

FIG. 4. Human no-decompression limits for hyperbaric respiration on air using precordial venous gas embolism detection as compared to bends (prediction from N_2 elimination curve and U.S. Navy Manual recommendation). Vge = venous gas emboli.

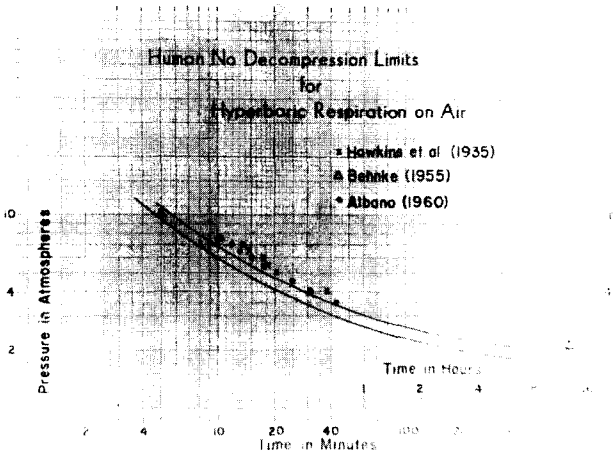

FIG. 5. Human no-decompression limits for hyperbaric respiration on air as determined by Hawkins et al. (8), Behnke (3a) and Albano (1) compared to the predicted curve determined from N_2 elimination data.

no-decompression lines of isoembolic occurrence follow a curvature less convex than that indicated by the nitrogen elimination data. There are three separate methods of defining the no-decompression tables: 1) safe diving experience as defined by the U.S. Navy no-decompression limits; 2) the theoretical prediction from experimental nitrogen elimination; and 3) the lines of isoembolism defining the no-decompression gradient; these methods are, however, in fair agreement. These data further indicate that a 2:1 supersaturation ratio is not sufficiently safe to prevent bends development. Further experiments should be performed to more precisely define the statistical probability of both bends and bubbles in terms of isocontour lines of equal bends and equal gas emboli occurrence.

BUBBLE PRONENESS

Table I, which gives the results on the experimental divers, is arranged according to decreasing order or proneness of the individual divers to form venous gas emboli. Three of the experimental divers consistently formed venous gas emboli on every experimental "dive," and three of the subjects never formed venous gas emboli on the same dives.* It was not surprising to find a difference in occurrence of bubbles between divers on the same dive, but the consistency with which each diver tended to form bubbles on any borderline exposure located on the no-decompression gradient was surprising. All venous gas emboli that were regionally localized came from the extremities with the exception of one diver in whom they were thought to come from somewhere within the chest or abdomen (Table I). In no instance were jugular vein venous gas emboli or arterial emboli detected.

OPEN-WATER TRIALS

Fifty-two open-water SCUBA dives were performed in the 50° waters of Puget Sound.

*The 150 feet for 10 minute and the 200 feet for 7 minute dives were preliminary experimental tests for conservatism before testing the predicted 150 feet/15 minute exposure which exceeded the presently accepted no-decompression limits.

These were training dives for the most part on beginning divers and ranged from 30 to 110 feet, and from 15 to 70 minutes. All dives were less in depth/duration than those prescribed by the present U.S. Navy limits except for three dives, two of which were at 70 feet for 50 minutes, and one at 80 feet for 40 minutes. No venous gas emboli were detected in divers immediately upon surfacing. In some repetitive dives, following initial conservative dives, venous gas emboli were detected. One especially interesting exposure was at 80 feet/40 minutes which produced no venous gas emboli although it surely would have if performed in the dry chambers. Open-water conditions, not present in the chamber, included psychological conditioning of open-water submergence, cold and exercise. The uptake and elimination of N_2 is undoubtedly altered by these conditions.

Discussion

Further data, using the method described here, could provide a complete definition of the isoembolic lines and isobends lines. This should define the limits of no-decompression respiration on air in terms of the gradients existing along the entire pressure-time function—from no bubbles and no bends to 100% bubbles and 100% bends. Such data when also available for working dives will then allow one to choose his own risk and balance it against the practical urgency of the exposure. In addition, these data may be useful in developing improved decompression procedures. With the precise baseline available such factors as age, obesity, exercise and water temperature might then be explored and their effects on diving tables be determined. If some venous gas emboli and no bends is an acceptable risk, this may safely allow 4 minutes at 300 feet and 2 minutes at 600 feet. This extrapolation of the data must, however, be confirmed.

Shallow Saturation Exposures

On the basis of the 30 feet/12 hour findings and the gradient of isobubbles and isobends lines that emerge from this study, 30-foot no-decompression exposures should be allowed for no more than 4 hours. A decompression stage or decanting on oxygen should be used for saturation dives after 2 ata exposures. The application of these results and the bubble detection technique in caisson work is apparent. The labor and other factors may produce a need for even greater conservatism. We believe that exposures which avoid venous gas emboli will eliminate bone necrosis as a risk for compressed air workers. The venous gas embolism detection technique may also provide a safe method of preselection of divers more resistant or less susceptible to bends. Since all bends symptoms of pain or discomfort appeared only after venous gas embolism signals and a time delay, a group of divers may be preselected in the wet or dry chamber by giving all subjects the same simulated borderline exposure selected on the no-decompression gradient. A possible extension of this concept is to develop individualized decompression tables.

Deep No-Decompression Exposures

From the experimental data now available it appears the lines of isobubbles and isobends follow a more gentle curvature than indicated by N_2 elimination data. At the deeper exposure venous gas emboli may appear first at exposures farther removed from the bends

gradient than in the shallower exposure. This is indicated by the animal data as well as in the preliminary data at exposure of 200 feet/7 minutes where three out of four subjects exhibited grade 1 bubbles for short periods of time without bends.

Probably the venous gas emboli appearing quickly after short deep exposures nucleate more from the blood and other short half-time compartments while the larger saturation exposures predispose to more delayed bubble origins from fat and other long half-time compartments.

The major difference between the no-decompression limits defined by venous gas emboli in this study and the limits of Hawkins' chamber and Albano's open-sea exposures is explained by the greater sensitivity of the ultrasonic detection method over recording of clinical bends. We have recorded 25% bends on the 5.5 ata for a 15-minute exposure which is an exposure indicated as safe by their results. All of the bends complaints (other than skin itching) in this study were very mild joint pains and possibly were below the level recorded by those investigators. All three sets of data above 3.5 ata agree with each other and with the N_2 elimination predicted limits more closely than with the deeper U.S. Navy limits. In the middle depths and around 3 ata there is good agreement between Albano, the U.S. Navy, and the 1.9:1 prediction as well as the data of this study.

Doppler Method

The method used here in the development of no-decompression respiration limits on air we believe, with variations, should be extended to the improvement of present decompression profiles for air diving and caisson work. It can easily be adapted to determination of the limits of altitude excursion both from the earth's surface as well as following exposure to hyperbaric atmospheres. In addition, it should be used for the development of helium tables and, in particular, for the deep diving schedules which are now necessary. With time a very useful body of knowledge concerning the meaning of venous gas emboli in terms of bends symptoms and complications will be developed. The Doppler surface detectors should also be more widely used in diagnosis and treatment of decompression illness and aeroembolism. Every decompression chamber used for human exposures should be equipped with it and have personnel available qualified in its use. The Doppler ultrasonic bubble detector in diving may be compared to the stethoscope in cardiology. The method also has many research potentials in elucidating mechanisms of bubble formation, distribution and dissipation.

The limitation of this present method is that it does not detect static bubbles in the tissues or blood. The venous gas emboli, however, appear to be dislodged into the circulation very early in the saturation state before symptoms develop; therefore, the limitation is primarily in research of static and tissue bubbles.

Other Ultrasonic Techniques

Other ultrasonic techniques for the detection of stationary bubbles in tissues and blood have been attempted (11,12,25,27). These techniques are based on through-transmission or reflected ultrasound using either continuous wave or pulse ultrasonic energy. The transducer is localized over an area of expected bubble formation and the subject decompressed until a change in transmission or reflectance is obtained. A serious limitation of these techniques presently is similar to that initially found with the Doppler peripheral searching: namely, that early bubble formation is unlikely to occur under the selected local

tissue. The randomness of early development of decompression bubbles, symptoms and signs means that a local detector has a very poor chance of giving an early warning because it necessarily limits the observer to one particular localized tissue site (usually on the accessible extremities or in the superficial tissues of the body). To detect the earliest developing static bubbles such transmission techniques will be required to scan all points simultaneously on the entire body or be so flexible and reliable that they can find use by moving rapidly from one suspect area to another.

Formation of Decompression Venous Gas Emboli

The question of whether or not tissue bubbles form extravascularly at the time of or before the detection of venous gas emboli remains a moot point. Present evidence, however, suggests that if they are formed at this early stage, they readily pass into the venous return through available channels. In open chest anesthetized dogs, random injections of air by means of a small hypodermic needle under the epicardium of the heart produce immediate gas emboli in the pulmonary artery. It therefore appears that in heart muscle, at least, a low resistance pathway exists for gas bubbles to traverse from interstitial spaces into the venous channels. It is presently believed that the earliest decompression bubbles in fact form and grow in the capillaries and small veins where they are dislodged into the central venous return. Furthermore, the precordial blood bubble detector is believed to be able to detect all physiologically significant sizes of blood bubbles which are considered to be greater than the diameter of a capillary. From in vitro studies it is known that bubbles smaller than those visible to the naked eye are detected by the present 5 MHz 10 milliwatt detector and it has been shown that gross injection of carbon dioxide into peripheral veins always produces showers of precordial bubble signals (23).

It now appears likely that the central nervous signs of decompression sickness are due to excessive venous gas embolization formation to the point of overload of the pulmonary capacity followed by passage through the lungs into the systemic arterial circulation. This belief is based on the fact that overload of the pulmonary bed capacity to handle venous gas emboli is exceeded before gross signs of heart or respiratory failure, in addition to the fact that the jugular blood in bends is not prone to venous gas emboli. It is known from clinical use of the blood bubble detector during cardiopulmonary bypass for open-heart surgery, that the brain and other body tissues tolerate the occurrence of hundreds of gas emboli per minute which are detectable with the 5 MHz blood bubble detector and do not cause overt central nervous system signs or symptoms (20). The limits of sizes and numbers tolerated remains to be determined.

Animal Models for Decompression Studies

It appears that both sheep and goats are good experimental animals for decompression studies using the Doppler ultrasonic methods, as the 50% venous gas embolism isoembolic limit line (2:1 supersaturation predicted limit) fits both in the range tested (5.5–7.5 ata) and the goat bends limit approximates the 100% range in the deeper exposures. Goats and sheep may therefore continue to be useful in leading the way for human studies. Swine appear from Gillis's experience (7) to be less appropriate for decompression emboli studies although the isobends lines from his data plotted in Fig. 3 roughly parallel, at greater exposures, the other animal data.

Summary and Conclusions

The precordial blood bubble detection technique using the Doppler ultrasonic principle was utilized in three selected exposures to define the human hyperbaric air no-decompression limits. The percentage of subjects exhibiting venous gas emboli after decompressing and the percentage developing minor bends was recorded with the objective of defining the limits in terms of isoembolic and isobends contour lines along the pressure-time limits. Three experimental protocols were selected on the basis of computed predictions from nitrogen elimination data. Thirty-six exposures were made on 13 subjects for 12 sets of data at 30 feet/12 hours, 70 feet/50 minutes and 150 feet/15 minutes each. The limits lines appear to be of multiexponential form similar in contour to the nitrogen elimination curve. No-decompression from 30 feet for 12 hours' exposures caused a 67% incidence of venous gas emboli and a 25% bends; 70 feet for 50 minutes produced 33% venous gas emboli and 8% bends, and 150 feet for 15 minutes produced 50% venous gas emboli and 25% bends.

Present U.S. Navy no-decompression schedules appear to be optimal for efficiency and safety in the middle ranges and 60–100 feet exposures, extra conservative for the greater depths, and not conservative enough for the shallower depths. Within the range studied the ratio of the percentage of subjects with venous gas emboli to the percentage of subjects demonstrating bends varied from 4:1 to 2:1. There was found a reproducible propensity in certain individuals to develop early decompression venous gas emboli and to produce them in the same extremity. At the same time other individuals appear to have a relative immunity to them. Venous gas embolism signals may be used as a guide for adequate recompression or oxygen therapy. Venous gas emboli were quickly dissipated by oxygen recompression therapy at pressures less than the no-decompression exposure producing them. No indications of adverse effects were observed with the use of the 5 MHz 10 milliwatts/cm^2 ultrasound. The method is considered to be safe for routine human use; it would be highly useful to develop decompression schedules, diagnose and treat decompression sickness and to investigate the process of bubble formation, distribution and dissipation. Venous gas emboli are reliably detected by sensors located over the right ventrical and pulmonary artery. Since their origin in the periphery develops first in specific localized areas without general distribution, they may be undetected unless peripheral scanning happens to be localized over the veins draining the area of bubble formation.

ACKNOWLEDGMENT

This research was supported by the Office of Naval Research Contract #00014-73-C-0094 with funds provided by the Naval Medical Research and Development Command.

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INERT GAS EXCHANGE AND BUBBLE FORMATION AND RESOLUTION IN THE EYE

S. Kronheim, C. J. Lambertsen, C. Nichols and P. L. Hendricks

Conventional approaches to the calculation of decompression tables for diving incorporate exponential functions to describe how gas tensions in a spectrum of hypothetical tissues change relative to changes in external pressure. The number of hypothetical tissues and their respective rate constants for exchange of a specific inert gas are usually arbitrarily assigned and then refined by empirical testing. Direct measurements of tissue gas tensions have been reported only by Campbell and Hill in 1933 (4, 5).

Study of inert gas exchange in the eye was undertaken because existing evidence indicated that the eye might be representative of the body's slowest gas exchange tissues. Such evidence included the anatomy of the eye and the results of the TEKTITE I and II nitrogen saturation dives (12, 13). From Fig. 1 it can be seen that the aqueous humor, the vitreous

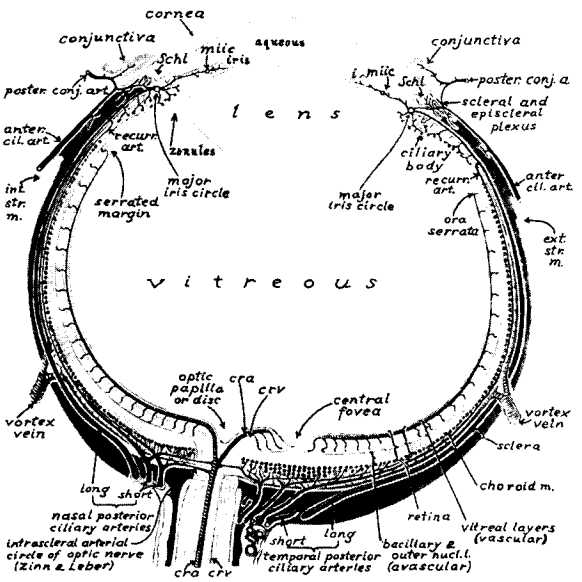

FIG. 1. Horizontal section through the human eye. Adapted from Polyak (14).

humor and the lens are avascular. The blood vessels of the eye are found in its peripheral coats and in the ciliary body and iris. The aqueous is continuously produced by the ciliary body and drained away by the venous system in the juncture of the cornea and sclera at the angle of the anterior chamber at a flow rate of approximately 0.013 minute^{-1} (8). However, neither the vitreous nor the lens has such a feature of flow. Even in the aqueous, gas exchange by diffusion may be faster than by mass flow of newly formed fluid. Gas exchanged by diffusion between the blood vessels of the eye and the vitreous, lens or aqueous must travel considerable distances. Some diffusion exchange should be considered to occur between the aqueous and the lens and the vitreous. The eye, therefore, appeared to offer an excellent opportunity for comparing tissue components which might represent fast and slow rates of inert gas exchange.

The second major reason for studying the eye was that asymptomatic bubble formation appears to have occurred in the lens (one of four subjects) and vitreous (three of nine subjects) of human aquanauts as a consequence of decompression from the TEKTITE I and II series of nitrogen saturation dives at 42 fsw (12, 13). The TEKTITE decompression tables had been developed on the basis of a 480-minute tissue halftime for nitrogen (equivalent to a rate constant of 0.0014 minute^{-1} [11]).

Experimental Approach

The experiments discussed below fall into three categories, including 1) in vitro determination of the *solubility* of ^{85}Kr in the aqueous, vitreous and lens of the New Zealand white rabbit eye at 34°, 37° and 40°C and 1 ata; 2) in vivo determination of the *rate constants* for ^{85}Kr uptake into and elimination from the same eye compartments at 1 ata; and 3) induction of *eye bubbles* in rabbits by varying simulated depth of exposure, bottom time and decompression time.

The in vitro gas solubility experiments, which utilized a temperature-controlled, microtonometric apparatus, were carried out first to develop the sampling and ^{85}Kr liquid scintillation counting methods to be employed in the in vivo exposures. These experiments demonstrated that the Bunsen coefficients for ^{85}Kr in aqueous, vitreous, lens and saline could be determined with precision on 0.2-ml samples (9). The solubility data obtained are shown in Table I and Fig. 2. The similarities in the solubilities of ^{85}Kr in saline and in the aqueous and vitreous are not unusual since these substances are 99% water (1, 3). The Bunsen coefficients for ^{85}Kr in the lens at 37° and 40°C are significantly smaller than the values at the same temperatures for the aqueous, vitreous and saline. It is not entirely certain that these

TABLE I

MEAN BUNSEN COEFFICIENTS FOR ^{85}KRYPTON IN EYE SUBSTANCES AND SALINE

Temperature (°C)	Aqueous	Vitreous	Lens	Saline
	(ml gas/ml substance)			
34	0.0420 ± 0.0008	0.0413 ± 0.0007	0.0409 ± 0.0010	0.0419 ± 0.0007
37	0.0387 ± 0.0014	0.0380 ± 0.0008	0.0349 ± 0.0009	0.0384 ± 0.0007
40	0.0350 ± 0.0005	0.0349 ± 0.0007	0.0324 ± 0.0007	0.0353 ± 0.0003

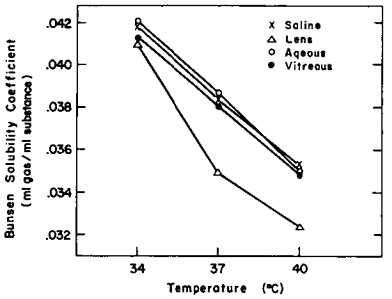

FIG. 2. Bunsen solubility coefficients for ^{85}Kr in eye substances and saline as function of temperature.

differences can be explained on the basis that the composition of the lens is approximately 65% water and 35% protein (10).

In the in vivo gas uptake and elimination experiments rabbits were exposed to constant concentrations of tracer amounts of less than 0.1% ^{85}Kr in air in a closed environmental glove-box system (9). In the gas uptake experiments rabbits were sacrificed in the ^{85}Kr environment after exposure times ranging from 15 minutes to 24 hours. The eye substances under study were quickly removed, placed in liquid scintillation counting vials, and the radioactivity counted, using the same method as for the in vitro studies. Mean rate constants for uptake and for elimination were obtained for each individual compartment by pooling data for each eye and determining the regression against time of individual measurements plotted on semilog paper.

Gas Uptake and Elimination

Analysis of the uptake data, shown in Fig. 3, demonstrated that 1) uptake by the aqueous, vitreous and lens was essentially complete in 2 to 4 hours; 2) uptake by the aqueous, vitreous and lens each followed monoexponential patterns; and 3) the mean rate constants for ^{85}Kr uptake are 0.112 minute^{-1} for the aqueous, 0.047 minute^{-1} for the vitreous and 0.029 minute^{-1} for the lens.

To determine the elimination rate constants for the aqueous, vitreous and lens, these eye compartments were initially saturated by an 8-hour exposure to ^{85}Kr in air. The rabbits were then removed to a normal room air environment and sacrificed at the same time intervals as for the uptake experiments, up to 24 hours. Analyses of the elimination data, shown

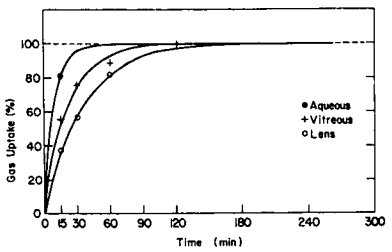

FIG. 3. Average mean uptake for aqueous, vitreous and lens across experiments.

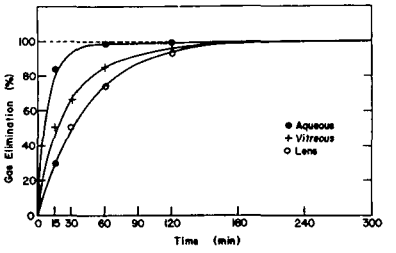

FIG. 4. Average mean elimination for aqueous, vitreous and lens across experiments.

in Fig. 4, demonstrated that: 1) elimination by the aqueous, vitreous and lens was essentially complete in 4 hours; 2) elimination by the lens followed a monoexponential pattern, while elimination by the aqueous and vitreous each followed at least two exponentials; and 3) the mean rate constants for ^{85}Kr elimination are 0.124 minute^{-1} for the fast component and 0.018 minute^{-1} for the slow component in the aqueous; 0.072 minute^{-1} for the fast component and 0.021 minute^{-1} for the slow component in the vitreous; and 0.023 minute^{-1} for the lens.

Since it was of interest to determine whether a rate constant of approximately 0.02 minute^{-1} might be representative of the slowest tissues in an animal the size of a rabbit, and because it was often found that rabbits dying after decompression from air dives contained macroscopic bubbles in perirenal fat but did not have bubbles in the lens, perirenal fat samples were analyzed for ^{85}Kr uptake and elimination in two experiments. Figure 5 **A** and **B** illustrates the preliminary findings that the ^{85}Kr gas exchange rate constants for perirenal fat and lens are similar. This is the first direct experimental evidence that two tissues with significantly different compositions—the one fat, the other water and protein—can have the same inert gas exchange rate constants. These results are too preliminary to confirm the possibility that the lens is representative of one of the slowest gas exchange tissues in the rabbit.

Rate constants for inert gas exchange in the vitreous and lens of the *human* eye were calculated on the basis of the experimental results obtained in rabbits. It was recognized that there are four factors which tend to make inert gas exchange in the human eye slower than that in the rabbit eye. These are differences in iris pigment (2), intrinsic flow of vitreous (3), vitreous volume (6, 17) (which is larger in the human) and blood circulation time (9). While the calculations of rate constants for human eyes, derived from the observations in rabbits, take into account differences in vitreous volume, vitreous flow could not be quantified. Differences in blood perfusion rates were ignored since the equations are based on the gas exchange being diffusion-limited. Of the two anatomic factors which tend to make the rate constants for the human eye larger than the rabbit eye, the volume of the lens (9) (which is larger in the rabbit) is a variable in the calculations. The pertinent diffusion distance for humans is between the retina and the vitreous rather than the distance between the choroid and the vitreous in the rabbit (9). The equations for the calculations are taken from Hills (7). The application to the eye makes the assumptions that gas exchange is by bulk diffusion and that rabbit and human vitreous and lens each approach the shape of a sphere. The equations are:

$$a\alpha = 3 \tag{1}$$

$$k = \alpha^2 D \tag{2}$$

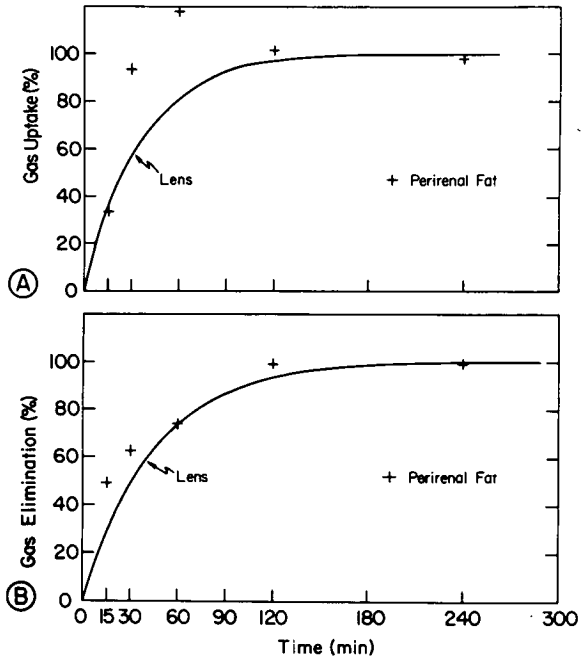

FIG. 5. **A**, Regression plot of lens uptake with fat uptake values shown. **B**, Regression plot of lens elimination with fat elimination values shown.

where:

$a\alpha$ = shape factor, which is 3 for a perfect sphere
a = the radius of the rabbit eye compartment
α = a constant with dimensions radius^{-1}
k = rate constant for ^{85}Kr elimination
D = ^{85}Kr diffusion coefficient.

The diffusion coefficients for ^{85}Kr are calculated to be 1.93×10^{-5} cm^2/sec in the rabbit vitreous and 3.41×10^{-6} cm^2/sec in the rabbit lens. Assuming these diffusion coefficients are applicable to the human, the rate constants for ^{85}Kr are calculated to be 0.0111 minute^{-1} in the human vitreous and 0.0720 minute^{-1} in the human lens. By applying Graham's law relating diffusivity to molecular weight, nitrogen rate constants of 0.0193 minute^{-1} in the human vitreous and 0.1255 minute^{-1} in the human lens are calculated. These calculated human rate constants for nitrogen are equivalent to tissue halftimes of approximately 35 minutes for the vitreous and 6 minutes for the lens, values which are not at all comparable to the 480-minute controlling halftime that led to eye bubbles during decompression in TEKTITE I and II. These differences may indicate that diffusion per se may not be the controlling factor in decompressing safely from saturation dives, that the eye may not be representative of one of the slowest gas exchange tissues in the human body, or that decompression itself may somehow modify gas exchange rate constants.

Bubble Formation In Vivo

By applying Graham's law to the experimentally determined rate constants for ^{85}Kr in the rabbit lens, high pressure exposure-decompression profiles were developed for rabbits breathing air to allow the animals to be decompressed with predictable, calculated inert gas-supersaturation in the lenses. The assumptions were made that: 1) the lens was representative of the slowest gas exchange tissues in the body; 2) the rate constants for gas exchange at increased ambient pressures were the same as at sea level, provided bubble formation did not occur; 3) since the lens was avascular, diffusion of gas out of the lens would be the controlling factor in bubble formation; and 4) diffusion of nitrogen out of the lens would be governed by Graham's law. Maximum depths of exposure were 366 fsw and maximum time at this pressure was 5 hours. Rabbits were examined ophthalmoscopically before, during and following decompression.

Early success in inducing lens bubbles in rabbits exposed to 300 fsw for 5 hours and decompressed in 22 to 26 minutes led to the hypothesis that bubble formation would occur in the lens whenever that tissue was returned to one atmosphere with 3.5 ata nitrogen in it. Later studies could not confirm this hypothesis, and failure to induce bubbles prior to death of the animals resulted in its abandonment in favor of a more empirical approach (9).

Bubbles were detected in the aqueous, lens (Fig. 6) and ciliary body of living rabbits as a result of decompression following pressurization with N_2–O_2 mixtures. Whereas the types of bubbles observed in the aqueous were transient, disappearing after no more than a period of minutes, lens bubbles were visible for as long as 50 to 60 hours postdecompression. In no cases were vitreous bubbles seen in the eyes of living rabbits. Although it is possible that bubbles were never present, it is more likely that the bubbles could not be viewed because they rose to the top of the eye (16), and because the unpigmented eye of the rabbit causes a great deal of light reflection and a poor red-orange background on ophthalmoscopic examination (9). Figures 7, 8 and 9 illustrate the types of bubbles seen in the aqueous, vitreous, and lens of rabbits which died within 1 hour of return to 1 atmosphere. It is of special interest to note that, with the exception of one case, all lens bubbles were found in the posterior aspect of the lens. This is the portion of the lens without any epithelium and having a thinner capsule or covering and a rounder aspect than the anterior segment (15).

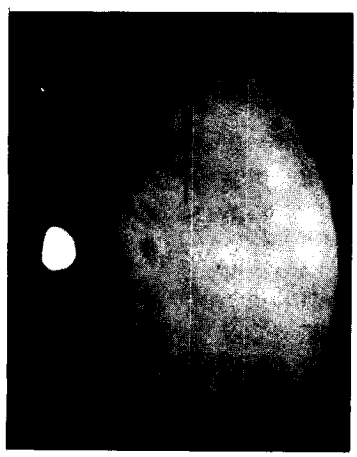

FIG. 6. Bubble seen in lens of living rabbit as a result of decompression following pressurization.

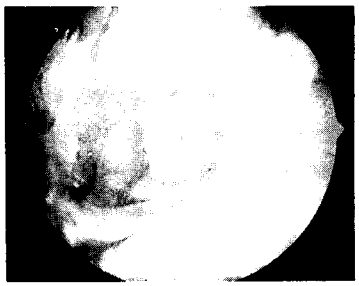

FIG. 7. Bubbles in aqueous of rabbit which died within 1 hour of return to 1 atmosphere.

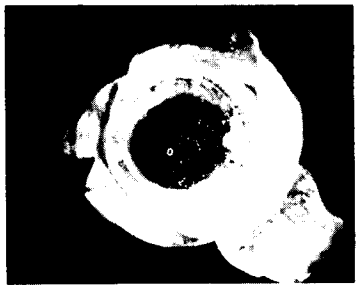

FIG. 8. Bubbles in vitreous of rabbit which died within 1 hour of return to 1 atmosphere.

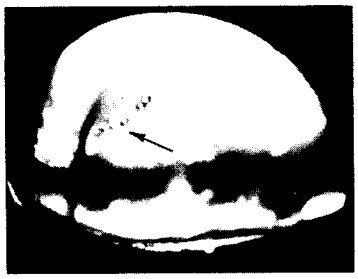

FIG. 9. String of bubbles in lens of rabbit which died within 1 hour of return to 1 atmosphere.

These results would indicate that gas exchange at the anterior half of the lens is much more efficient than at the posterior—a reasonable deduction if one considers that the anterior surface is continuously perfused by aqueous while the posterior surface abuts directly on and adheres to the anterior vitreous.

Conclusions from the Study

The findings described demonstrated that: it is possible to make quantitative measurements of inert gas uptake and elimination in vivo in individual tissues of the body; gas uptake and elimination in the eye tissues studied follow exponential patterns of exchange; gas uptake and elimination in specific tissues are not necessarily symmetrical; even at 1 ata, gas elimination appears to be slower than gas uptake in individual tissues; intertissue gas exchange appears to occur in the tissues of the eye; it is possible to induce reversible bubble formation in the aqueous, lens and ciliary body-zonule area of the eye by varying exposure time on

air, exposure pressure, and/or decompression rate or time; it is possible to apply rate constants determined for ^{85}Kr at 1 atmosphere to nitrogen at high pressure by application of Graham's law, but the experimental testing of such extrapolation indicates either that this approach in itself is not valid, or that other factors interfere at high pressure and/or during decompression to obscure the approach; by calculation, the rate constants for gas exchange in specific tissues of the human eye are roughly 10 (for the vitreous) to 100 (for the lens) times faster than what was anticipated on the basis of results of human nitrogen-oxygen saturation dives.

ACKNOWLEDGMENTS

The work reported here was performed at the Institute for Environmental Medicine, University of Pennsylvania, as a component of its Predictive Studies III program. This study was supported in part by contract HL 08899-08 from the National Institutes of Health, and contract N00014-67-A-0216-0026 and N00014-67-A-0216-0024 with the Office of Naval Research.

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BUBBLE FORMATION RESULTING FROM THE STEADY COUNTERDIFFUSION OF TWO INERT GASES

J. Idicula, D. J. Graves, J. A. Quinn and C. J. Lambertsen

Following discovery of severe consequences of isobaric inert gas counterdiffusion (7), specific analyses and in vitro studies of the counterdiffusion process have been carried out. The occurrence of skin lesions during nitrogen or neon breathing in a helium environment was reported by Blenkarn et al. (1), who considered gas-induced osmotic forces and water flux rather than gas bubble evolution to be responsible for the phenomenon. In the next observations, during Predictive Studies III (6), it was developed that the gross dermal lesions and vestibular changes were most likely both generated by bubble formation, due to a previously unrecognized mechanism related to unequal rates of gas diffusion between skin surface and skin capillaries.

~~In the description which follows it is shown~~ by use of mathematical and physical models of stable isobaric counterdiffusion that true gas supersaturation and bubble formation can be expected to occur, even without pressurization and subsequent decompression. These findings relate to the now well-demonstrated development of dermal gas lesions and vestibular derangement in circumstances where nitrogen or neon is breathed while the subjects are exposed to a helium–oxygen environment at increased ambient pressure.

Analysis of Mechanism

Analysis of stable-state counterdiffusion of any two gases across a single, homogenous liquid phase shows that in the presence of only a single phase gas supersaturation in the liquid does not occur. However, in a two- or more layered system supersaturation and bubble development is to be expected.

Counterdiffusion supersaturation in a more complex situation can be illustrated mathematically by assuming constant diffusivities and ideal solubility relationships for the gases involved (3, 4). These restrictions are not necessary but simplify the analysis considerably and lead to the possibility of developing interesting quantitative conclusions and criteria via a mathematical model. The linear partial pressure profiles which are calculated to result during gas counterdiffusion under the conditions described are illustrated in Fig. 1. The properties of the layers and permeants have been chosen in such a way that the resistance to gas transport is low in the first layer which a permeant traverses and high in the second

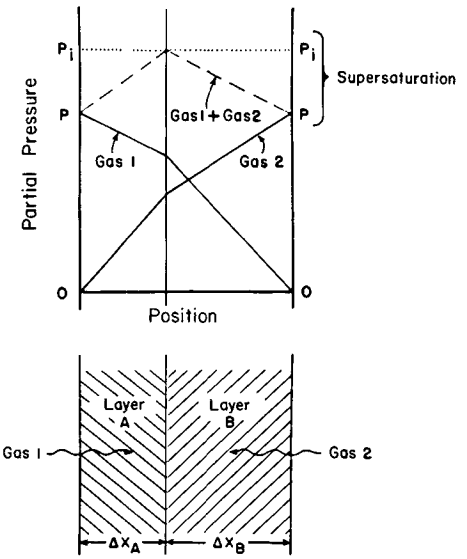

FIG. 1. Isobaric gas counterdiffusion. Analysis of gas partial pressure profiles resulting from steady counterdiffusion of two inert gases through a two-layer system composed of materials having different permeabilities for the two gases.

layer. The sum of the partial pressures of the two gases is shown as a broken line, which in this case is always above the ambient pressure and is a maximum at the two-layer interface.

More specific understanding can be obtained by starting with a flux equation based on a combination of Fick's law for diffusive flux and Henry's law for the gas solubilities (7):

$$J_{1A} = -K_{1A}(\Delta P_{1A}/\Delta X_A) \tag{1}$$

where:

J_{1A} = the flux of species 1 through membrane layer A;
K_{1A} = the permeability coefficient of 1 in A (the product of diffusivity and solubility);
ΔP_{1A} = the partial pressure difference of 1 across layer A;
ΔX_A = the thickness of A.

By writing the four equations of this type for the two permeants and the two layers and invoking restrictions such as the continuity of fluxes, it is possible to find an expression for the sum of the partial pressures at the interface between the two layers (P_i). For pure permeants 1 and 2 at equal pressure P on opposite sides of layers A and B, the following result is obtained:

$$\frac{P_i}{P} = \frac{\Delta X_B K_{1A}}{\Delta X_B K_{1A} + \Delta X_A K_{1B}} + \frac{\Delta X_A K_{2B}}{\Delta X_A K_{2B} + \Delta X_B K_{2A}} \tag{2}$$

The first term in Eq. (2) is the relative partial pressure of component 1 and the second term that of component 2.

Several aspects of this analysis are particularly pertinent to human pressure exposure. The first is that two adjacent layers of tissue or membrane do not have to exhibit opposite semipermeability for the two counterdiffusing gases to produce supersaturation ($P_i/P > 1$). A necessary and sufficient condition is simply that the layers exhibit *different* semipermeabilities and that they be arranged in *proper sequence.* Specifically, the condition is given by

$$K_{1A}/K_{2A} > K_{1B}/K_{2B} \tag{3}$$

A second relevant aspect is that, for a given pair of permeants and of materials, a maximum supersaturation is obtained at a certain ratio of layer thicknesses:

$$\Delta X_A/\Delta X_B = \sqrt{K_{1A}/K_{1B} \cdot K_{2A}/K_{2B}} \tag{4}$$

The absolute thicknesses are immaterial at steady state. In isobaric supersaturation, if at least one of the layers is liquid and if suitable nuclei are present, bubbles will form and grow continuously. With poorly adherent solid layers, gas blebs or gross separation of the layers could result from forces of gas accumulation between the layers. If mechanical restraints are imposed which prevent layer separation, an increase in gas phase pressure within the layers could result.

Although this analysis has emphasized behavior of dissolved gaseous permeants, its importance is by no means limited to gases. For example, in the case of two solutes which participate in a common chemical reaction, the *product* of chemical activities (or, in the ideal case, concentrations) may be of primary importance rather than the *sum* of partial pressures. Counterdiffusion in this instance might drastically alter the velocity of a chemical reaction. Specifically, the phenomenon affects a situation in which two different reactants are maintained at equal concentrations in separate compartments (e.g., intra- and extracellular) and the reaction involving them takes place between two sequential membranes separating the compartments. If each membrane is perfectly permeable to the reactant in contact with it and impermeable to the second reactant, the concentrations within the intramembrane space would be the same as in each external compartment. If a second system is prepared which has two identical membranes with some finite permeability, however, the intramembrane concentrations would be half those in the two compartments. For a reaction which is first order in each reactant, the rates in those two cases would differ by a factor of four. This example illustrates how reaction rates in a multi-membrane system such as a cell might be quite different from those anticipated.

Physical Systems

The initial prediction of a counterdiffusion supersaturation leading to bubble formation has been further confirmed through a series of related experimental studies in model physical systems (4). With the physical model (Fig. 2) it was possible to demonstrate bubble evolution in an oil–water system seeded with crushed glass for nuclei, with counterdiffusing nitrogen and helium (4). The diffusion cell had two chambers separated by a water-repellent filter paper (Aquapel). The paper allowed a water–oil layer to be supported while helium diffused through from the water side, to and through the oil, and nitrogen diffused through

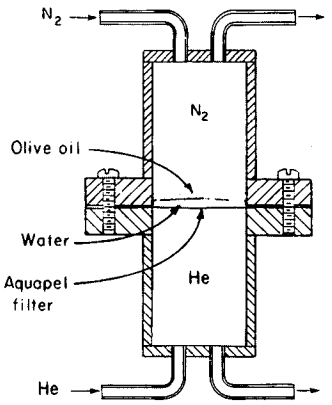

FIG. 2. Gas counterdiffusion cell. A two-piece diffusion cell such as that illustrated was used in diffusion experiments. For pressure measurements in two-membrane experiments, a washer-like ring with a hypodermic needle connection was clamped between the two halves and a membrane was sealed between either face of the washer and a cell half.

from the oil side, to and through the water. Bubble formation occurs at the oil-water interface. Bubbles were not generated when the directions of counterdiffusion were reversed. Two solid membranes have also been used as the layers (XD1 silicone copolymer [2], and ethyl cellulose or polyethylene) with the same counterdiffusing gases (nitrogen on the silicone side). A small gas space was provided between the two membranes with an outlet so that either gas flow into the space or gas pressure buildup could be monitored. Even with this relatively crude apparatus, both a continual flow of gas into the space and a pressure buildup to 25 Torr (3.4×10^4 dynes/cm^2) when the outlet was closed off in the silicone-polyethylene system, were measured. A very satisfactory confirmation of Eq. (2) was obtained in the silicone-ethyl cellulose system (see Fig. 3) with the substantial pressure of 336 Torr being measured.

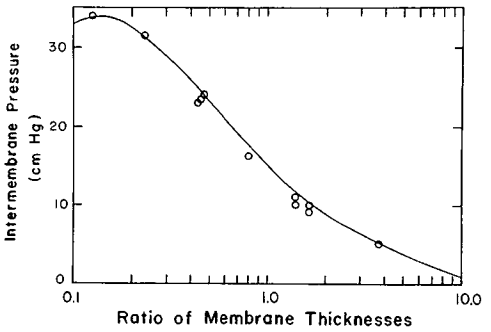

FIG. 3. Confirmation of Eq. (2) (solid curve) was obtained with an ethyl cellulose-silicone copolymer two-membrane system (data points). The ordinate shows the steady-state pressure rise (between the membranes) over ambient pressure (outside the membranes) as a function of the thickness ratio of ethyl cellulose to silicone. The solid curve was calculated from measured permeabilities by using Eq. (2); measurement of the data points was carried out using a dual-membrane cell. Pressure goes through a maximum as predicted by Eq. (3).

Isobaric Counterdiffusion Bubble Formation in Animals

A series of studies of isobaric counterdiffusion in vivo has also been carried out and will be reported in detail elsewhere (5). In it young pigs were used, anesthetized to prevent itching, breathing through an endotracheal tube, and exposed at various stable ambient pressures to inert gas counterdiffusion to determine occurrence and character of dermal lesions.

Table I summarizes the occurrence in animals breathing one inert gas with oxygen while surrounded with another. Exposures were conducted at ambient pressures to 10 ata, sometimes with helium as the ambient gas and sometimes with the heavier inert gas ambient. In the simplest interpretation it is evident that, as with the mathematical analysis and the physical model, gas lesion development occurred only with a specific direction of the counterdiffusion (i.e., with helium surrounding the animal and the second inert gas respired, to diffuse from the skin capillary bed through the skin). In no case did dermal lesions occur when these conditions were reversed. A striking aspect of these observations was that dermal lesions could be generated even at 1 atmosphere of ambient pressure, without prior compression, when the counterdiffusing gas respired was nitrous oxide. No explanation yet exists for failure of dermal lesions to occur when SF_6 was breathed.

TABLE I

BUBBLE FORMATION IN COUNTERDIFFUSION EXPOSURES

Depth	Breathing Ne / Chamber He	Breathing N_2 / Chamber He	Breathing Ar / Chamber He	Breathing N_2O / Chamber He	Breathing SF_6 / Chamber He	Breathing He / Chamber N_2O	Breathing He / Chamber Ar
Surface		+	+	+	0	0	
33 fsw		+	+		0		
66 fsw		+	+		0		
100 fsw	+	+	+		0		
200 fsw	+	+	+				
300 fsw	+	+	+				

Severity of lesions for any given depth and time: $N_2O > Ar > N_2 > Ne$.
"+" indicates that dermal lesions developed.
"0" indicates that dermal lesions did not develop.

Implications in Undersea Medicine

These analyses of gas supersaturation under isobaric conditions, and demonstrations of actual bubble formation in skin and subcutaneous tissues clarify the inevitable hazard of the counterdiffusion process. This process must be considered capable of generating bubbles in dermal tissues, with transport of bubbles in the circulation to distant vital organs. It should in addition be considered capable of adding to or exaggerating the effects of gas bubbles formed during actual decompression. Prevention should include avoidance of exposure of skin, ear canal and eyes to counterdiffusion. Rational therapy could be considered equivalent to that employed for decompression sickness itself.

ACKNOWLEDGMENTS

The work reported here was performed at the Institute for Environmental Medicine, University of Pennsylvania, as a component of its Predictive Studies III program. This study was supported by contract HL 08899-08 from the National Institutes of Health, and contract N00014-67-A-0216-0026 with the Office of Naval Research.

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INNER EAR DISORDERS RESULTING FROM INADEQUATE DECOMPRESSION—"VERTIGO BENDS"

A. A. Bühlmann and H. Gehring

Experimental Procedure

From June, 1965 to April, 1972, the Deep Diving Research Laboratory of the Department of Internal Medicine, University Hospital, Zürich carried out a total of 478 decompressions ranging in depth from 30 to 350 meters (4–36 ata) with 160 different subjects. For 163 exposures the breathing gas was air; in the remaining 315 it consisted of O_2–He mixtures. Some of the subjects, mostly amateur divers, took part in various exposures. Table I gives a summary of the decompressions used.

Results

Table II summarizes data from 10 different experiments carried out with a total of 24 subjects in depths of 45–300 meters (5.2–31 ata). Twelve subjects exhibited symptoms indicating inner ear disorder. The nature of these symptoms is shown in Table III.

All 12 subjects suffered from vertigo as the predominant symptom, which always appeared as a first sign, mostly in the form of rotary vertigo with a constant turning course. The

TABLE I

SUMMARY OF EXPOSURES CARRIED OUT FROM 1965 TO 1972

Pressure (ata)	Decompressions	Decompressions With Inner Ear Disorders
4.0–5.5	252	1
5.6–13.0	165	0
16.0–23.0	52	7
31.0–36.0	9	4
Total	478	12

TABLE II

SUMMARY OF THE EXPOSURES ASSOCIATED WITH INNER EAR SYMPTOMS

Trial No.	Pressure (ata)	Time at Bottom (hrs)	Number of Subjects	Inner Ear Disorders	
				Subjects With	Subjects Without
1[a]	23	6	1	1	0
2[a]	23	6	2	1	1
3[a]	23	3	2	1	1
4[a]	23	4	4	2	2
5[a]	23	5	4	1	3
6[a]	16	1	2	1	1
7[a]	31	2	2	1	1
8[a]	31	3	2	2	0
9[a]	31	4	2	1	1
10[b]	5.2[b] air	30[b] min	3	1	2
Total			24	12	12

[a]Trials 1 to 9 with O_2–He mixtures.

[b]Trial 10: with air on a simulated altitude of 3000 m above sea level, corresponding to pressure of 0.7 ata. The breathing mixture was air; the bottom time, 30 minutes.

TABLE III

SYMPTOMS OF INNER EAR DISORDERS

Trial No.	Name (Age in years)	Vertigo	Nausea, Vomiting	Loss of Hearing	Murmurs	Appearance of Symptoms		Recompression to ata	Residual Effects[a]
						ata	Minutes After Beginning of Decompression		
1	Arnold S. (23)	1	0	0	0	12.0	100	14.0	0
2	Rino G. (45)	1	1	0	0	10.7	170	14.5	0
3	Rino G. (45)	1	0	0	0	11.7	169	14.0	0
4	Luigi G. (27)	1	1	0	0	12.5	161	14.5	0
4	Enricó L. (28)	1	1	0	0	12.5	161	14.5	0
5	Rino G. (45)	1	1	1	1	13.5	143	14.5	v,h,m
6	Rolf B. (21)	1	1	1	0	5.8	124	7.5	v,h
7	August K. (23)	1	1	1	1	16.6	100	21.0	v,h,m
8	Rolf B. (22)	1	1	0	1	18.5	140	21.0	No control
8	Ernst L. (23)	1	1	1	1	16.0	325	19.7	h,m
9	Rino G. (47)	1	0	1	1	21.0	135	28.0	h,m
10	Karl K. (25)	1	1	0	0	0.7	152	3.0	v
Total		12	9	5	5				6

[a]Residual effects: **v**, vertigo and light equilibrium disturbances; **h**, loss of hearing; **m**, murmurs.

vertigo decreased in the horizontal position and with eyes closed, but increased with head movement, sitting and standing. The elapsed time from the beginning of the decompression until the appearance of the vertigo varied between 100 and 170 minutes; in one case it was 325 minutes.

Nine subjects experienced nausea and vomiting in addition to vertigo. In two cases (Rino G., trial no. 5, and Rolf B., trial no. 8) the vomiting appeared with the vertigo; in the remaining seven cases it occurred 5–25 minutes later than the vertigo, so that vomiting coincided partly with the phase of therapy. Some of the subjects described it as explosive vomiting. Five subjects exposed to depths of 16, 23 and 31 ata complained about loss of hearing or murmurs or both, either after they had left the pressure chamber or during decompression.

Case Histories—Five Subjects

After decompression, the Department of Otolaryngology at University Hospital examined five subjects with the following results:

1) *Rino G.* (45 years, trial no. 5, 23 ata, 5 hours bottom time, Table III). Symptoms were inner ear deafness (left), murmurs (left), vestibular hyperreflexia upon vestibular function examination (left), and light vertigo and light equilibrium disturbance.

The vestibular hyperreflexia and the equilibrium disturbances disappeared within 6 weeks during treatment with vasodilators; the murmurs showed a slight improvement but continued to disturb the patient considerably. The inner ear deafness persisted.

In the course of trial no. 9 (31 ata, 4 hours bottom time) the same symptoms appeared during decompression as did in trial no. 5, but they did not bring about a lasting alteration such as murmurs and deafness on the left.

2) *Rolf B.* (21 years, trial no. 6, 16 ata, 1 hour bottom time, Table III). Symptoms were of spontaneous nystagmus to the right when sitting, lying and in various positions of the body and the head; no change in the nystagmus after caloric stimulation. Weber experiment showed lateralization to the right. The audiogram showed a loss to about −80 dB in the range of frequencies from 4000 to 8000 Hz; this applies to both bone and air conduction.

3) *August K.* (23 years, trial no. 7, 31 ata, 2 hours bottom time, Table III). Symptoms before the experiment were a partial loss of hearing on both the right and left sides. After the experiment, symptoms were partial loss of hearing on both right and left sides, lateralization to the left (Weber experiment), murmurs (right), head nystagmus (right), and no nystagmus upon symmetrical vestibular function examination. The experiment did not influence the deafness which, in the subject's opinion, spontaneously diminished within 2 months.

4) *Ernst L.* (23 years, trial no. 8, 31 ata, 3 hours bottom time, Table III). Symptoms before the experiment were a loss of hearing, symmetrical on both sides, for low and high sounds, and murmurs on both sides. After the experiment, the loss of hearing remained unchanged, and murmurs continued on both sides.

5) *Karl K.* (25 years, trial no. 10, 5.2 ata, diving trial of 45 meters in an altitude of 3000 meters over sea level, 30 minutes bottom time, air, Table III). The following symptoms were evident: falling tendency to the left; normal finger-to-finger and finger-to-nose tests; no cochlear disturbances.

Therapy

Therapy begun at the onset of vertigo consisted of interruption of the decompression and the recompression until the vertigo had disappeared. Nausea and vomiting always disappeared as well. During the next decompression six subjects again suffered from vertigo and vomiting, or vertigo alone, so that four cases again required recompression. The interrupted decompressions and the therapeutic recompressions considerably increased the total decompression time.

Residual Effects

After reaching zero meters, six subjects still complained about loss of hearing, murmurs and/or slight equilibrium disturbances with vertigo (Table III). The equilibrium disturbances disappeared over a period of a few days to a maximum of 6 weeks (in the case of Rino G., trial no. 5). The ear noise intensity was unchanged for Ernst L. after the experiment, but he did not perceive it as very disturbing. Disturbing ear noises, however, persisted over a period of years for Rino G. Therefore, a transtemporal neurectomy of the vestibular nerve was first carried out on the left side after approximately 3 years. The operation, which did not have any beneficial effect on the spontaneous auditory noises, showed that the vessels supplying the vestibular nerve were distinctly reduced. In order to eliminate the murmurs, a translabyrinthal neurectomy of the cochlear nerve was later performed on the left side. Rino G. has lost the disturbing ear noises, but the operation caused a slight disturbance in the equilibrium which appears mainly on walking and standing. His left ear is practically deaf.

Evidence of inner ear deafness or partial loss of hearing can be positively attributed to one of the exposures referred to here only in the cases of Rino G. and Rolf B. Two divers, August K. and Ernst L., showed a slight loss of hearing before the experiments which neither positively nor negatively influenced the deficiency.

Discussion

The symptoms such as vertigo, nausea and vomiting which point to disturbances in the inner ear (so-called vertigo bends), can be observed in the course of decompressions after exposures with air as well as with O_2–He mixtures used as breathing gases (1, 4, 9). On 417 decompressions in depths corresponding to 4.0–15.9 ata only one case of inner ear disorder was recorded, whereas such troubles occurred 11 times in seven subjects in the course of 61 decompressions after exposures between 16 and 36 ata. Mechanisms which may lead to the appearance of inner ear disturbances are either related to overpressure or dependent on insufficient decompression.

Related to Overpressure

1) Insufficient pressure equalization between middle ear and ambient pressure (barotrauma).
2) Increased endolymphatic fluid volume caused by osmotic pressure gradients related to the dissolved inert gases.

DEPENDENT ON INSUFFICIENT DECOMPRESSION

1) Bubble formation in the endolymphatic system.
2) Intravascular bubble formation or gas embolization in the inner ear vessels.
3) Extravascular bubble formation in the inner ear tissues.

Irritation of the labyrinth and the hearing loss caused by an impeded or missing pressure equalization through the eustachian tube has been described (5). This type of barotrauma with inner ear disorders can be observed after dives within no-decompression limits. The coincidence of head nystagmus, nausea and vomiting with loss of hearing and ear noises is known as Ménière's disease. This disease depends on an endolymphatic hydrops (8). It is unlikely that the vestibular cells are influenced by a massive increase of the endolymph owing to the additional inert gases dissolved, this being of a very low rate. The appearance of inner ear symptoms during decompression in the cases described here does not favor such a mechanism.

The symptoms described above may result from an increase of the endolymphatic pressure by bubble formation in the endolymph; this is caused by a too rapid decompression in reference to bottom time and oversaturation factors of the tissues concerned. Other authors, who also refer to Ménière-like symptoms during decompression (2, 6, 7) consider as causal intravascular bubble formation in the arteria auditiva interna and their vestibular branches or bleeding of these vessels with a consecutive ischemia and damage to the sensory cells. A further possibility is that bubbles formed locally in the tissues may directly irritate the sensory cells or may bring about ischemic damage by compression of small vessels from outside.

Mathematical analysis of the decompressions with inner ear disorders showed that, for the tissues with He half-time values of 45, 60 and 90 minutes (corresponding to N_2 half-time values of 120, 160 and 240 minutes), there are critical oversaturation factors which 12 other subjects tolerated without showing any symptoms during the same experiments. Figure 1 shows the critical oversaturation factors above the curved lines. The inner ear symptoms occurred within an hour after a decompression phase with one of these high oversaturation factors. On 454 decompressions, of which 294 were carried out with an O_2–He mixture, including 40 exposures between 16 and 36 ata, such disturbances never occurred with the use of lower factors according to the curves.

Considering the correlation between the oversaturation of tissues, which the inner ear can be considered also to represent, and the appearance of the symptoms, it can be concluded that some local bubble formation may play a leading causal part in the origin of so-called vertigo bends after deep diving using O_2–He mixtures or long lasting exposures with compressed air. This view is supported by the following arguments: The acute inner ear symptoms such as vertigo, nausea, vomiting and loss of hearing were not often observed until O_2–He mixtures were used for deep diving. Since tissues are saturated by He about 2.6 times faster than by N_2 (3), there is a much greater risk that the tissue concerned will reach a critical point of oversaturation, e.g. on short exposures with air at conventional depths. Only on tunnel exposures lasting for hours are critical values reached by N_2 which can then lead to accidents. Insufficient decompression after air diving in high altitude (trial 10) causes the same symptoms. When, however, adequate oversaturation factors were used during decompression, no such symptoms occurred on deep diving with O_2–He mixtures. Recompression was very effective.

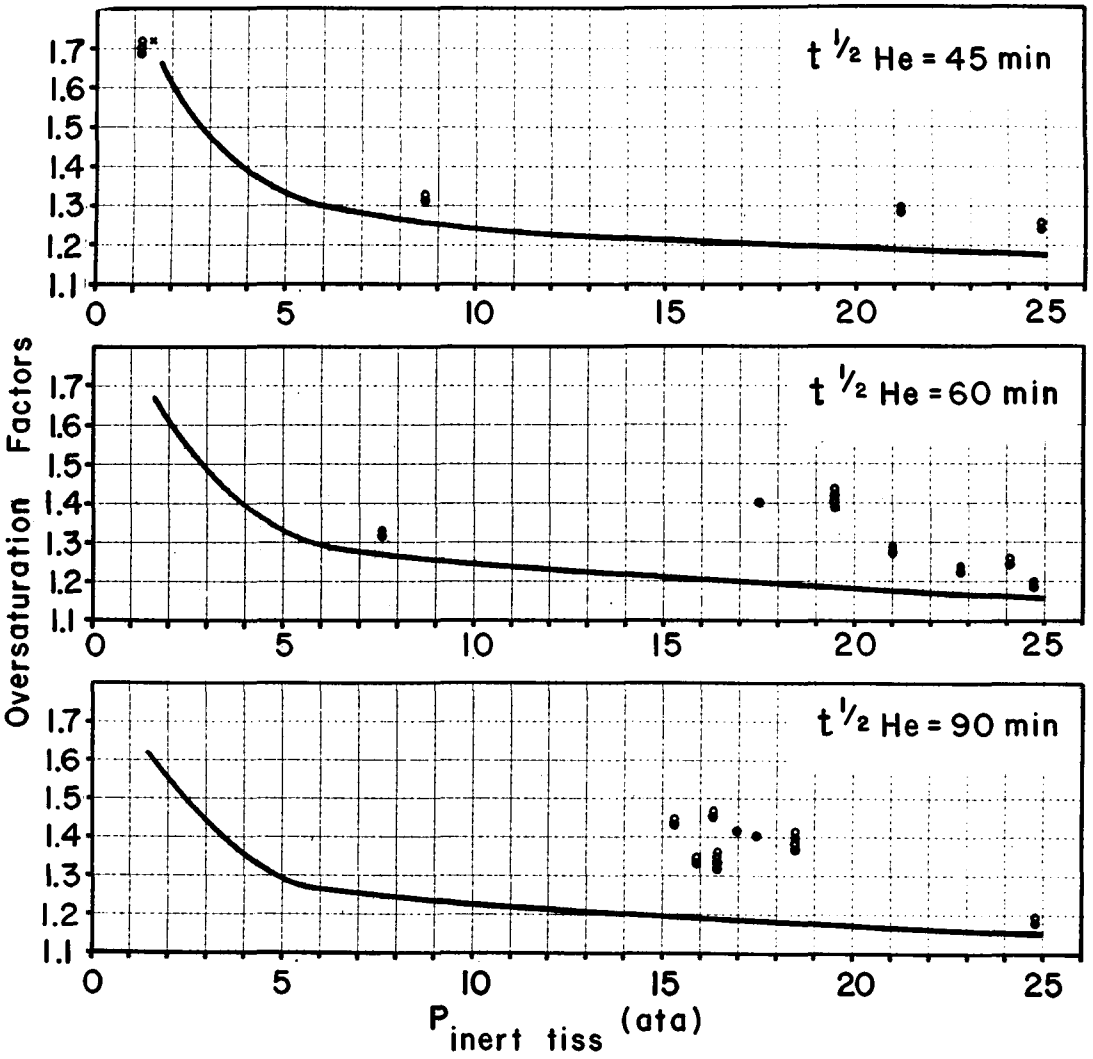

FIG. 1. Oversaturation factors of tissues with He half-times of 45, 60 and 90 minutes; 24 decompressions (21 with O_2-He, 3 with air [x] at an ambient pressure of 0.7 ata) with higher oversaturation factors showed vertigo bends in 12 cases; 454 decompressions with oversaturation factors on or below these lines showed no inner ear disorders. ● = vertigo bends; O = no symptoms; —— = tolerated oversaturation factors.

Summary

During 24 decompressions following exposures with pressure in the range of 5.2-31 ata, symptoms of inner ear disorders such as vertigo, nausea and vomiting were observed in 12 cases, as well as deafness and murmurs in 5 cases. These symptoms disappeared as a result of adequate recompression after some minutes. In one case, without full recompression, loss of hearing and murmurs have persisted for years.

The mathematical analyses of these decompressions showed high oversaturation factors for tissues with He half-times of 45, 60 and 90 minutes. These factors were tolerated without symptoms by 12 other subjects during the same experiments. During 454 decompressions (294 with O_2–He mixtures including 40 experiments between 16 and 36 ata) no "vertigo bends" were observed by using lower oversaturation factors for these same tissues. It was concluded that vertigo bends result from bubble formation in the cochlear and vestibular region, and perhaps in the endolymph.

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MEASUREMENT OF UPTAKE AND ELIMINATION OF NITROGEN IN TISSUE, IN VIVO

K. N. Ackles, D. E. Holness and C. A Scott

Despite some success in the computation of decompression schedules, there is still little knowledge of the actual laws governing the uptake and elimination of inert gases by tissues. Current difficulties in decompressing from deep subsaturation dives, and the wide differences between saturation-decompression schedules used by different American and European groups, create a pressing need for information on the basic factors governing tissue-gas exchange.

Usually each diving laboratory has its unique decompression schedules, based on principles of gas exchange which are believed to be close approximations of true values. Most of these schedules are based on Haldane's original ideas and have been modified through the years as a consequence of cases of decompression sickness occurring with current schedules. Mathematical models have been applied to the empirical schedules and these models have simplified the calculation of schedules for which empirical data are not available. This procedure has created problems. For example, air schedules which appear completely adequate for 30 minutes at 150 feet are inadequate for 30 minutes at 250 feet, indicating that some of the factors which must govern gas exchange at the tissue level have not been considered. Furthermore, there is still disagreement as to whether tissue gas exchange is diffusion- or perfusion-limited.

The availability of a mass spectrometer capable of measuring tissue inert gas tension directly in vivo makes it possible now to begin to acquire the information necessary to place the calculation of decompression schedules on a firm scientific basis. This paper reports the preliminary experiments which have been completed with such application of the mass spectrometer, using anaesthetized dogs. The threefold purpose of these experiments has been: first, to gain experience in measuring tissue gas tensions with the mass spectrometer; second, to identify any difficulties in data collection and analysis; and third, to collect preliminary data on gas exchange.

Methods

The Mass Spectrometer-Sampling System

When operated for study of gas tensions in the tissues, the mass spectrometer* was

*Medspect MS-8, manufactured by Scientific Research Instruments Corporation, Baltimore, Maryland.

capable of sampling from two tissue sites simultaneously, with the digital and analog output automatically switching from one to the other every 50 seconds. Tissue gases were sampled by means of a Teflon catheter which passed through a 15 gauge, thin-wall needle. The sampling length was 1 inch, and sampling rate was less than 10^{-6} ml/second. The internal vacuum of the mass spectrometer, approximately 1×10^{-6} mm Hg, enabled gas to be drawn directly from the tissue fluid. Fluid was not taken up by the nonthrombogenic catheters. The mass spectrometer was calibrated before each experiment using water-filled tonometers equilibrated with appropriate gas mixtures at 37 °C. Long-term stability studies of the mass spectrometer have shown that there was negligible drift over the time of these experiments (less than 1% over 6 hours). In addition, there was no evidence of tissue-gas depletion by the catheters.

General Procedure

Dogs, ranging in weight from 10 to 20 kg, were anaesthetized with intravenous sodium pentobarbital. A tracheal cannula was inserted and the animals breathed spontaneously but were assisted in their respiration by a "Bird" Mk8 respirator.* A catheter was inserted and secured in the gracilis muscle of the thigh and another one in the abdominal fat. All catheter positions were checked post-mortem at the conclusion of the experiment. In the post-mortem check, a small number of catheters were found to be in connective tissue instead of muscle (i.e., between muscle sheaths).

Several different experimental gas-exchange procedures were used. In nine experiments, at 1 ata, following equilibration of the catheters in the animal breathing air, the breathing gas mixture was switched to 20–80 oxygen–helium mixture and nitrogen washout recorded for 2 hours, following which the breathing mixture was switched back to air and nitrogen uptake recorded for 2 more hours. Because the mass spectrometer is unable to measure helium, only nitrogen curves were obtained. In a second group of five experiments, the same procedure was followed but, instead of a 20–80 oxygen–helium mixture, the animals were ventilated with a 20–80 oxygen–argon mixture. The mass spectrometer detects argon and consequently uptake and elimination curves for argon were obtained simultaneously with the nitrogen elimination and uptake curves. In other experiments the dogs were pressurized at 60 feet/minute to 2 or 3 ata while breathing air. In all cases, decompression was directly back to 1 ata at 50 feet/minute.

Results and Discussion

Analysis of the results demonstrated several difficulties with this type of experiment which were not apparent when the experiments were planned and carried out.

All individual tissues measured demonstrated a multi-exponential form of uptake and elimination of inert gas. This presented difficulties in the final analysis because, in the preliminary experiments, gas exchange was not measured to saturation and arbitrary extrapolations had to be made.

Uptake and elimination curves were first plotted linearly and extrapolated to 250 minutes (Fig. 1). These curves were then replotted as percentages on semilogarithmic paper, the

*The "Bird" respirator was also used to ventilate the animals with gas mixtures other than air.

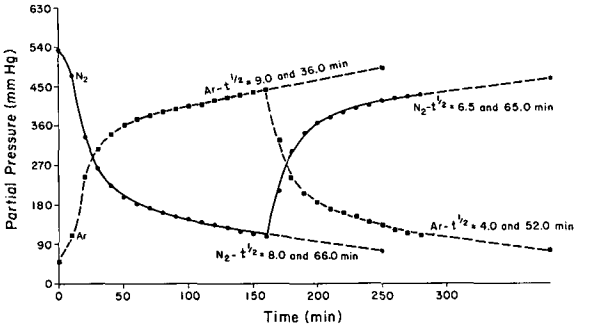

FIG. 1. Nitrogen and argon exchange in dog muscle while the breathing mixture was switched from air to a 20–80 oxygen–argon mixture, then back to air at 1 ata.

components being separated graphically by the peel-off method (Figs. 2 and 3). With this method, one or two separate half-times were identified in every tissue measured. In muscle, two components were always found: the first with a half-time of less than 15 minutes, and a second component greater than 15 minutes. From the results summarized in Table I, it can be seen that the second component, in particular, has a great variation in time constants in different animals. Indeed, the variation is so great that average values have little meaning. In fat and connective tissues, the first component is not always present in either the uptake or elimination phase or sometimes in both.

As can be seen in Table I, 15 minutes was taken arbitrarily as the division between the two components. Despite the great individual variations between animals, the important

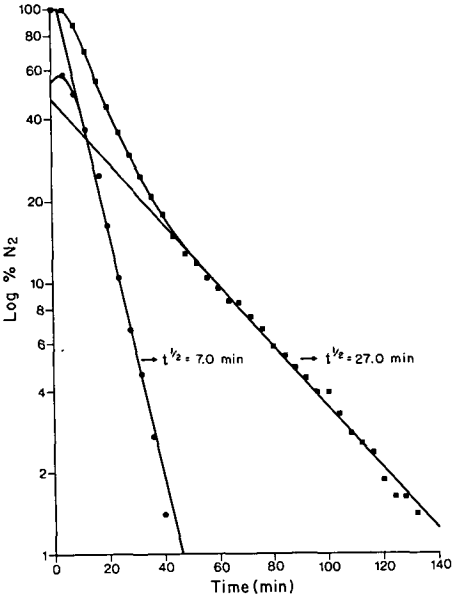

FIG. 2. Semilogarithmic plot of nitrogen uptake at 1 ata in dog muscle during air breathing following nitrogen washout with a 20–80 oxygen–helium mixture. Two separate half-times are present.

TABLE I

INERT GAS EXCHANGE—TISSUE HALF-TIMES (MINUTES)
STATISTICAL SUMMARY OF DATA

	N_2-Muscle Uptake		*N_2-Muscle Elimination*	
	$t^{1/2}$-C_1	$t^{1/2}$-C_2	$t^{1/2}$-C_1	$t^{1/2}$-C_2
x	6.8	47.6	7.5	51.5
N	12	12	12	12
SEM	±0.4	±7.8	±0.7	±9.7
	N_2-Connective Tissue Uptake		*N_2-Connective Tissue Elimination*	
	$t^{1/2}$-C_1	$t^{1/2}$-C_2	$t^{1/2}$-C_1	$t^{1/2}$-C_2
x	8.5	58.8	7.5	56.4
N	4	4	3	4
SEM	±1.4	±17.0	±0.3	±6.7
	N_2-Fat Uptake		*N_2-Fat Elimination*	
	$t^{1/2}$-C_1	$t^{1/2}$-C_2	$t^{1/2}$-C_1	$t^{1/2}$-C_2
x	8.4	52.3	5.5	56.3
N	7	12	4	11
SEM	±1.0	±5.3	±1.4	±4.9
	Ar-Muscle Uptake		*Ar-Muscle Elimination*	
	$t^{1/2}$-C_1	$t^{1/2}$-C_2	$t^{1/2}$-C_1	$t^{1/2}$-C_2
x	6.7	43.2	5.6	50.2
N	5	5	5	5
SEM	±1.0	±10.4	±0.6	8.1
	Ar-Fat Uptake		*Ar-Fat Elimination*	
	$t^{1/2}$-C_1	$t^{1/2}$-C_2	$t^{1/2}$-C_1	$t^{1/2}$-C_2
x	4.5	53.1	7.5	57.3
N	1	4	1	4
SEM	-	±12.1	-	9.7

finding of these experiments is the fact that more than one time component characterized individual tissues. The first component is not due solely to the arterial half-time which has been measured at about 4 minutes, although this obviously contributes to it (unfortunately, in these preliminary experiments it was not possible to demonstrate a difference between the half-times of nitrogen and argon exchange). Another finding of interest is the confirmation that all tissues are inherently unsaturated; that is, the total of all gas partial pressures is always less than ambient atmospheric pressure (Table II).

It is apparent from these experiments that perfusion plays a major role in the gas exchange process. Often, while recordings were made during steady-state conditions, the nitrogen tension slowly increased, then decreased to its original value after about 4–5 minutes. These slow blips were interpreted as indications of a temporary change in capillary perfusion. In addition, although the level of anesthetic was kept as constant as possible, some slow changes were associated with the administration of anesthetic.

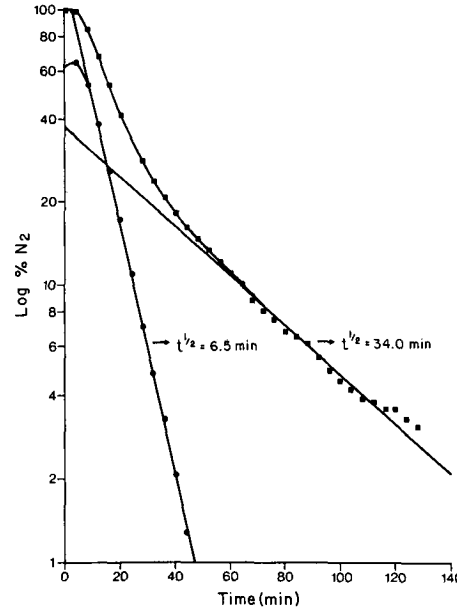

FIG. 3. Semilogarithmic plot of nitrogen uptake in dog muscle following pressurization from 1 ata to 2 ata in 30 seconds.

Further evidence was gained concerning the importance of perfusion. Following the re-establishment of equilibrium with air at 1 ata, two animals were compressed on air to 10 ata at 60 feet/minute, held at 10 ata for 10 minutes and then decompressed directly back to 1 ata at 50 feet/minute. In these supplementary experiments only oxygen was monitored. In the first animal the arterial and venous oxygen levels were recorded and in the second, muscle tissue P_{O_2} was monitored.

The arterial P_{O_2} increased slowly at first so that it was only 3.3 times the surface value when 300 feet was reached, then increased rapidly to 14.5 times the surface value after 5 minutes at 300 feet. Thereafter it remained stable until decompression was started. Following decompression it was 4.6 times the starting surface value when the surface was reached and had returned to starting value after 5 minutes on the surface.

TABLE II

INHERENT UNSATURATION IN TISSUE. REPRESENTATIVE VALUES AT 1 ATA (MM HG)

Tissue	Animal #	P_{O_2}	P_{CO_2}	P_{N_2}	P_{Ar}	P_{H_2O}	P_{total}	P_{atm}	Unsaturation
Muscle	1	36	66	550	7	47	706	752	−46
	2	21	60	552	7	47	687	742	−55
	3	34	68	509	7	47	665	765	−100
Fat	1	45	56	586	7	47	741	752	−11
	2	37	57	552	7	47	700	742	−42
	3	48	70	504	7	47	676	765	−89

This is what would be expected for the arterial system with a relatively short time constant. However, the venous and tissue readings were unexpected. By the time 300 feet was reached during the compression phase, the venous P_{O_2} was only 1.7 times the surface value. There was a gradual increase while at 300 feet, until after 10 minutes the venous P_{O_2} was 3.6 times the surface value. This value remained stable during decompression until approximately 20 feet was reached: a rapid increase in P_{O_2} then occurred until, at 2 minutes after surfacing, the venous P_{O_2} was about 20 times the starting surface value. This value then decreased rapidly so that 2 minutes later it was 3 times the starting value, and it returned to normal after a further 2-3 minutes.

The muscle P_{O_2} in the second dog followed essentially the same pattern as the venous P_{O_2} in the first dog but went off scale at over 30 times the starting value after 4 minutes of decompression (at about 60 feet) and was 6 times the starting value after 4 minutes at the surface.

From these findings it can be inferred that massive vasoconstriction takes place in the tissue due to the oxygen and apparently is not released until the surface is almost reached during decompression.

The results described here raise more problems than they answer. However, it has been demonstrated that this type of mass spectrometer has the potential for making a major contribution to understanding gas exchange processes during diving. It is hoped that future experiments will yield data which will allow sound calculations to be made of gas exchanges in tissues and the establishment of reliable decompression profiles based on the findings.

ACKNOWLEDGMENTS

The technical assistance of Mr. A. Brock in the execution of this work is gratefully acknowledged.

INERT GAS ABSORPTION AND ELIMINATION KINETICS IN SPINAL CORD

G. Albano and M. Columba

The knowledge of inert gas exchange kinetics is of fundamental importance for the application of preventive decompression and for the decompression therapy, either for the whole body or for the organs which are disease targets.

Experiments performed on the whole body and theoretical studies have not, even until now, succeeded in establishing whether gas exchange rate is determined only by blood circulation (4, 7, 11, 15) or, as considered by others (9, 10), the diffusion through the tissues is of prevailing importance.

We believe both mechanisms of exchange should be taken into account simultaneously. This is especially true when relatively large volumes of nonvascularized tissues or spaces (joint cartilages, ocular and labyrinthine fluids, cerebrospinal fluid [CSF]) are adjacent to well-vascularized tissues and constitute reservoirs from which the inert gas slowly diffuses toward the latter. This results in a tissue inert gas pressure much higher than the value that could be expected if only the exchange due to circulation were considered. In such cases the problem of evaluating the inert gas exchange rate can be analytically solved if adequate physical models of the organs involved could be developed.

This study focuses on the spinal cord which plays a fundamental role in the study of decompression diseases. In the spinal cord gray matter and white matter—both well-vascularized tissues—are surrounded by a thick layer of cerebrospinal fluid in which no circulation at all takes place. The CSF can exchange gas only by diffusion with the spinal capillary network and in a quite negligible quantity with the outside.

To calculate the time transient pressure distribution of an inert gas in the spinal cord, the model shown in Figs. 1 and 2 has been assumed. The figure shows that the assumed contour of gray matter is somewhat different from true gray matter contour. However, for the white matter and for the CSF the real shape is similar enough to that of the model. Due to the high exchange rate and the small cross-sectional area, the assumption of a circular cross section for the gray matter should not introduce a significant error in the evaluation of exchange times. For all three substances the cross-sectional areas have been maintained in the model.

It has also been assumed that no gas exchange takes place through the thick membrane of the dura mater, and CSF circulation has been neglected due to the very low value of turnover (0.005 ml/ml min) (8). Capillary networks in gray and white matter have been con-

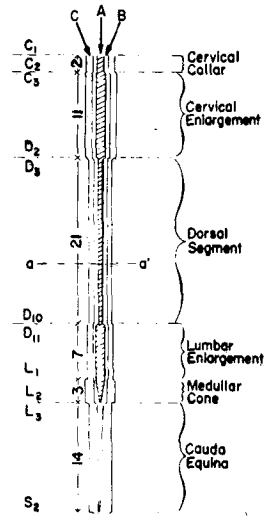

FIG. 1. Schematic longitudinal section of the model of the spinal cord according to the data in Table I. **A**, gray matter; **B**, white matter; **C**, cerebrospinal fluid.

sidered as distributed sources of uniform densities. In other words, a cylindrical symmetry of the spinal cord cross section has been postulated which greatly simplifies the mathematical treatment of the problem.

The sizes of the spinal cord cross sections at various heights, and the blood flow rate in resting conditions are reported in Table I. Table II reports solubility and diffusion coefficients of five inert gases (N_2, He, A, Ne, H_2) for which the calculations have been carried out.

The inert gas pressure as a function of radius (r) and time (τ) could be obtained by solving the three following simultaneous differential equations (where subscripts *G, W, F,* refer to gray matter, white matter and CSF, respectively):

$$\begin{aligned} K_G[(\partial^2 P_G/\partial r^2) + (1/r)(\partial P_G/\partial r)] + \sigma_G(P^* - P_G) &= a_G(\partial P_G/\partial \tau) \\ K_W[(\partial^2 P_W/\partial r^2) + (1/r)(\partial P_W/\partial r)] + \sigma_W(P^* - P_W) &= a_W(\partial P_W/\partial \tau) \\ K_F[(\partial^2 P_F/\partial r^2) + (1/r)(\partial P_F/\partial r)] &= a_F(\partial P_F/\partial \tau) \end{aligned} \tag{1}$$

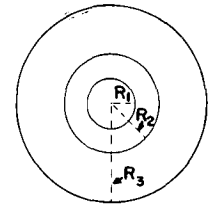

FIG. 2. Cross section of the model of the spinal cord at the level a-a' of Fig. 1.

TABLE I

AVERAGE ANATOMO-PHYSIOLOGIC DATA OF THE HUMAN SPINAL CORD

			Gray Matter			White Matter			CSF	
Spinal Cord Segment	Vertebral Topography[a]	Length[a] (cm)	Area[a] (mm^2)	Volume[a] (cm^3)	Blood flow at rest[b] (ml/cm^3 min)	Area[a] (mm^2)	Volume[a] (cm^3)	Blood flow at rest[b] (ml/cm^3 min)	Area[a] (mm^2)	Volume[a] (cm^3)
Cervical collar	C_1-C_2	2	15.90	0.32	0.540	47.70	0.95	0.096	190.9	3.82
Cervical enlargement	C_3-D_2	11	28.27	3.11	0.540	66.70	7.34	0.096	188.5	20.73
Dorsal	D_3-D_{10}	21	7.07	1.48	0.540	43.13	9.06	0.096	103.8	21.79
Lumbar enlargement	D_{11}-L_1	7	28.27	1.98	0.540	35.33	2.47	0.096	137.4	9.64
Medullar cone	L_2	3	28.27	0.85	0.540	21.93	0.66	0.096	194.7	5.84
Cauda equina	L_3-S_2	14	—	—	—	9.60	1.30	0.096	145.4	20.36

[a]Testut and Latariet (17).
[b]Albano (1).

TABLE II

CHEMICAL COMPOSITION AND CONSTANTS OF GAS SOLUBILITY AND DIFFUSION IN THE SPINAL CORD

		Gray Matter	White Matter	CSF
Chemical composition of cord[a]	Water (%)	84.0	70.0	99.00
	Lipids (%)	5.6	18.3	0.01
	Other solids (%)	10.4	11.7	0.99
Solubility a (bar^{-1})	Argon[c]	$2.940 \cdot 10^{-2}$	$4.323 \cdot 10^{-2}$	$2.586 \cdot 10^{-2}$
	Nitrogen[c]	$1.382 \cdot 10^{-2}$	$2.082 \cdot 10^{-2}$	$1.253 \cdot 10^{-2}$
	Neon[c]	$0.888 \cdot 10^{-2}$	$0.997 \cdot 10^{-2}$	$0.928 \cdot 10^{-2}$
	Helium[c]	$0.799 \cdot 10^{-2}$	$0.868 \cdot 10^{-2}$	$0.859 \cdot 10^{-2}$
	Hydrogen[b]	$1.549 \cdot 10^{-2}$	$1.826 \cdot 10^{-2}$	$1.579 \cdot 10^{-2}$
Diffusibility K (cm^3/bar min cm)[d]	Argon	$1.993 \cdot 10^{-5}$	$3.089 \cdot 10^{-5}$	$3.227 \cdot 10^{-5}$
	Nitrogen	$1.115 \cdot 10^{-5}$	$1.776 \cdot 10^{-5}$	$1.875 \cdot 10^{-5}$
	Neon	$0.849 \cdot 10^{-5}$	$0.997 \cdot 10^{-5}$	$1.628 \cdot 10^{-5}$
	Helium	$1.707 \cdot 10^{-5}$	$1.964 \cdot 10^{-5}$	$3.395 \cdot 10^{-5}$
	Hydrogen	$4.668 \cdot 10^{-5}$	$5.803 \cdot 10^{-5}$	$8.803 \cdot 10^{-5}$

[a] Tower (19).
[b] Bennett (6).
[c] Behnke and Yarbrough (5).
[d] Calculated from Krogh (12) and Thews (18).

together with the boundary value conditions:

$$\begin{aligned} (\partial P_G/\partial r)_{r=0} &= 0; \qquad (\partial P_F/\partial r)_{r=R_3} = 0 \\ (P_G)_{r=R_1} &= (P_W)_{r=R_1}; \qquad (P_W)_{r=R_2} = (P_F)_{r=R_2} \\ K_G(\partial P_G/\partial r)_{r=R_1} &= K_W(\partial P_W/\partial r)_{r=R_1} \\ K_W(\partial P_W/\partial r)_{r=R_2} &= K_F(\partial P_F/\partial r)_{r=R_2} \end{aligned} \tag{2}$$

and the initial value conditions:

$$\begin{aligned} P_G(r, 0) &= F_1(r) \\ P_W(r, 0) &= F_2(r) \\ P_F(r, 0) &= F_3(r) \end{aligned} \tag{3}$$

where:

σ = the exchange coefficient between tissues and blood ($bar^{-1}min^{-1}$)
K = the diffusibility (Krough's) coefficient (cm^3/bar cm min)
a = the solubility (bar^{-1})
P = the inert gas partial pressure in tissues (bar)
P^* = the inert gas partial pressure in arterial blood (bar).

The analytical solution of this mathematical problem is quite a difficult task, and even if it could be obtained, it would be in a form of series expansion and would require a large number of calculations to be carried out on a computer.

Thus it was preferable to perform a finite difference integration, since the simplified shape of the cross section which was assumed makes the attempt to obtain an "exact" solution out of proportion to the effort needed to obtain it.

In Fig. 3 and in the following equations $P_{i+1,j}$, $P_{i,j}$, $P_{i-1,j}$ represent the inert gas pressures at time τ_j on three adjacent layers of thickness Δr and of medium radii r_{i+1}, r_i, r_{i-1} for a single substance. The pressure $P_{i,j+1}$ at radius r_i and at the time τ_{j+1} can be obtained from the continuity equation written in form of finite differences for the layer across radius r_i for unit length:

$$\begin{aligned} -K[(P_{i,j} - P_{i-1,j})/\Delta r]\cdot 2\pi[r_i - (\Delta r/2)] + K[(P_{i+1,j} - P_{i,j})/\Delta r]\cdot 2\pi[r_i + (\Delta r/2)] \\ - \sigma(P_{i,j} - P^*)\cdot\pi\{[r_i + (\Delta r/2)]^2 - [r_i - (\Delta r/2)]^2\} \\ = a[(P_{i,j+1} - P_{i,j})/\Delta\tau]\cdot\pi\{[r_i + (\Delta r/2)]^2 - [r_i - (\Delta r/2)]^2\} \end{aligned} \tag{4}$$

Hence:

$$\begin{aligned} (P_{i+1,j} + P_{i-1,j}) - 2P_{i,j} + (P_{i+1,j} - P_{i-1,j})(\Delta r/2r_i) - \sigma(P_{i,j} - P^*)(\Delta r)^2 \\ = (a/K)[(\Delta r)^2/\Delta\tau](P_{i,j+1} - P_{i,j}) \end{aligned} \tag{5}$$

If we put

$$a(\Delta r)^2/K\Delta\tau = \alpha \tag{6}$$

the last expression becomes:

$$\begin{aligned} P_{i,j+1} = P_{i,j}[1 - (2/\alpha) - (\sigma/\alpha K)(\Delta r)^2] + (1/\alpha)(P_{i+1,j} + P_{i-1,j}) \\ + (1/\alpha)(P_{i+1,j} - P_{i-1,j})(\Delta r/2r_i) + (\sigma/\alpha K)P^* \end{aligned} \tag{7}$$

In order to satisfy Dusinberre's stability rule (16), the value of α is obtained from the limiting condition:

$$\alpha - 2 - (\sigma/K)(\Delta r)^2 = 0 \tag{8}$$

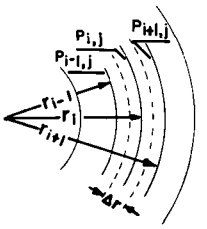

FIG. 3.

and the equation becomes:

$$P_{i,j+1} = (1/\alpha)[(P_{i+1,j} + P_{i-1,j}) + (P_{i+1,j} - P_{i-1,j})(\Delta r/2r_i) + (\alpha - 2)P^*] \tag{9}$$

Introducing the dimensionless pressure:

$$p = P/P^* \tag{10}$$

Eq. (9) can be written in the adimensional form:

$$p_{i,j+1} = (1/\alpha)[(p_{i+1,j} + p_{i-1,j}) + (p_{i+1,j} - p_{i-1,j})(\Delta r/2r_i) + (\alpha - 2)] \tag{11}$$

For the other two substances an iterative equation like Eq. (11) can be obtained in the same way with the only difference of the value of α and Δr. From Eq. (6) and (8):

$$\alpha = (a/K)[(\Delta r)^2/\Delta\tau] = 2 + (\sigma/K)(\Delta r)^2 \tag{12}$$

and

$$\Delta r = \sqrt{\frac{2\Delta\tau}{(a/K) - (\sigma/K)\Delta\tau}} \tag{13}$$

For the CSF, since $\sigma = 0$, $\alpha = 2$.

Both quantities depend on the value of $\Delta\tau$—which must be the same for every substance and can be chosen independently—and on the values of a, σ and K.

The number of layers into which every substance has to be subdivided is then given from the ratios (rounded to the closest integer value):

$$g = R_1/(\Delta r)_G\,; \qquad w = (R_2 - R_1)/(\Delta r)_w\,; \qquad f = (R_3 - R_2)/(\Delta r)_F \tag{14}$$

Three iterative equations can now be written:

$$(p_{i,j+1})_G = (1/\alpha_G)\{(p_{i-1,j} + p_{i+1,j}) + (p_{i+1,j} - p_{i-1,j})[(\Delta r)_G/2r_i] + (\alpha_G - 2)\} \tag{15}$$

$$(p_{i,j+1})_w = (1/\alpha_w)\{(p_{i-1,j} + p_{i+1,j}) + (p_{i+1,j} - p_{i-1,j})[(\Delta r)_w/2r_i] + (\alpha_w - 2)\} \tag{16}$$

$$(p_{i,j+1})_F = (1/2)\{(p_{i-1,j} + p_{i+1,j}) + (p_{i+1,j} - p_{i-1,j})[(\Delta r)_F/2r_i]\} \tag{17}$$

Eq. (15) holds for $0 < i < g$; Eq. (16) holds for $g < i < (g + w)$; and Eq. (17) for $(g + w) < i < (g + w + f)$. The values of $p_{i,j+1}$ for $i = 0$; $i = g$; $i = g + w$ and $i = g + w + f$ can be obtained from boundary conditions.

For $i = 0$, along the axis of the spinal cord, the value of p has been calculated connecting the values of p at $i = 1$ and $i = 3$ with a second order curve that orthogonally intersects the axis; the analogue procedure has been adopted for the outer boundary at $r = R_3$. For

the boundary between gray and white matter ($i = g$) the pressure p_g may be obtained from Eq. (18):

$$K_G(p_g - p_{g-1})/(\Delta r)_G = K_W(p_{g+1} - p_g)/(\Delta r)_W \tag{18}$$

and at the boundary between white matter and CSF the pressure p_{g+W} is given from Eq. (19):

$$K_W(p_{g+W} - p_{g+W-1})/(\Delta r)_W = K_F(p_{g+W+1} - p_{g+W})/(\Delta r)_F \tag{19}$$

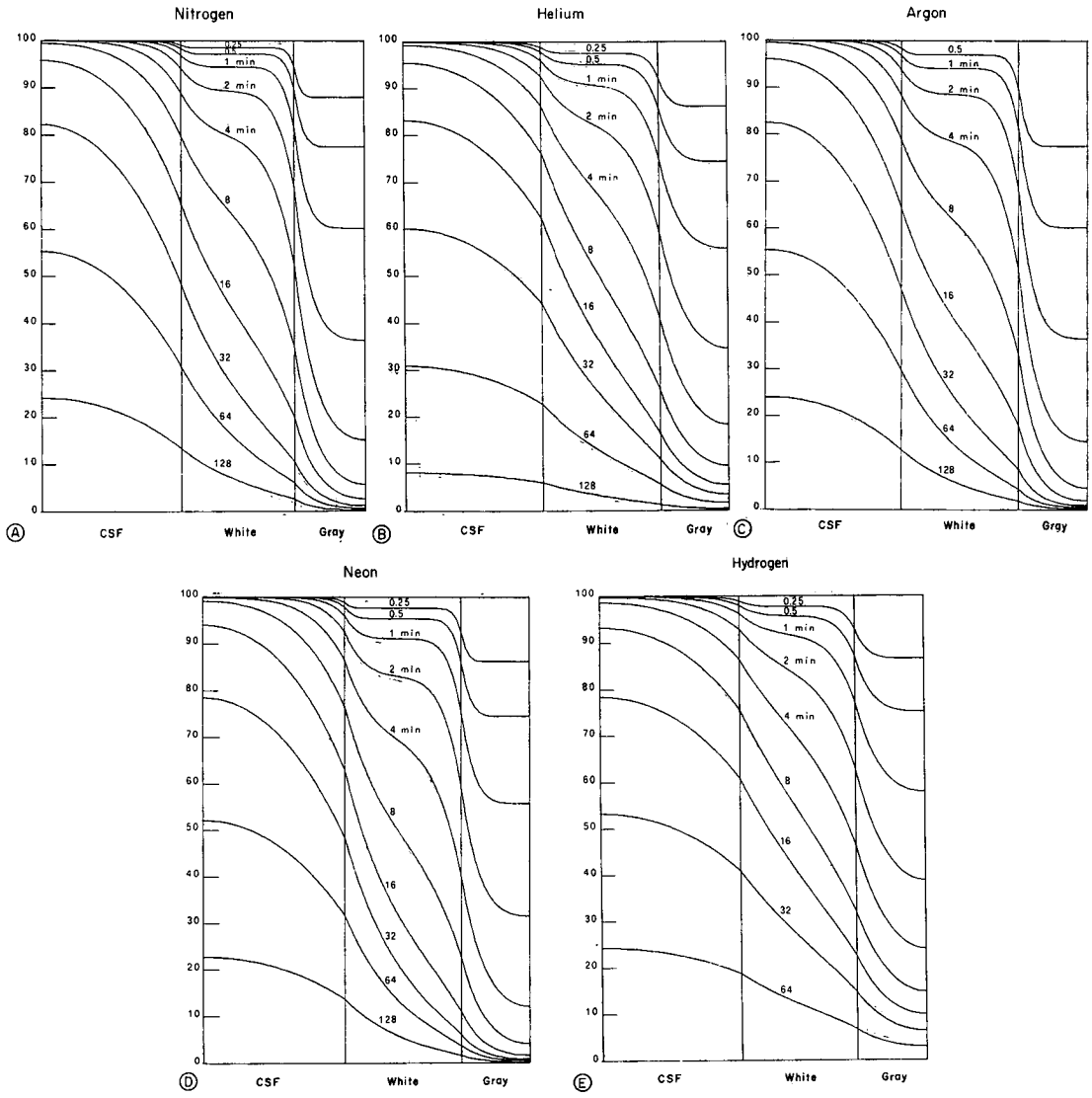

FIG. 4. Pressure distribution at different times for five inert gases. Dimensionless pressure is shown on the vertical axis; radius on the horizontal. Axis of spinal cord is on the extreme right.

Calculations have been carried out on an electronic calculator. The output has been plotted directly on percentage of the inert gas vs. radius diagrams. Calculations have been limited to the thoracic tract of the spinal cord (Fig. 1, a-a′ and Fig. 2) since the maximum number of decompression embolisms occurs at this level. This probably happens because at this level the volume of gray matter, strongly vascularized, reaches its lowest absolute and relative values (Table I) and therefore the elimination of the inert gas contained in CSF has to take place almost exclusively through the less vascularized white matter.

All of the results presented in this paper refer to decompression in a hyperbaric chamber starting from saturation, for subjects in a condition very near to rest.

The curves in Fig. 4 show the pressure distribution at different times for the five inert gases already mentioned. A strong difference of inert gas pressure along the radius can be observed, particularly for the white matter at intermediate times. This may explain why decompression embolisms occur more often in lateral and in posterior bundles where white matter has the maximum thickness.

Elimination times are very close together for the more diffusible gases (He, H_2) on the one hand, and for the less diffusible gases (N_2, A, Ne) on the other. To represent the two groups, values of average pressure in CSF and in white matter are plotted vs. time in Figs. 5 and 6 for the more commonly used gases (N_2, He). The difference between the two curves in Fig. 6 clearly demonstrates the delay in the elimination of nitrogen for a large zone of white matter in the first part of decompression.

As shown in Fig. 7, McArdle's (14) experimental data (dots) fit very well with calculated average pressure of nitrogen in CSF (solid line). This is a significant confirmation of the theory and of the good approximation of the calculations made.

The theory outlined above and the solution presented for the spinal cord are, we believe, powerful tools that make it possible to evaluate the pressure of inert gas at every level

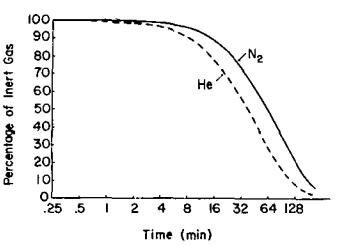

FIG. 5. Average dimensionless pressure of N_2 and He in CSF vs. time.

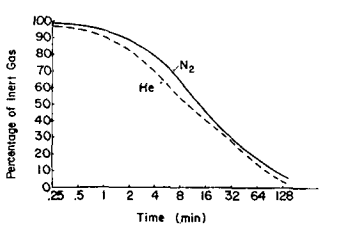

FIG. 6. Average dimensionless pressure of N_2 and He in white matter vs. time.

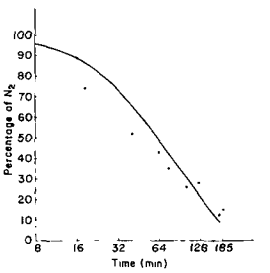

FIG. 7. Average dimensionless pressure of N_2 in CSF (solid line) and McArdle's data (dots).

of the spinal cord during saturation or desaturation for each gas breathed, and also for ternary or quaternary mixtures; it should also correctly explain decompression sickness cases with spinal localization.

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EVIDENCE AGAINST THE METABOLIC PRODUCTION OF MOLECULAR NITROGEN IN SUBSTANTIAL QUANTITIES BY MAN*

J. M. Herron, H. A. Saltzman, B. A. Hills and J. A. Kylstra

Since the time of Lavoisier, respired molecular nitrogen has been generally assumed to be metabolically inert in animals. As a consequence, relevant calculations have expressed or implied that in the steady state the inspired volume of nitrogen gas equals the expired volume.

Some scientific observations in recent times have been at variance with this traditional concept, however. One example was the observation that long-term nitrogen retention occurred in animals with no proportionate increase in body weight (3, 4). Other observers have reported that the expired gas of man often contains more nitrogen than inspired gas in the steady state, particularly after ingesting protein or while exercising (2, 4, 5). It was inferred from these studies that a previously unrecognized metabolic pathway might exist for metabolic production of molecular nitrogen by the body with subsequent elimination in expired gas (4, 5, 10).

If substantial quantities of molecular nitrogen are produced metabolically, the consequences would be of great significance to those concerned with respiration physiology and the practical problems of decompression. For this reason, steady-state volumes of inspired and expired nitrogen gas were measured directly in healthy volunteers at rest, before and after ingesting protein, and during exercise. The gaseous environment was adjusted so that differences between inspired and expired nitrogen of less than 2 cc per minute could be measured with precision. These results form the basis of this report.

Methods

Physiologic measurements were obtained from each of 10 healthy male volunteers after an overnight fast. During the entire course of each experiment, the subject and investigators were confined within a large polyethylene bag containing approximately 6000 liters of 21% oxygen in helium. The temperature was maintained within 1 °C. The relative humidity and carbon dioxide levels were maintained below 60 and 0.2%, respectively, by an environmental control unit. A slight positive pressure of 6 mm water within the bag reduced in-

*For a more detailed description of this data, see: Herron, J. M., H. A. Saltzman, B. A. Hills and J. A. Kylstra. Differences between inspired and expired minute volumes of nitrogen in man. *J. Appl. Physiol.* 35: 546–551, 1973.

gasing of nitrogen to a minimum. Measurements were begun after an equilibration period of at least 2½ hours and after the ambient concentration of nitrogen was 0.15% or less. In two studies, measurements were obtained after more than 12 hours equilibration with the oxygen-helium gas mixture.

Each experiment consisted of four or more collections in the steady state of inspired and expired gas from the seated subject. The order of collection was 1) at rest and then during work while in the fasting state, and 2) at rest and then during work, at least 1 hour after ingesting 2 liters of a liquid milk and food concentrate containing 36 gm protein with a total caloric value of 1100 calories. Pure helium bubbled through the liquid food for at least 12 hours before ingestion. After 2 minutes of breathing on the respiratory assembly, resting measurements were obtained over an elapsed time of approximately 5 minutes. Collections during work were shorter, averaging 2 minutes at the end of a 7-minute effort. The level of work was such as to raise oxygen consumption approximately fourfold.

Each subject respired through a low resistance directional breathing valve (Hans Rudolph high velocity valve) and plastic tubing (3.81 cm internal diameter). Ambient gas was inspired from a 120-liter water sealed Tissot gasometer. Expired gas was vented simultaneously into a 100-liter Douglas bag. Samples of inspired and expired gas were collected and stored in sealed glass syringes and the remaining gas in the bag was vented back into the spirometer.

Inspired and expired gas samples were analyzed by gas chromatography for the fractional composition of oxygen (F_{O_2}), carbon dioxide (F_{CO_2}) and nitrogen (F_{N_2}). The helium concentration (F_{He}) was derived by subtraction. The precision of measurement for F_{N_2} was estimated conservatively to be 1 part in 10,000. Minute volumes of inspired ($\dot{V}_I$) and expired ($\dot{V}_E$) gas were determined from gasometer spirograms and expressed as STPD values after making standard corrections for sample removal, temperature, barometric pressure and water vapor pressure at full saturation. For a 5-minute collection of respired gas, the volumetric error was estimated to be 60 ml.

From measured data, respired volumes of nitrogen ($\dot{V}_{EN_2}$, $\dot{V}_{IN_2}$) and helium ($\dot{V}_{EHe}$, $\dot{V}_{IHe}$) were calculated according to the following equations:

$$\dot{V}_{EN_2} - \dot{V}_{IN_2} = \dot{V}_E \cdot F_{EN_2} - \dot{V}_I \cdot F_{IN_2} \tag{1}$$

$$F_{He} = 1 - (F_{O_2} + F_{CO_2} + F_{N_2}) \tag{2}$$

$$\dot{V}_{EHe} - \dot{V}_{IHe} = \dot{V}_E \cdot F_{EHe} - \dot{V}_I \cdot F_{IHe} \tag{3}$$

The experimental method employed in this study was selected in order to reduce the error of measurement for nitrogen by minimizing the amount of this gas being respired. The rationale for enhanced accuracy of measurement is outlined as follows. If the inspired volume of nitrogen gas equals the expired volume, then Eqs. (4) and (5) are true:

$$\dot{V}_{IN_2} = \dot{V}_{EN_2} \tag{4}$$

$$\dot{V}_{IN_2} \cdot F_{IN_2} = \dot{V}_{EN_2} \cdot F_{EN_2} \tag{5}$$

If an inequality exists between inspired and expired nitrogen, the difference, $\dot{v}_{N_2}$, may be represented algebraically as in Eq. (6).

$$\dot{v}_{N_2} = \dot{V}_E \cdot F_{EN_2} - \dot{V}_I \cdot F_{IN_2} \tag{6}$$

The $\dot{v}_{N_2}$ must be greater than the sum of maximum possible errors in Eq. (6) if significance is to be established with certainty. The maximal error in the measurement of nitrogen $\Delta\dot{V}_{N_2}$ can be calculated as in Eq. (7):

$$\Delta\dot{V}_{N_2} = (\dot{V}_I \cdot \Delta F_{I_{N_2}}) + (\dot{V}_E \cdot \Delta F_{E_{N_2}}) + (F_{I_{N_2}} \cdot \Delta\dot{V}_I) + (F_{E_{N_2}} \cdot \Delta\dot{V}_E) \tag{7}$$

where $\Delta F_{I_{N_2}}$ and $\Delta F_{E_{N_2}}$ represent the error in measuring the fractional concentration of nitrogen in the inspired and expired gas, respectively, and $\Delta\dot{V}_I$ and $\Delta\dot{V}_E$ represent the error in measuring the minute volume of inspired and expired gas.

By using the same parameters in measuring the fractional concentration of nitrogen and minute volume, it may be assumed that $\Delta F_{I_{N_2}} = \Delta F_{E_{N_2}} = \Delta F_{N_2}$ and that $\Delta\dot{V}_I = \Delta\dot{V}_E = \Delta\dot{V}$.

By substitution, Eq. (7) becomes:

$$\Delta\dot{V}_{N_2} = (\dot{V}_I + \dot{V}_E)\Delta F_{N_2} + (F_{I_{N_2}} + F_{E_{N_2}})\Delta\dot{V} \tag{8}$$

Eq. (8) can be used to demonstrate the numerical consequences of high and low values for F_{N_2}. In a resting subject breathing 10,000 ml of air each minute, the following calculation of $\Delta\dot{V}_{N_2}$ is a reasonable estimate of maximal possible error:

$$\begin{aligned} \Delta\dot{V}_{N_2} &= (10{,}000 \text{ ml} + 10{,}000 \text{ ml})\,(0.0001) + (0.79 + 0.79)\,(60 \text{ ml}) \\ \Delta\dot{V}_{N_2} &= 2.0 \text{ ml} + 94.8 \text{ ml} \\ \Delta\dot{V}_{N_2} &= 96.8 \text{ ml} \end{aligned} \tag{9}$$

If a gas containing little or no nitrogen is breathed, however, the maximal possible error is minimized.

$$\begin{aligned} \Delta\dot{V}_{N_2} &= (10{,}000 + 10{,}000 \text{ ml})\,(0.0001) + (0.0015 + 0.0015)\,(60 \text{ ml}) \\ \Delta\dot{V}_{N_2} &= 2.0 \text{ ml} + 0.18 \text{ ml} \\ \Delta\dot{V}_{N_2} &= 2.18 \text{ ml} \end{aligned} \tag{10}$$

With a large F_{N_2}, the error in computing $\dot{v}_{N_2}$ is closely related to the error in measuring volume. For example, with exercise, the collection time is usually shorter and $\Delta\dot{V}$ increases. Eq. (9) for a halved collection time and doubled $\dot{V}$ becomes:

$$\begin{aligned} \Delta\dot{V}_{N_2} &= (20{,}000 \text{ ml} + 20{,}000 \text{ ml})\,(0.0001) + (0.79 + 0.79)\,(120 \text{ ml}) \\ \Delta\dot{V}_{N_2} &= 4.0 \text{ ml} + 189.6 \text{ ml} \\ \Delta\dot{V}_{N_2} &= 193.6 \text{ ml} \end{aligned} \tag{11}$$

With a low F_{N_2}, the effect of exercise is much less. For the previously cited conditions, Eq. (10) becomes:

$$\begin{aligned} \Delta\dot{V}_{N_2} &= (20{,}000 \text{ ml} + 20{,}000 \text{ ml})\,(0.0001) + (0.0015 + 0.0015)\,(120 \text{ ml}) \\ \Delta\dot{V}_{N_2} &= 4.0 \text{ ml} + 0.36 \text{ ml} \\ \Delta\dot{V}_{N_2} &= 4.36 \text{ ml} \end{aligned} \tag{12}$$

By design, in this experiment there was a continuous washout of nitrogen from the body and more nitrogen would be anticipated in expired gas than in inspired gas. The increment due to washout would be expected to diminish with continued exposure to the oxygen-helium gas mixture, however, and has been reported to be approximately 2 ml/minute after 2.5 hours of breathing a nitrogen-free gas in past studies of normal subjects (7).

Results

In 43 of 44 steady-state measurements, more nitrogen was expired than inspired (Table I, Fig. 1). The difference between $\dot{V}_{E_{N_2}}$ and $\dot{V}_{I_{N_2}}$ ($\dot{v}_{N_2}$) was small, however, and under otherwise comparable circumstances, was always less during the later portions of each exposure to the oxygen-helium gas mixture (Table I and Fig. 2). After 12 hours of equilibration, the $\dot{v}_{N_2}$ was approximately 0.5 ml/min STPD. The ingestion of food was not followed by any discernible increase in $\dot{v}_{N_2}$ (Table I, Fig. 2). Instead, values for postprandial $\dot{v}_{N_2}$ (obtained after the fasting determinations) were significantly lower. Exercise was associated with an

TABLE I

TIME COURSE OF ($\dot{V}_{E_{N_2}} - \dot{V}_{I_{N_2}}$)

Condition	Number of Subjects	Number of Studies	Range of Exposure Times to O_2-He Prior to Measurement (min)	Average $\dot{V}_{E_{N_2}} - \dot{V}_{I_{N_2}}$ (ml/min STPD)	Range of $\dot{V}_{E_{N_2}} - \dot{V}_{I_{N_2}}$ (ml/min STPD)
Fasting, rest	8	8	105–165	2.54	1.44–4.40
Fasting, work	8	8	130–210	4.89	1.05–7.56
Postcibum, rest	8	8	210–305	1.49	0.15–2.57
Postcibum, work	8	8	240–330	2.04	0.77–3.40
Fasting, rest	2	6	760–875	0.56	0.46–0.87
Postcibum, rest	2	6	900–1000	0.47	0.23–0.72

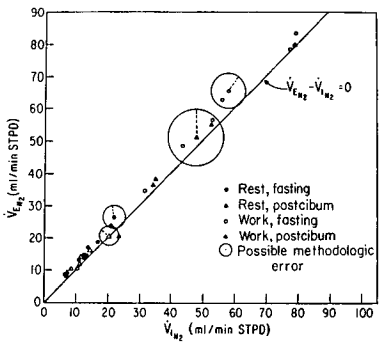

FIG. 1. Each individual symbol depicts $\dot{V}_{I_{N_2}}$ on the abscissa and $\dot{V}_{E_{N_2}}$ on the ordinate for one collection of respired gas. All 32 measurements obtained within the first 330 minutes of exposure to the special environment are included. The volumes of respired nitrogen increased with the larger minute ventilations associated with exercise. The error of measurements increased as well.

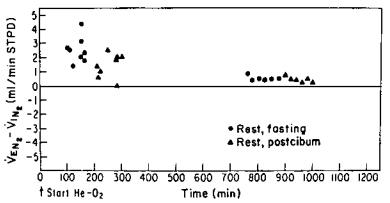

FIG. 2. All 28 individual resting measurements of $\dot{V}_{E_{N_2}} - \dot{V}_{I_{N_2}}$ on the ordinate decreased as the duration of exposure to O_2-He on the abscissa increased. There was no discernible effect after ingestion of protein. $\dot{V}_{E_{N_2}} - \dot{V}_{I_{N_2}}$ was small in all instances.

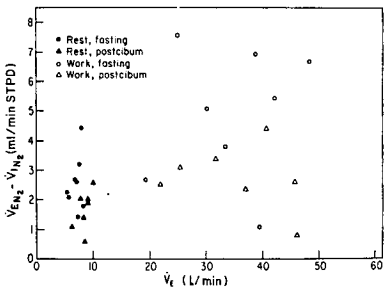

FIG. 3. The larger respired volumes during exercise, on the abscissa, correlate roughly with larger values for $\dot{V}_{E_{N_2}} - \dot{V}_{I_{N_2}}$ on the ordinate. The greatest observed value for $\dot{v}_{N_2}$ was 7.5 ml/min STPD.

increase in $\dot{v}_{N_2}$ that correlated roughly with the increase in respired gas volumes (Fig. 3). As was true for the measurements obtained at rest, $\dot{v}_{N_2}$ was lower during the later postprandial exercise measurements than during the earlier fasting exercise measurements (Table I).

Values for computed ($\dot{V}_{E\mathrm{He}} - \dot{V}_{I\mathrm{He}}$) varied greatly but were positive on the average (Fig. 4, Table II). After more than 12 hours of exposure to the oxygen-helium gas mixture, the mean ($\dot{V}_{E\mathrm{He}} - \dot{V}_{I\mathrm{He}}$) in 12 resting measurements averaged 80.5 ml/minute STPD.

TABLE II

TIME COURSE OF ($\dot{V}_{E\mathrm{He}} - \dot{V}_{I\mathrm{He}}$)

Condition	Number of Subjects	Number of Studies	Range of Exposure Times to O_2-He Prior to Measurement (min)	Average $\dot{V}_{E\mathrm{He}} - \dot{V}_{I\mathrm{He}}$ (ml/min STPD)	Range of $\dot{V}_{E\mathrm{He}} - \dot{V}_{I\mathrm{He}}$ (ml/min STPD)
Fasting, rest	8	8	105-165	50.6	−59.1 to 108.49
Fasting, work	8	8	130-210	26.4	−519 to 171
Postcibum, rest	8	8	210-305	14.2	−79 to 106
Postcibum, work	8	8	240-330	−91.0	−363 to 222
Fasting, rest	2	6	760-875	73.0	31 to 137
Postcibum, rest	2	6	900-1000	88.0	30 to 289

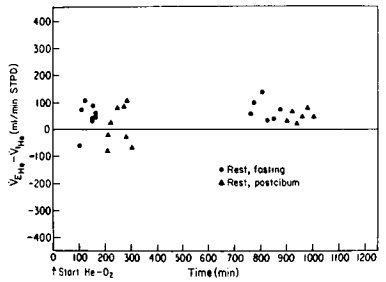

FIG. 4. Individual values for computed $\dot{V}_{E_{He}} - \dot{V}_{I_{He}}$ are shown for 28 resting measurements on the ordinate. Positive and negative values were observed during the first 305 minutes of breathing oxygen and helium. After 12 hours of equilibration, all values were positive. Inspection revealed a large random scatter of results as well as a systematic excess of helium in expired gas.

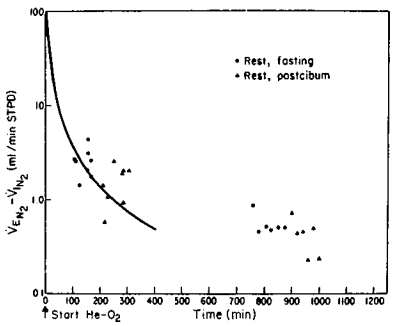

FIG. 5. The smooth curve is taken from the data of Jones (7) who measured the elimination of nitrogen in normal volunteers, breathing oxygen. The 28 superimposed resting measurements from this experiment are similar in magnitude where data can be compared directly.

Discussion

Under the conditions of this study, expired gas contained slightly more nitrogen than inspired gas, in the steady state. Possible explanations for this finding include: 1) methodologic error; 2) washout of dissolved nitrogen from body tissues; and 3) metabolic production of molecular nitrogen.

Since all values for $\dot{v}_{N_2}$ were less than the computed maximal errors, individual differences between $\dot{V}_{E_{N_2}}$ and $\dot{V}_{I_{N_2}}$ cannot be attributed with certainty to factors other than the limitations of the technique for measurement. The apparent systematic increment of nitrogen in expired gas cannot be attributed to random methodologic error, however.

If measurements of $(\dot{V}_{E_{N_2}} - \dot{V}_{I_{N_2}})$ in this experiment are superimposed on the nitrogen washout curve obtained by Hardin Jones in normal volunteers breathing oxygen (Fig. 5), the similarity is apparent despite substantial differences in experimental design (7, 12). Comparable values for $\dot{v}_{N_2}$ were also reported by Willmon and Behnke (1, 13). These earlier studies have generally been accepted as yielding quantitative data for nitrogen elimination consistent with the anticipated physical storage of molecular nitrogen by body tissues in equilibrium with air at sea level. These similarities support but do not prove the hypothesis

that the small excess of expired nitrogen observed in this experiment was due to a simple washout of inert gas dissolved in body tissues.

This experiment fails to support recent observations that volumes of molecular nitrogen, sometimes in excess of 100 ml/minute STPD, are produced metabolically by the body. The small values for ($\dot{v}_{N_2}$), the decrement in $\dot{v}_{N_2}$ with prolonged equilibration, the apparent lack of a discernible response after ingesting protein, and the small effect of exercise are far more consistent with the classic hypothesis that molecular nitrogen is metabolically inert. It is possible, however, that metabolic production of molecular nitrogen does occur at low levels that cannot be detected with certainty by the techniques employed in this study. Another possibility is that the substantial metabolic production of molecular nitrogen is largely concealed by simultaneous metabolic assimilation. Despite these uncertainties, it seems clear that if metabolic production of nitrogen does occur, the small excess volumes in expired gas are unlikely to significantly affect either decompression or classical computations of respiratory gas exchange.

The computations of ($\dot{V}_{E\mathrm{He}} - \dot{V}_{I\mathrm{He}}$) reveal a random variation consistent with the predicted errors associated with attempts to measure small differences between large volumes (Table II, Fig. 4). The obvious systematic excess of helium in expired gas cannot be ascribed to random methodologic error, however.

There are five possible explanations for the excess of expired helium in these steady-state experiments: 1) metabolic production; 2) diffusion through the skin; 3) gastrointestinal absorption of ingested gas with transport to the lungs via the blood stream or by eructation into expired gas; 4) systematic methodologic error; and 5) inadvertent inhalation of environmental gas other than that in the gasometer.

Metabolic production of helium by the body is most unlikely. No biochemical models or relevant supporting experimentation are known to the authors.

Small molecules of helium will diffuse through the skin, down a gradient of pressure into the body and will be detected ultimately in expired gas. The volumes transported across the intact skin are small, however, and cannot account for the observed difference between $\dot{V}_{E\mathrm{He}}$ and $\dot{V}_{I\mathrm{He}}$ in this experiment (8).

It has been recognized for many years that some environmental gas is ingested normally by man. Diffusion gradients lead to absorption and transport via venous blood to the lung (9, 11). Alternatively, some swallowed environmental gas may be eliminated in eructate or flatus. Previous observations suggest an ingested gas volume of approximately 1–2 liters in 24 hours (9, 11). If it is assumed that the most reliable steady-state determinations of excess expired helium in this experiment were after 12 hours or more of equilibration and if all excess expired helium is assumed due to gastrointestinal absorption or eructation, then it can be calculated that approximately 146,700 ml of 21% oxygen in helium must be ingested in a 24-hour period in order to maintain the observed difference between $\dot{V}_{E\mathrm{He}}$ and $\dot{V}_{I\mathrm{He}}$ of 80.5 ml/min STPD. This level of gas ingestion is not compatible with past measurements or the known physical capacity of venous blood to transport inert gas at sea level (6, 9).

Nevertheless, a portion of excess expired helium in this study may be the result of gas ingestion. This mechanism could also account in part for the findings that humans breathing air exhale more nitrogen than they inhale in the steady state (2). Gastrointestinal absorption of air might also invalidate the assumption of equilibrium between the environment and the body for argon on which Muysers has based his computations of excess expired nitrogen (10). If significant amounts of nitrogen are transported from the gastrointestinal tract of

man to expired gas, then the classic assumption on which the respiratory equations are based merits challenge, albeit on a different basis.

Another explanation for the calculated systematic excess of helium in expired gas in this study is that inspired gas within the water-sealed gasometer was assumed incorrectly to be saturated with water vapor. By subtracting a computed value for water vapor pressure in excess of true pressure, the inspired dry gas volume would be underestimated. An average volumetric error of approximately 1% would result from an 8 mm Hg overestimate of water vapor pressure in inspired gas and would account for the calculated excess expired helium of 80.5 ml/minute STPD. Since the nitrogen concentration in this study was less than 0.15%, however, the computed $\dot{v}_{N_2}$ would not be affected significantly. For a 100 ml volumetric error, the calculated $\dot{v}_{N_2}$ would be overestimated by only 0.15 ml.

Finally, $\dot{V}_{E\mathrm{He}}$ could be greater than $\dot{V}_{I\mathrm{He}}$ because environmental gas other than that within the gasometer is inhaled during the period of measurement. By implication, an approximate 1 percent constant rate of helium entry through the walls of the apparatus would account for the excess expired helium and would not perceptibly alter calculations of metabolic exchange for oxygen and carbon dioxide. ($\dot{V}_{E\mathrm{N}_2} - \dot{V}_{I\mathrm{N}_2}$) would be overestimated by approximately 0.15 ml/minute STPD.

ACKNOWLEDGMENTS

This study was supported in part by contracts N00014-67A-0251-0015 with the Office of Naval Research and #H1 07896 of the National Institutes of Health.

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PART V. INERT GAS EXCHANGE AND BUBBLE FORMATION*

DISCUSSION

R. C. Bornmann, Chairman

Dr. Walder: Dr. Spencer, I take exception to your statement that your results indicated it is logical to conclude that decompression sickness is due to bubbles. The argument put forward seemed to me to be: no bubbles detected, no decompression sickness; bubbles detected and no decompression sickness; and a third possibility, many bubbles and decompression sickness; and therefore decompression sickness is due to bubbles.

This is, of course, not the logical conclusion because there are alternative possibilities: that the situation in which many bubbles occur also leads to some other situation or runs concurrently with some other situation which itself causes decompression sickness. We have to be careful here. At this conference so far there have been many very interesting suggested alternatives to the bubble theory. Although I subscribe to this theory myself, I still think this precaution is very necessary.

Dr. Spencer: I have not heard any paper at this conference that in my opinion has talked about a phenomenon involved in decompression where bubbles could not have been the initiating factor.

Dr. Saltzman: I will confine my remarks to the superb presentation by Dr. Idicula from the University of Pennsylvania. In the best traditions of science they have evaluated an interesting phenomenon by formulating a sound hypothesis and presenting strong experimental evidence to support it. I will make two comments and ask a rather obvious question.

The first relates to what I believe to be a very important difference between the experimental studies in pigs and the actual exposures of men, in both the Duke experiment and the Pennsylvania experiment. Very properly in the experimental model, the pigs continuously breathe, as I understand it, a normoxic gas—for example, oxygen in nitrogen—and are continuously exposed at a given constant pressure isobarically to a different inert gas around them, such as helium. And so the experimenters had an opportunity to observe in a single-variable situation the clash or interface of the inert gases extending from the external environment into the tissues through the skin or through the operative wounds of the experimental model. In the men, however, the interface must have occurred not only at the surface of the body, but within the body, since initially they breathed normoxic helium and then breathed normoxic nitrogen.

As an obvious expression of that difference, instead of a several-hour period being required for the full evolution of manifestations, as was true in the pig, in the men (at least at Duke) the manifestations occurred within a very few minutes (perhaps 5 minutes) of beginning to breathe the normoxic nitrogen gas after breathing normoxic helium.

The distinction between animal and man is of much more than pedantic interest because it relates to where this phenomenon may occur and, of course, to the very important differences in clinical manifestations. If the phenomenon can occur independently of the surface interface, as seems probable to me, then we can see manifestations not only at the skin and not only proximal to the tympanic membrane, but in the central nervous system and in any portion of the body. I think one needs to be exceedingly cautious about changing gases under real circumstances in man. This was a great concern to us—to such an extent that we modified our planned experiment in 1970 because of this concern, as well as our own ignorance of what we might have been seeing.

The second comment I would like to make concerns the nature of the skin lesion in man, and it may also bear on the difference between the experiments in the pig and the experimental setting at Pennsylvania and at Duke.

**Panelists*: K. N. Ackles, A. A. Bühlmann, M. Columba, D. J. Graves, J. M. Herron, J. Idicula, S. Kronheim, J. Smith-Sivertsen, M. P. Spencer.

We saw lesions in many parts of the body. Our subjects were immersed in helium. The only place they were not immersed in helium was beneath the head tent and where they were breathing nitrogen under those particular conditions.

The lesions had the typical appearance of hives to two experienced physicians within the chamber and to several physicians outside the chamber, including a dermatologist. We assumed that they were largely liquid-filled and had no evidence other than visual as to whether this was so. But I would like to suggest that one could well evoke hives of the classical form by the occurrence of bubbles beneath the skin and whatever neurohumoral processes these would initiate.

Let me conclude by asking why did SF_6 not provoke this fascinating phenomenon? What is the explanation for the difference in the different gases?

Dr. Idicula: With regard to the sulfahexafluoride paradox—our physical cell model consisted of a two-layer system and when we used it with sulfahexafluoride the lipid layer of the cell was in direct access to the sulfahexafluoride. However, when sulfahexafluoride was administered via the lung of the pig it had to be carried through the blood before it reached any of these membranes or the lipid interface.

With the low solubility of sulfahexafluoride probably hours, or maybe even days might be required before sufficient concentration in the membrane is reached to produce the isobaric supersaturation.

Dr. Quinn: You would probably be better off in the case where you are switching in one direction than in the other, because, of course, helium diffuses faster than the nitrogen. If you are going from helium to nitrogen the helium is liable to come out faster than the nitrogen goes in, so you would not have a supersaturation effect. It would not be as likely as under the reverse situation when going from nitrogen to helium.

Secondly, with regard to the SF_6, the extent of supersaturation is dependent on the relative thickness of the two layers in our model. For any given set of permeabilities for the two gases in the two layers there exists an optimum thickness ratio for these two layers to give the highest degree of supersaturation. All the parameters are not known yet, but one very likely possibility is that we were just not near the optimum with SF_6, whereas we might have been closer with some of the other combinations.

Dr. Saltzman: Contrary to my anticipation, or what would have been my anticipation and your suggestion, in our experiment the lesions occurred on switching from normoxic helium breathing to normoxic nitrogen breathing and not the other way around.

Dr. Quinn: I was pointing out that in the deep tissues, and not in the interface phenomenon you would not get the situation that leads to trouble.

Dr. Bornmann: Dr. Saltzman, in the film it was quite clear that there was gas in the vesicles seen in this demonstration. Are you saying you feel the pheonmena you saw at Duke were different?

Dr. Saltzman: I think they were. Yes, I think they probably did not contain gas in the lesions that we saw.

Dr. Smith: If one takes gas transport through these phases, which is the product of the diffusion coefficient times the solubility, then your table is beautifully rationalized by the asymmetry. If the breathing gas has a higher coefficient of permeability than the external gas, then one gets symptoms—but not in the other case. I would think this would also be true if one examined the more plausible physical parameter of the ratio of the permeability coefficient in water to fat.

Dr. Bornmann: You did not describe the phenomenon as occurring when you had the subject in air breathing helium. Did you not demonstrate it or did you not try?

Dr. Idicula: We have had such situations but did not find the lesions. When the animals breathe helium and the body is surrounded by nitrogen, or when the animals breathe helium and the body is surrounded by nitrous oxide you do not find the lesions. In fact, it would be just the reversal of the model we have. An undersaturation would be predicted; instead of the total tension going up it would go down. So the mathematical model predicts an undersaturation and we did not find any lesions at all.

Dr. Bornmann: So if the operational diver stays in the phase he generally is in and avoids the one you are describing, he should not have much problem. Most dives are done with the diver in air or in helium when he is breathing helium, very seldom in helium breathing air.

Dr. Idicula: That is correct.

Dr. Behnke: I do not want to take the Pope's attitude toward Galileo's findings here, but I have never seen so many bubbles in my life--more bubbles than if all the gas I ever saw in bubble form was gas evolved under isobaric condition.

For many years we have switched gases. Divers were on helium at deep depths—divers were not on equilibrium with the ambient helium, but they may have been exposed for 20 minutes or a half-hour—and coming up were switched to air. If the switch to air was made quickly, divers had symptoms; but if the air was let in slowly they did

not. The point is, this has been a routine practice and we certainly have missed a very fundamental phenomenon, but nevertheless the skin lesions did not occur. We switched routinely and Dr. Bühlmann does it routinely—switching on the helium dive, saturation dive, to air beginning at 150 feet and coming to the surface. I would like to know whether Dr. Bühlmann observed any of these skin effects.

But the main point is that your pig was under hyperbaric conditions and your gas was coming out at normal pressure, correct? When you had the pig's tissue underwater it was 1 atmosphere?

Dr. Idicula: The switching which Dr. Bühlmann has done is exactly the reverse of the situation we are talking about now. If you go from a helium-saturated condition to a nitrogen one, you should find an undersaturation. After the ocurrence of the bubbles at increased pressure we decompressed the animals in some experiments. However, they occur without any alteration of pressure—you do find the process occurring on the surface also.

Dr. Bühlmann: We have never seen such symptoms on the skin, but our conditions were not the same as in your experiments. We have used three conditions.

First condition: under sustained pressure, for the first hour with the chamber filled with air and the diver breathing helium; the second hour under the same pressure with the diver breathing air from the chamber. No symptoms were seen.

Second condition: the chamber always filled with air (that is our normal technique for our professional diver in the sea) and with the diver breathing oxyhelium with breathing apparatus and then changing to the air in the chamber. No symptoms on the skin were observed.

Third condition: in experimental dives the chamber is always filled with the same mixture. If the diver is breathing helium he has helium in the chamber and then we flush the chamber to air and he is breathing air.

But we have never used the conditions of your experiments, and with our situation we have never observed skin lesions.

Dr. Farmer: Dr. Bühlmann has shown again that the organ of hearing seems to be a uniquely sensitive organ in deep helium-oxygen diving. In our collected series of up to 20 cases we now have noted a very high correlation between the lack of residual defects and prompt treatment, as though this was decompression sickness even though there were no other classical signs of decompression sickness.

I would suggest in our follow-up of the residual symptoms, that the classical clinical history of the sudden destruction of an end organ consists of a 2- to 3-week period of vestibular and spatial orientation problems with ambulation, and then gradually compensation takes place and such individuals usually do not have symptoms unless they are put to stress or tested under specific conditions. So in our follow-up I would hope that we would test such individuals and make note of that phenomenon.

Dr. Bühlmann, did you notice any vestibular problems with changing inert gases?

Dr. Bühlmann: On the last point, no. During changing gases we have never noted vertigo symptoms. I agree that we have to study all the different functions of the inner ear over many years.

Dr. Bornmann: Dr. Bühlmann, you seemed at first to get good results in recompression therapy with small increases in depth; treatment was successful. Then you began to get some residuals and in one case you even recompressed from 21 to 28 atmospheres in therapy and still had a residual after the man surfaced; this is quite different from the 1- or 2-atmosphere recompression which was completely successful at the beginning.

Do you have any comments or suggestions for doctors who may have to treat divers with vertigo?

Dr. Bühlmann: In case 9 we made the recompression to 28 atmospheres and the subject lost his hearing. This has been the third experiment with him. In the last experiment with these disturbances we made practically a full recompression, and I think it was the best. He actually had had symptoms of problems before on three previous dives: the first time was in 1966 and for that we made only a little recompression, and that is not good.

Dr. Walder: I was surprised that, in Dr. Ackles' studies, he was surprised that he got a two-component uptake and clearance curve from muscle. He tended, it seemed to me, to be thinking of muscle as though it were a uniform tissue. The muscle in the man or the animal is not, of course, a uniform tissue. It consists of muscle cells, which I suppose one could think of as uniform material, but between them there is a considerable amount of connective tissue which varies in amount from muscle to muscle.

In some experiments in another context we looked at the uptake and clearance of isotopes from muscle and we were able to convince ourselves that with muscle (such as the biceps) you are dealing with two separate tissues and these have two quite different half-times.

Dr. Ackles: We were initially surprised, until we started to think of it in detail—because everyone just talks about half-time as though it is something related to aqueous half-time and fat half-time. But I think the slow half-time is due, as I said, to the blood interface, the blood-capillary interface. The biggest surprise to us was the wide variation. We could not come up with an average half-time of muscle, for example—it is just not possible

because of the wide variation between animals. There is also such a variation in one animal between the uptake and elimination in that same muscle, at the same site. That we have not explained yet.

I think the first half-time, the fast one, is due to the arterial blood supply as it saturates.

Dr. Barnard: We have been doing gas washout studies with the mass spectrometer and at very low levels there are things that can interfere. One is the possibility of diffusion of gas through the skin. Some assumptions can be made here that lead to the conclusion for our experiments that this must be less than about 1 ml per minute because otherwise you cannot get down to a baseline. The second possibility was that we got some sort of cyclic variations of the sort that Dr. Ackles talked about and we wondered whether to call this background noise or what to do with it.

Dr. Bornmann: I would like to go back to the very clear and very beautiful illustrated presentation on bubbles in the eye by Miss Kronheim, and I would like her or her clinical colleagues to assure me about the significance of bubbles in the eye. What happened to the bubble in the lens? Did it go on to scarring or did it disappear?

Miss Kronheim: The bubbles that we have seen in the lenses of rabbits do disappear. However, we did no histology on the animals. It did not look like there was anything unusual remaining. Dr. Nichols and Dr. Lambersten found a bubble in the lens of a human subject following nitrogen saturation, and that resolved completely.

Dr. Bornmann: You are not concerned, then, about bubbles in the eye which you have demonstrated as being a problem for divers?

Miss Kronheim: There are certain aspects of the rabbit eye that are quite different from man. For example, the rabbit lens is larger than the human lens. The vitreous is smaller in the rabbit than in the human. It demonstrates such things as posterior flow, while there seems to be no evidence for this in the human. Based on the results obtained in the rabbit, we can estimate what the rate constant or half-time would be, for example, in the human vitreous. This can be done by using Hills' equations for gas exchange in perfect geometric forms, and if you assume that the vitreous approaches the shape of a sphere, you can introduce the diffusion coefficient which you can calculate from having determined experimentally the half-time for krypton uptake in the rabbit vitreous. You can calculate what the half-time would be in the human vitreous based on the increased size. And assuming a square root of the molecular rate relationship for nitrogen, and also assuming that the perfusion rate of the rabbit is perhaps four times that of the human, you can come out with a half-time for the vitreous of about 430 minutes as a preliminary calculation. So it is quite possible that this is a factor. I do not think it has really been considered in the past.

Dr. Bornmann: I accept that bubbles may appear in divers under conditions analogous to what you have described, but I would for the record like to get assurance that they do no harm.

Dr. Barnard: With regard to prevention and therapy of decompression sickness, we are not at all sure of what we ought to be doing. We have considered whether or not we should be using heparin, for instance. We have had this sort of argument about oxygen for a long time. Dr. Spencer, for instance, in using bubble detectors, missed out on the possibility of random assortment between bubbles and bends—the possibility that he might have bubbles and no bends. We did not miss this point. We looked at this and we came to the conclusion, in fact, that we often did see bubbles and no bends.

It is a rather awkward situation, if you are a diving supervisor and you use some device which will show whether or not bubbles occur.

I think it is rather important for us to make up our minds what new developments we push out to the practical diving field and what we say are very much still under test. And I feel that very much of what we have talked about to the moment is quite unproven.

Dr. Walder: I hope the diving supervisor will bear in mind that heparin may lead to bleeding, that dextran blocks the kidneys, that cortisone gives us bone necrosis, and if he is thinking of turning to aspirin—which most of us tend to think of as a pretty innocuous drug—we see in hospital many people suffering from peptic ulcers as a result of taking aspirin.

Dr. Bornmann: Could Dr. Ackles speculate on a tantalizing phenomenon that has been very poorly understood for many years: the so-called "off effect" for oxygen—in which a person exposed to hyperbaric oxygen after decompression, sometimes convulses when he is no longer breathing oxygen. When I noted Dr. Ackles' comments about the rise in tissue of venous oxygen after decompression I wondered if there was some possible relationship between the two.

Dr. Ackles: That did not occur to me until just now, but I think it probably could because I cannot explain where this fantastic store of oxygen is being stored; but in two cases measuring two different sites it consistently came out as we came very close to the surface, and it went very, very high. We were not expecting anything that

high and did not have the machine scaled properly. I mention these since they confirmed each other—the venous in one animal and the muscle in the other.

Dr. Spencer: May I go back to Dr. Barnard's statement for just a moment? There is a finding I did not mention. We were grading the bubbles that we hear in the precordium because we cannot at the moment count them or size them ultrasonically; this works pretty effectively though. When we hear bubble frequency in which most of the heart cycles have bubble signals coming, and in which they are very loud and obvious (even to someone who has had no training whatsoever) then very frequently bends will ensue.

In Dr. Smith's lab this has been observed particularly on the decompression experiments using a detector, and I am sure that if you use it on your men and listen routinely you will find the same thing: that they will go on to bends; you will then perhaps come to the same conviction that we have—namely, that the bubbles should be treated before the bends come on.

Dr. Lundgren: Now that bubble detection by means of ultrasonic devices is apparently becoming more important I would like to ask Dr. Spencer, how can we be sure that we do not introduce bubbling by the ultrasonic devices?

Dr. Spencer: I think that is a very good question and a very important one. We know that ultrasonic frequencies—20 to 60 kilohertz—can produce bubbles in unsupersaturated or normally equilibrated water. As the frequency goes up it takes a great deal more energy to produce this cavitation. We also are worried about the fact that we have a supersaturated solution, however, in which it may take less energy at any given frequency.

The safety factor, from a theoretical standpoint, can be viewed this way: the amount of energy that we are putting in through the skin is 10 milliwatts on the surface of the skin. We have calculated—through an attenuation that will take place through muscle (which is looking at it conservatively, because we also have lung and bone between the heart, for example) and our transducer—that by the time this ultrasound reaches the heart it is attenuated to 5 microwatts.

For purposes of comparison, the stresses which the heart itself generates within itself are several orders of magnitude greater than the stresses which the ultrasound pressures produce.

Dr. Farmer: When you compare it with the forces generated by heart sounds—actual sound pressure levels there—that is at an entirely different frequency spectrum. Is is legitimate to compare this with the forces generated by your ultrasound?

Dr. Spencer: I think it is. These are the forces that it takes to compress and expand tissue in a local region—the force involved at any particular frequency.

Dr. Farmer: I thought you were still discussing the forces necessary to cause cavitation.

Dr. Spencer: I am, but this would have to be something that pulls the tissue apart, would it not?

Dr. Farmer: No. Maybe Dr. Lundgren, who is more of a physicist than I am, can help us here. My initial thought is that you are talking about two different things: that heart sounds are at a different frequency spectrum, a different energy level entirely. I thought you were trying to say that the energy level in the chest would be so low from your ultrasound device that it would not cause cavitation.

Dr. Spencer: That is what I was saying, yes.

Dr. Farmer: Can you say that without trying to talk about heart sounds?

Dr. Spencer: I think it is useful to compare. We do have compressional energy and expansion energy caused by the ultrasound in the heart; so I am saying that if the heart is supersaturated in a certain situation it should be far more likely that it will produce bubbles within its own cell than that the ultrasound will. This is just one argument on the theoretical side.

Dr. Farmer: I do not agree.

Dr. Spencer: I do not think those arguments are completely satisfying, certainly not to me. We have done some experimental work as a second consideration of the safety factor, in which we shine ultrasonic energy upstream to a dowstream detector; by turning this on and off we do not hear any change in bubble formation or formation of bubbles when they were not present.

Finally, we think the final proof will not be useful until we have used it in a number of individuals. We have done this locally and we have not seen any adverse effect. For example, we have not seen any skin lesions immediately below the transducer, even though we have skin itching in these people.

Dr. Bornmann: I would like to emphasize the point that in an operational diving organization like a Navy we insist on tested procedures to prevent problems. We are aware of the existence of new machines. However, we want the sailor to follow tested procedures to stay out of trouble and to restore him if he does by chance get into trouble. We recognize the value of new machines in exploration of ways to improve our procedures—we are very interested in them. I might comment, however, that if you use these machines (and they are perfectly acceptable in an

experimental mode) then you have a greater obligation to the diver in treating him as soon as possible.

I will close now, remarking that a few years ago the possibility of silent bubbles was emotionally disputed by so many people—even the possibility that it would occasionally exist (as Dr. Behnke is well aware) was just absolutely refused. Now we have evidence that silent bubbles occur so often we do not know what to do with them.

We are now better equipped to detect and to visualize these bubbles. We are better equipped to measure inert gas exchange in the body, even in the tissue itself. We have a better understanding of the processes that may go on after a bubble has been formed. It is now necessary to integrate all of this information toward establishing the definitive causes of decompression sickness in divers.

Part VI. HYDROSTATIC PRESSURE

INFLUENCES OF HYDROSTATIC PRESSURE ON BIOLOGICAL SYSTEMS

A. M. Zimmerman and S. Zimmerman

Below the surface of the sea, all life is subject to hydrostatic pressure in excess of 1 atmosphere (equivalent to 1.01325×10^5 Newtons/meter^2 or 14.696 p.s.i.). Almost 90% of the ocean floor is covered with water at a pressure exceeding 100 atm; moreover, 57.5% of the marine environment is subjected to pressures exceeding 400 atm (65). Thus, if man is to explore the secrets of the ocean, it is essential for him to understand the influences of high hydrostatic pressure.

Although divers have reached a depth of 850 ft where the hydrostatic pressure is in excess of 400 p.s.i., it is conceivable that within the next 5 to 10 years much greater diving depths will be achieved. A group at Comex has attained a chamber pressure equivalent to a depth of 2,000 ft. At these depths, the hydrostatic pressure would be equivalent to approximately 1,000 p.s.i., a pressure at which changes in cellular events start to manifest themselves as alterations of biochemical and physiological activity. It is most probable that, as a result of the recent advances in the physiology of inert gases, man will be able to descend to greater depths, where hydrostatic pressure may be the limiting factor. However, the biological alterations which may occur in man at these depths are still to be resolved. If man is to descend beyond the limits of the continental shelf (approximately 300 meters), it is necessary to acquire sufficient knowledge of the effects of hydrostatic pressure on biological systems so that appropriate precautions will allow for a successful manned dive.

Among the earliest reports concerned with the influences of high pressure on biological systems were those of Regnard (40-45) and Certes (7, 8) who, from 1884 to 1887, published a series of reports on their investigations; a few years later Regnard (46) published a monograph in which he developed his ideas on the effects of pressure on oceanic life. These studies were inspired by the voyage of the *Talisman* (1882-1883) in which organisms were recovered from oceanic depths as far down as 6,000 meters, where the hydrostatic pressure is about 8,800 p.s.i. These early findings were influential in directing Regnard to study cellular systems in the laboratory. He noted that deep-sea animals were considerably different from surface-dwelling varieties in their response to hydrostatic pressure. Certes (7) was able to demonstrate that marine microorganisms recovered from great depths were more resistant to pressure than microorganisms living near the surface.

During the next half century, research on the effects of high pressure on biological

systems was relegated to a relatively small group of dedicated scientists. A review of the literature to the year 1936 concerning the physiological effects of pressure can be found in the report of Cattell (6). At this time, pioneering programs were initiated by Brown, Johnson, Kitching, Marsland and ZoBell; their work stimulated further interest in the effects of pressure on biological systems.

Throughout the last several decades, investigators have been interested in evaluating the effects of high hydrostatic pressure, not only on intact plant and animal life, but also on isolated chemical systems. It is essential to understand the effects of pressure on chemical reactions in order to comprehend fully the influence of pressure on biological systems. For example, although high pressure directly influences biological systems within the ocean, high pressure also alters biological systems by affecting the physical characteristics of sea water. It has been established that the density, ionization constant, viscosity and pH of sea water are altered by increasing hydrostatic pressure (cf. 10, 13). A comprehensive treatise, *The Kinetic Basis of Molecular Biology* (14), analyzes and evaluates the kinetic basis for pressure studies. Recently, several monographs have been published which review the effects of high hydrostatic pressure on biological systems (4, 20, 48, 53, 57).

The purpose of this article is to acquaint the reader with the influences of high hydrostatic pressure on morphological, physiological and biochemical events in a variety of biological systems and to speculate on the effects of hydrostatic pressure in diving.

Morphological Studies

The effects of pressure on the morphology of single cells and multicellular organisms can be readily observed in the laboratory. By viewing the cells through ports in pressure chambers which have been adapted to accommodate high-power microscopes, it is possible to observe changes in shape and behaviour during compression. There are several chambers (Fig. 1) which have been designed and have proven to be most useful for studying pressure effects (24, 31, 32, 34, 59).

Pressure-induced morphological changes have been reported on numerous cellular systems including bacteria (cf. reviews 33, 35, 66, 67), algae (cf. review 52), protozoa (cf. reviews 18, 64), marine eggs (cf. reviews 32, 58), and cultured cells (cf. reviews 21, 22).

In order to illustrate some of the morphological changes which result from hydrostatic pressure, a series of photomicrographs are shown of various protozoa and marine embryos which were subjected to compression (Fig. 2). The photographs were taken while the cells were in the pressure chamber. Morphological changes are readily seen following pressure treatment of protozoa; for example, pressures of 5,000-7,000 p.s.i. (340-476 atm) induce amoebae to retract pseudopodia. After 10-20 minutes of compression the cells become spherical and quiescent. Within 1-2 minutes following decompression, cytoplasmic streaming is again evident and new pseudopodia form (Fig. 2a-f). The minimum pressure which induces cell rounding in *Amoeba proteus* and *Amoeba dubia* is a function of the temperature. Whereas 6,000 p.s.i. causes cell rounding at 25°C, comparable form changes occur following compression to 3,000 p.s.i. at a temperature of 10°C. Pressure-centrifugation studies indicate that the pressure-induced cell rounding results from a solation of plasmagel, which at atmospheric pressure is responsible for maintaining the amoeboid form. Another protozoan, *Tetrahymena pyriformis*, also undergoes a morphological change at high pressure. This ciliated protozoan, which has a pyriform shape, develops a teardrop shape and

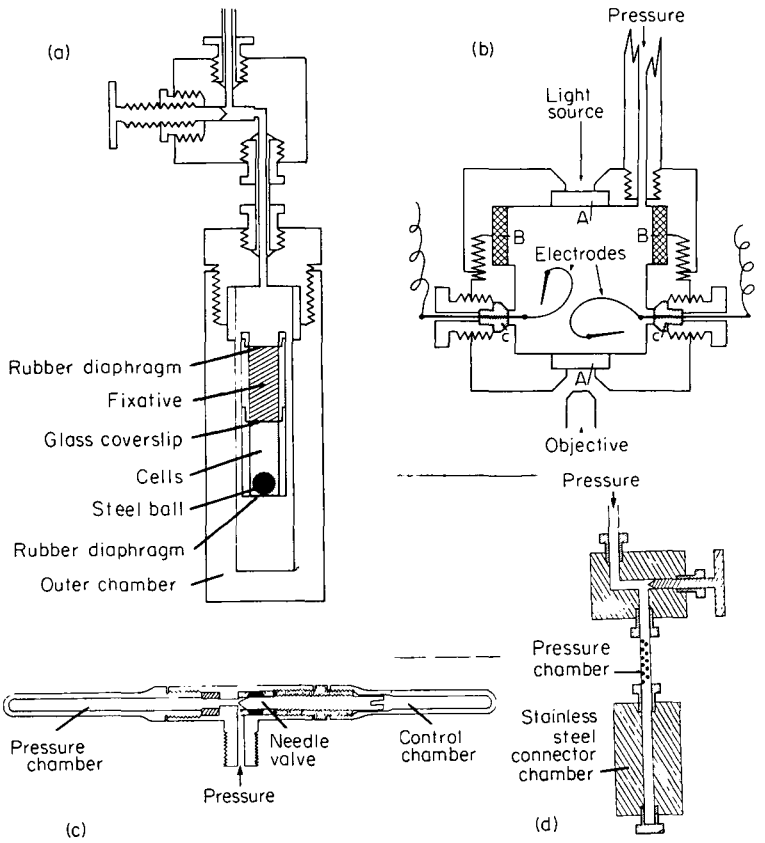

FIG. 1. Schematic drawing of several pressure chambers. **a**, Pressure chamber designed by Landau and Thibodeau (24) which may be used for fixation of cells under hydrostatic pressure. The steel ball breaks the cover glass separating the cell from the fixative when the outer chamber is inverted. **b**, Microscope pressure chamber containing window ports which accommodate a high-power compound microscope. This chamber is a modification of one designed by Marsland (31). **c**, Pressure-centrifuge head, originally designed by Brown (5), contains a central T-shaped unit which is attached to the pressure line. The cells are placed in the pressure chamber; the pressure is applied and the needle valve is sealed. The control chamber is attached and the unit is connected to a high-speed centrifuge. **d**, A simple pressure chamber originally employed by Landau and Peabody (23). The cells are placed in pressure tubing and, following compression, the entire unit is frozen and the frozen core of cells is removed. (From Zimmerman [59].)

loses its ability to display translational movement soon after pressurization (7,500-10,000 p.s.i. for 10 minutes) (Fig. 2g-h). Following decompression the cells recover and display translational movement; 5-10 minutes after return to atmospheric pressure large vacuoles appear in the cells (64).

Although cilia do not break away from the surface of *Tetrahymena* under high pressure, the surface cilia of sea urchin blastulae are sensitive to pressure treatment. Sea urchin blastulae under moderate pressure lose their swimming capabilities due to a loss of cilia. In several experiments, Young et al. (54) report that most cilia are lost from *Arbacia* embryos following 2,000 p.s.i. for 20 minutes. *Strongylocentrotus* embryos are more resistant to

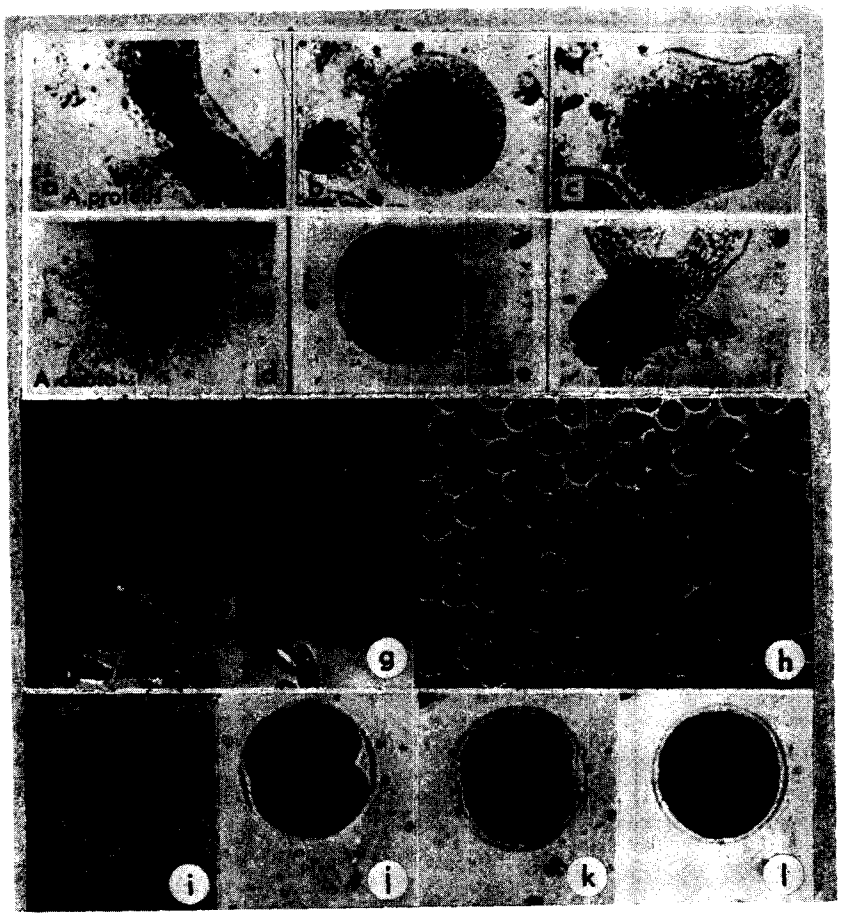

FIG. 2. The effects of high pressure on protozoa and marine eggs.

Amoeba proteus. **a**, Photomicrograph of amoeba at atmospheric pressure as seen through the window of the pressure chamber. **b**, After 15 minutes at 6,000 p.s.i. (25°C) the cell has retracted its pseudopodia and becomes quiescent. **c**, The same cell 7 minutes after decompression displays new pseudopodia. (From Landau et al. [25].)

Amoeba dubia. **d**, Photomicrograph of cell at atmospheric pressure. **e**, The cell observed 5 minutes after compression of 6,000 p.s.i. at 25°C. The cell is almost completely spherical and there is negligible protoplasmic activity. **f**, The same cell 5.5 minutes after decompression, displays vigorous amoeboid activity. (From Landau et al. [25].)

Tetrahymena pyriformis. **g**, Log growth culture at atmospheric pressure. **h**, Photomicrograph of cells under 10,000 p.s.i. for a duration of 10 minutes. There is very little translational movement. Ciliary activity continues but there is no effective swimming. (From Zimmerman [59].)

Arbacia punctulata. **i**, Fertilized sea urchin egg at atmospheric pressure has just started to cleave. **j**, Photomicrograph of the same cell 30 seconds after compression to 7,000 p.s.i. The furrow continues to progress. **k**, Three minutes after initiation of compression, furrow is receding. **l**, At 4.5 minutes after compression, furrow has aborted.

pressure-deciliation; however, with sufficiently high pressure (10,000 p.s.i.) most of the cilia can be removed from these embryos within 10 minutes. In other experiments, *Strongylocentrotus* embryos are deciliated with hypertonic sea water and then subjected to different levels of compression for short durations. A high pressure (> 7,500 p.s.i.) completely blocks cilia regeneration for the duration of pressure treatment (Fig. 3). These studies show a direct relationship between cilia regeneration and the magnitude of applied pressure.

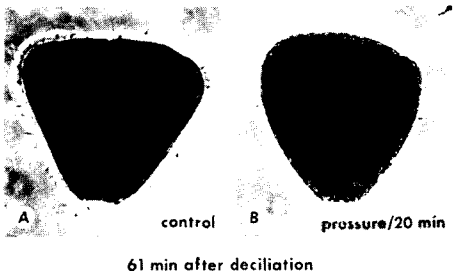

FIG. 3. Effects of pressure on cilia regeneration. Prismatic stage *Arbacia punctulata* embryos were deciliated with hypertonic sea water. Immediately after deciliation, cells were compressed for 20 minutes at 10,000 p.s.i. **A**, Control cell, 61 minutes after deciliation. The cilia regenerated to a length of 12μ. **B**, Experimental cell which received pressure treatment immediately after deciliation. The growth of cilia in pressure-treated cells is 5μ. (From Young et al. [54].)

Transmission and scanning electron microscopic analysis of pressure-treated cells reveals marked structural changes accompanying the shape changes observed under the light microscope. One of the most striking effects of hydrostatic pressure is revealed in an analysis of the fine structure of the axopodia of the heliozoan, *Actinosphaerium nucleofilum*. Hydrostatic pressure causes a reversible disintegration of the normal double spiral array of microtubules which is accompanied by an instability of the axopodia (51). In *Tetrahymena*, high pressure treatment (7,500-10,000 p.s.i.) for durations of 2-10 minutes results in a disruption of longitudinal microtubules (Fig. 4). Thin sections through the cell show progressive disorganization of the longitudinal microtubules and amorphous dense granular material replaces the disintegrated tubules. The unpaired central ciliary tubules distal to the axosome also undergo dissolution under pressure (16). Recent studies using the scanning electron microscope reveal that pressure causes *Tetrahymena* cilia to become shorter and thicker (3). Not all the microtubular elements in cilia and flagella are sensitive to pressure disintegration. The cilia which remain on sea urchin blastulae following 6,500 p.s.i. for 1 hour display normal fine structure (50). In a variety of sperm cells, the flagella microtubules retain their normal arrangement at 10,000 p.s.i. for 10 minutes (32).

In fertilized marine eggs the mitotic apparatus (spindle-aster-chromosome complex) is readily disorganized by high hydrostatic pressure (60). Electron microscopic analysis reveals that high pressure (10,000 p.s.i., for 1-minute duration) causes a dissolution of microtubules in metaphase *Arbacia* eggs (Fig. 5) (61).

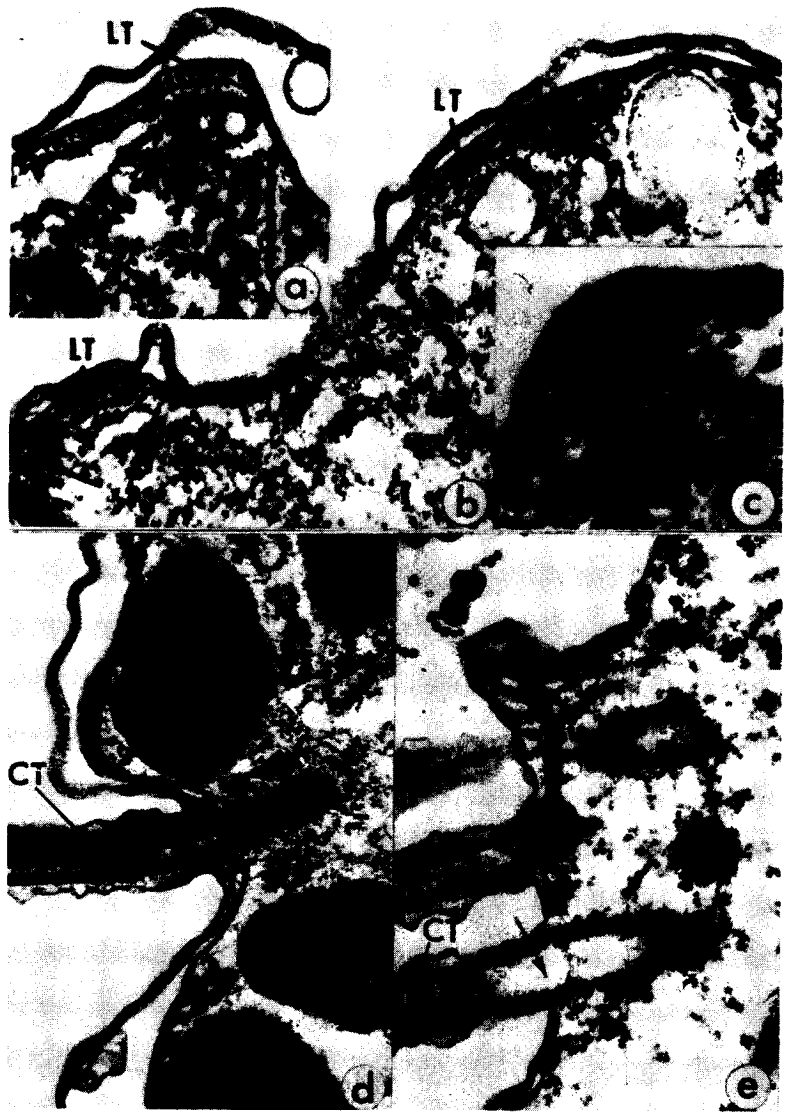

FIG. 4. The effects of pressure on the fine structure of *Tetrahymena pyriformis*. **a**, Cross section through a cell at atmospheric pressure. The longitudinal microtubules (LT) are shown just below the surface (X 72,000). **b**, Section through a cell subjected to 7,500 p.s.i. for 2 minutes. There is an increased amount of granular material (arrows) in the region normally containing longitudinal microtubules (LT) (X 72,000). **c**, Cross section through a cell subjected to 7,500 p.s.i. for 2 minutes. The longitudinal microtubules show some disruption. Both normal and disrupted (arrows) microtubules are evident (X 144,000). **d**, Cilium from a control nonpressurized *Tetrahymena*. The central tubules (CT) of the cilium appear unaltered (X 52,000). **e**, Section through surface of a *Tetrahymena* which was subjected to a pressure of 10,000 p.s.i. for 10 minutes. A longitudinal section through the cilium shows breakdown (arrow) of the central ciliary microtubules (CT). Swelling of the ciliary shaft appears to be at the junction of the basal body (arrow) (X 52,000). (From Kennedy and Zimmerman [16].)

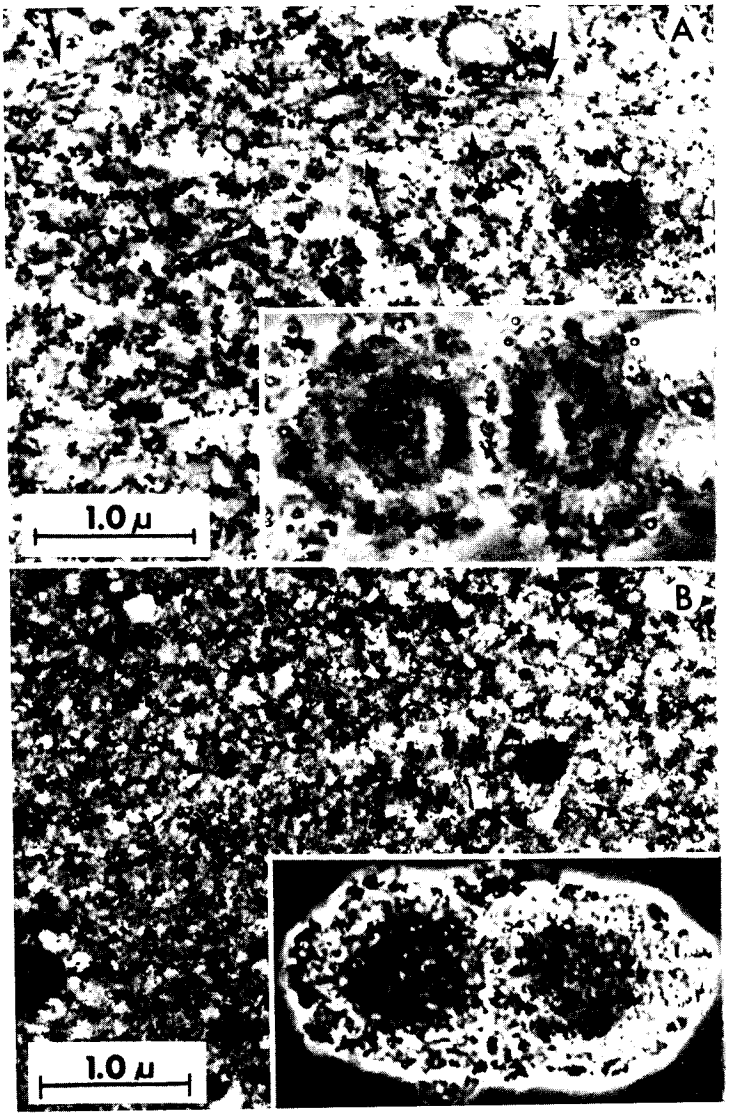

FIG. 5. Pressure studies on mitotic apparatus. **A**, Electron micrograph through *Arbacia* egg at atmospheric pressure. The microtubules in the aster region of metaphase egg are shown (see arrows). The insert shows an isolated mitotic apparatus at metaphase. **B**, Electron micrograph of an egg which was subjected to 10,000 p.s.i. for 1-minute duration. Note absence of microtubules. Insert shows isolated mitotic apparatus with loss of linear and radial orientation in the spindle and aster. (From Zimmerman and Philpott [61].)

Physiological Studies

Physiological alterations induced by high pressure are extensive and encompass a wide spectrum of activities. Protoplasmic streaming in animal and plant cells is markedly affected. Ciliary beat, heart rate and the electrical activity of muscles and nerves respond dramatically to high pressure in a variety of cellular organisms (cf. reviews 9, 18, 19, 37, 47, 59).

The action of pressure on mitosis and cytokinesis can be used as examples to illustrate how cellular activities are affected at high pressures. Hydrostatic pressure reversibly blocks cytokinesis in a variety of marine cells (see Fig. 2i-l). Mitosis is also delayed through disruption of the mitotic structure which is essential for chromosome activity (32, 60).

In addition to the above-mentioned stages at which pressure can initiate a blocking or delaying effect on cell division, other more subtle effects are found following short pulses of pressure at varying stages during the cell cycle. A short pulse of pressure (7,500 p.s.i. for 1 minute) applied to sea urchin zygotes results in a cleavage delay which is dependent upon the time at which pressure treatment is initiated. A maximum cleavage delay (13 minutes) is reported when treatment is initiated at prophase; minimum delay occurs just prior to division and at the time of pronuclear fusion (Fig. 6B) (62). The division-delaying effect of pressure on synchronized populations of *Tetrahymena* appears to be quite different from that found with sea urchin embryos (see Fig. 6A). Whereas in the division cycle of sea urchin cells, sensitivity to pressure fluctuates throughout the cell cycle, in division-synchronized *Tetrahymena*, sensitivity to pressure increases linearly through the first half of the cycle. With *Tetrahymena* high pressure (10,000 p.s.i. for 2-minute duration) results in division

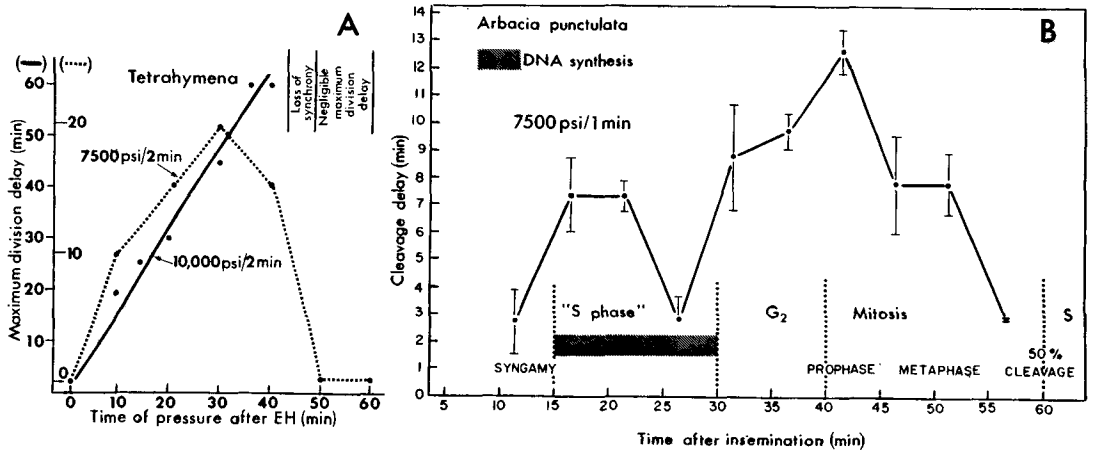

FIG. 6. Effects of short pulses of pressure on division schedule. **A**, Heat-synchronized *Tetrahymena* were subjected to short pulses of pressure (either 7,500 p.s.i. for 2 minutes or 10,000 p.s.i. for 2 minutes at various times during the cycle). Division delays are plotted as a function of time after the last heat shock (EH). Nonpressurized control cells divide at 75 minutes after EH. (From Lowe-Jinde and Zimmerman [27].) **B**, Sensitivity of fertilized *Arbacia* eggs to short pulses of pressure during the cleavage cycle. The cleavage delays, following pressure treatment (7,500 p.s.i. for 1 minute) at various times after insemination, are shown. DNA synthesis occurs during the period 15-30 minutes after insemination. (From Zimmerman and Silberman [62].)

delays which directly correspond to the age of the synchronized culture. Maximum division delays of 60 minutes occur during the middle of the cell cycle; negligible division delays occur just prior to furrowing (27).

Biochemical Studies

Although much of the early work on high pressure was concerned with changes in the structural and physiological activities of cells, in recent years there has been an emphasis on the biochemistry of cellular systems. Pressure-induced biochemical changes can provide a basis for understanding the effects of pressures on cell structure and physiological activity. Recent studies include the influences of high pressure on isolated biochemical systems, the activity of enzymatic systems from organisms which are indigenous to the deep sea, and macromolecular synthesis from organisms which inhabit shallow waters (cf. review 35).

For the past several decades Chieko and Keizo Suzuki and co-workers have conducted extensive research on the denaturation of proteins and the inactivation of enzymes under high pressure. In recent work they report that solutions of calf thymus DNA are quite stable to extremely high pressures (9,000 atm) (49). Other investigations include the effects of pressure on: multimeric enzymes (39); growth and glycolysis (30); protein synthesis (2); the uptake and respiration of amino acids in microorganisms (38). Paul and Morita (38) who studied a facultatively psychrophilic marine bacterium—designated MP-38—reported that hydrostatic pressure and low temperature inhibit glutamate transport more than glutamate respiration. These investigators studied several amino acids, and they conclude that the inhibition of amino acid uptake is the cause for the inability of these cells to grow under pressure.

Following the 1970 expedition of the *Alpha Helix* to the Galapagos Archipelago, Hochachka and co-workers (12) reported on the influence of pressure on selected systems isolated from abyssal species of fish. These elegant biochemical studies must be carefully interpreted in view of the extensive structural and physiological artifacts which accompany the retrieval of benthic organisms from their natural habitat where there are low temperatures and high pressures; the retrieval of such organisms over extended periods of time will be accompanied by structural, physiological and biochemical artifacts.

Perhaps one of the best ways to illustrate the influence of pressure on the biochemistry of cells is to review the effects of pressure on macromolecular synthesis in the ciliated protozoan *Tetrahymena*. This protozoan, which ordinarily lives in a shallow water environment, has been extensively used as a model for investigational purposes.

The conformational alterations in macromolecules are probably responsible for numerous cellular effects which culminate in physical and structural changes. In general, macromolecular synthesis (RNA, DNA and protein synthesis) is progressively reduced with increasing magnitudes of hydrostatic pressure. This has been demonstrated in marine cells as well as in protozoa. In *Tetrahymena*, DNA synthesis is reduced approximately 10% at 2,000 p.s.i. (for a 10-minute pulse), and as much as 70% at 4,000 p.s.i. as compared to atmospheric controls (36). RNA and protein synthesis are inhibited by high pressure in a similar fashion (28, 56). An investigation of the protein synthesizing system has shown that hydrostatic pressure directly disrupts polysomes. For example, the amount of polysomal material in *Tetrahymena* is reduced by pressures of 5,000 p.s.i. (Fig. 7). The disruption of the messenger RNA-ribosome complex may be the factor causing reduction of protein

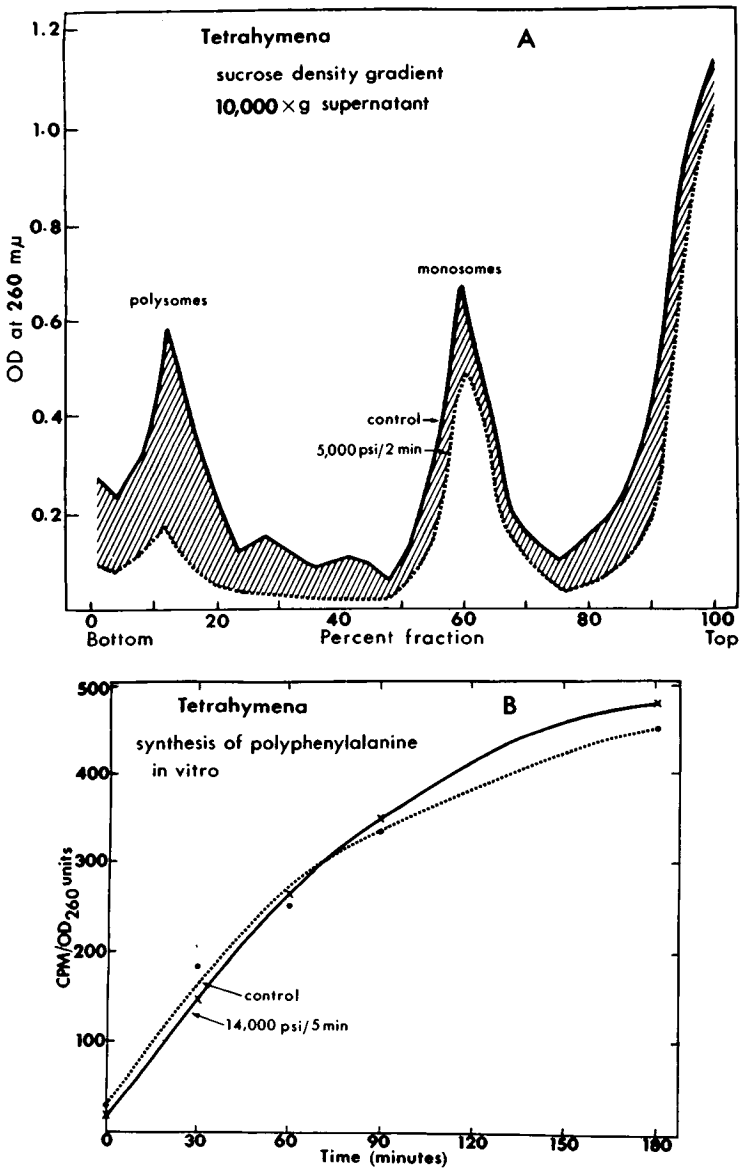

FIG. 7. Effects of pressure on ribosomes from *Tetrahymena pyriformis*. A, Sucrose gradient analysis of 10,000 x *g* supernatant fraction, obtained from pressure-treated *Tetrahymena,* is compared to nonpressurized controls. The pressure-treated cells were subjected to 5,000 p.s.i. for 2 minutes at 60 minutes EH. The hatched area illustrates the amount of material that could not be recovered from pressure-treated cells. (Data of Hermolin and Zimmerman [11].) B, A comparison of the activity of microsomes isolated from pressure-treated *Tetrahymena* (14,000 p.s.i. for 5 minutes) and atmospheric control cells. The microsomal material was isolated from the control and pressure-treated cells; the ability of these preparations to synthesize polyphenylalanine is an index of their translational activity. The microsomes were incubated in an incubation medium containing an "energy-generating system," exogenous messenger (Poly-U), 14.5 m*M* Mg^{++} and "supernatant fraction." (From Letts and Zimmerman [26].)

synthesis in vivo (11). The ribosomes themselves, however, are not affected by the pressure treatment. In cell-free systems, these ribosomes are as effective as control ribosomes in translational activities, e.g. synthesis of polypeptides (26). Hydrostatic pressure does, however, influence the synthetic activity of the cell which is responsible for the formation of messenger RNA (mRNA) and ribosomal RNA (rRNA). It has been demonstrated (55) that messenger synthesis, as well as ribosomal precursor material, are reduced following a 10-minute pressure pulse of 5,000 p.s.i. (Fig. 8).

These studies clearly demonstrate that the pressure-inducing effects on the structural and physiological activities of the cells have a biochemical basis. The inhibition of cell division and the reduction of ciliary activity, which affects the movement of the cells, are manifestations of structural and biochemical processes.

High pressure has been used as a tool for investigational purposes in a large number of studies. Many of these studies are concerned with the effects of chemical agents on cellular activities. It has been reported that agents such as demecolcine (Colcemid), heavy water, mercapto-ethanol and adenine nucleotides influence the response of cells to pressure. These studies have advanced our knowledge of the action of hydrostatic pressure and have aided our understanding of the nature of cellular activity (cf. reviews: 32, 58, 63).

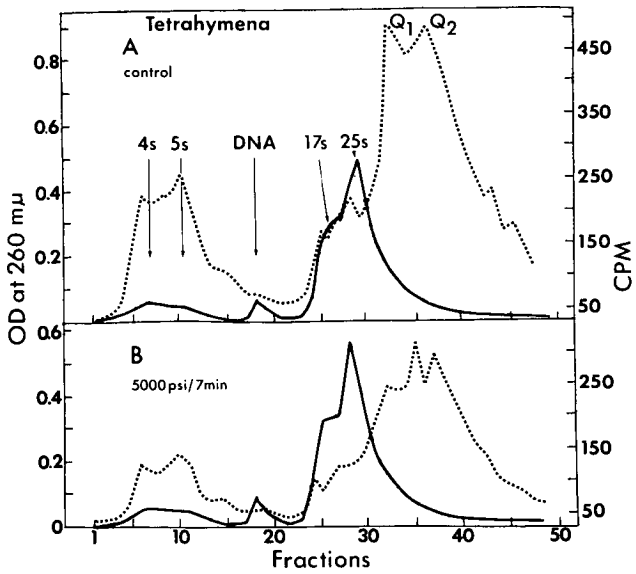

FIG. 8. Influence of pressure on RNA synthesis in synchronized *Tetrahymena*. MAK chromatographic profiles of nucleic acids from synchronized *Tetrahymena* following pressure treatment. The solid line represents the optical density at 260 mμ. The dotted line represents the radioactivity (counts per minute). The RNA species are labelled in the atmospheric control experiment. The 5, 17 and 25S RNA are ribosomal components. The 4S RNA is transfer RNA. The Q_1 and Q_2 RNA represent ribosomal precursor RNA (35S RNA) and DNA-like RNA (messenger RNA), respectively. A, Control cells were pulsed with ^{3}H-uridine (10 μCi ml) from 40-50 minutes EH. B, Experimental cells were placed into ^{3}H-uridine at 40 minutes EH and 5,000 p.s.i. pressure was applied from 43-50 minutes EH. (From Yuyama and Zimmerman [55].)

Conclusion

A study of the action of hydrostatic pressure on cellular systems provides a vehicle for investigating the structure and function of organisms that inhabit great oceanic depths. The biochemical studies on pressure-treated organisms complement and add new significance to the physiological and morphological investigations. In order to illustrate the effects of pressure on several different cellular systems a pressure chart has been prepared (Fig. 9). This chart indicates the approximate relationship between depth (expressed in meters) and hydrostatic pressure (expressed in atmospheric pressure units and in pounds per square inch) and includes examples of morphological, physiological and biochemical activities that are modified at various oceanic depths. For example, although the lethal pressures for several organisms (*Gammarus, Cyprina, Asterias* and *Mytilus*) occur at 10,000 p.s.i. or greater (about 7,000 meters), a large number of physiological and morphological alterations occur at depths of 3,000-4,000 meters (4,000-6,000 p.s.i.). Even at a depth of 1,000 meters where the pressure is approximately 1,500 p.s.i., biochemical and physiological activity of cells are affected. The various effects observed for different organisms may be related to the ecosystems of these organisms. The fact that certain marine forms inhabit the oceanic floor where high pressures prevail is indicative of their adaptive evolution of biological systems that are able to cope with high pressure.

The problems that confront a diver using an inert gas under hydrostatic pressure may be more complex than has been envisioned in the past. It is conceivable, although as yet untested, that the action of pressure and inert gas may be synergistic and cause a detrimental effect on human tissues under conditions where each alone has negligible effects. Inhalational anaesthetic agents affect various cellular activities such as cell division, protoplasmic streaming and ciliary movement; they can cause a disaggregation of microtubular elements, at concentrations proportional to those required to anaesthetize man (1). Johnson and Miller (15) reported that pressure and anaesthesia have an antagonistic action. They found that pressure reverses the anaesthesia in animals. The interaction between anaesthetics and hydrostatic pressure has also been investigated in dividing *Tetrahymena*. At low partial pressures, individually acting anaesthetics have no effect; however, when combined with pressure, these anaesthetics cause reversible inhibition of division (17, 29).

It is difficult to speculate on the possible changes which may affect a diver at a depth of 500-1,000 meters; however, the wealth of published literature on the influence of high pressure on plant and animal tissue offers some insight into this problem. On a molecular level, changes in DNA synthesis and chromosome replication may be found. Although these changes may not be significant to somatic cell tissue, alterations in reproductive tissue may indeed be more serious. Thus oogenesis and spermatogenesis may be modified and hence affect genetic expression. In view of the pressure effects on polysomal material which begin to become noticeable at this depth, long exposures to pressure may alter protein synthesizing systems and affect other cell functions. Accompanying the biochemical changes, physiological alterations might be expected. On physiological grounds ciliary activity may be altered; interference with the coordinated ciliary activity in the human epithelial tissue may interfere with respiration. Although effects on muscle and nerve activity are observed at higher hydrostatic pressures, it might be expected that subtle neural aberrations would be recorded. Perhaps within the next decade further experimentation will yield answers to some of these speculations.

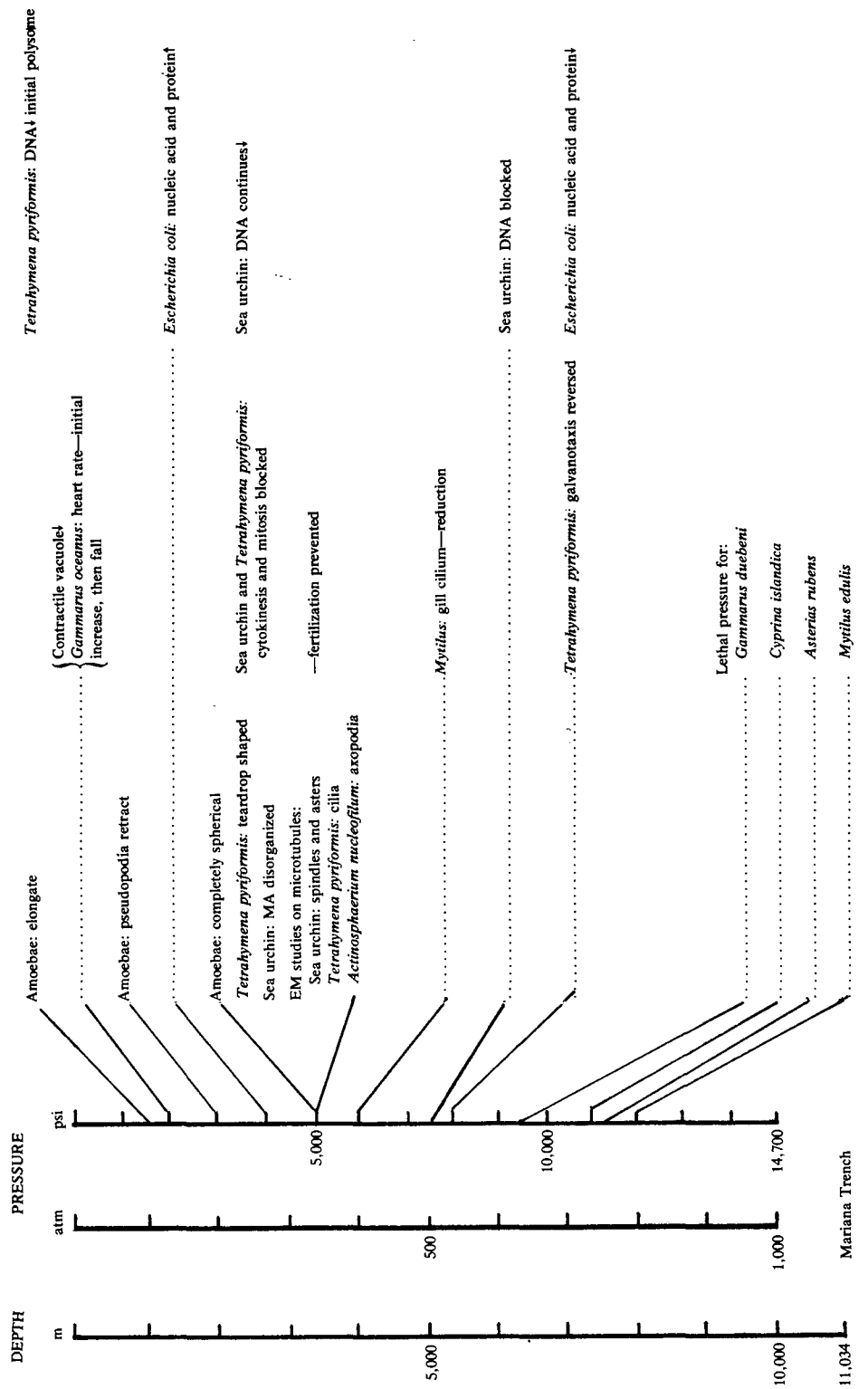

Fig. 9. A summary of high pressure effects on various organisms. MA, mitotic apparatus; ↓, decreasing amounts; ↑, increasing amounts; EM, electron microscope.

ACKNOWLEDGMENTS

The research from the authors' laboratory was carried out with the support of research grants from the National Research Council of Canada.

The authors wish to express their appreciation to Mrs. Helen Laurence for her technical assistance.

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HYDROSTATIC PRESSURE TOLERANCE IN LIQUID-BREATHING MICE

C. E. G. Lundgren and H. C. Örnhagen

Most studies of hydrostatic pressure tolerance made so far have been performed on single-cell organisms, tissue preparations or cool-blooded animals (c.f. 2, 9). These studies have been concerned mainly with dynamics of biochemical reaction and metabolism. Experiments performed on gas-breathing mammals indicate that pressure effects per se on muscles and nerves can be masked by the pharmacological effects of inert gases at high pressure (1). True hydrostatic compression of liquid-breathing mice was performed by Kylstra and co-workers in 1966 (3).

This work was aimed at investigating the influence of body temperature and compression rate on pressure tolerance in mice, spontaneously breathing oxygenated fluorocarbon liquid.

Material and Methods

Female NMRI mice, weighing between 25 and 35 gm, were used. The fluorocarbon liquid used was FC-75.* The compression chamber (Fig. 1) consisted of a steel chamber (volume 1.7 liters) with an acrylic glass window. The pressure in the chamber was increased by a motor-driven hydraulic piston pump operating at a frequency of 17 beats/min. The compression rate was changed by adjusting the stroke volume. The pressure increase with each pump cycle varied between 0.03 and 0.4 atm. A thermostat-controlled heat exchanger inside the chamber was used to control liquid temperature. Continuous, rapid stirring of the liquid was obtained by a motor-driven propeller in the chamber. The unanesthetized animal, retained in a cage (not shown in Fig. 1), was fitted with a rectal thermistor probe and a three-lead EKG-cable; the electrodes, which consisted of copper threads, were sutured into the skin. The EKG was continuously recorded on a Mingograf 800.† Gross electromyographic activity from respiration and convulsions appeared superimposed on the EKG-tracing. Using a high precision manometer‡ as a standard, pressure was recorded by two independent systems—namely, the manometer just mentioned and an electrical transducer§

*3M-Company, St. Paul, Minnesota.

†Elema-Schönander, Stockholm, Sweden.

‡NAF 315660 Nordarmatur, Linköping, Sweden.

§NAF 350422 Nordarmatur, Linköping, Sweden.

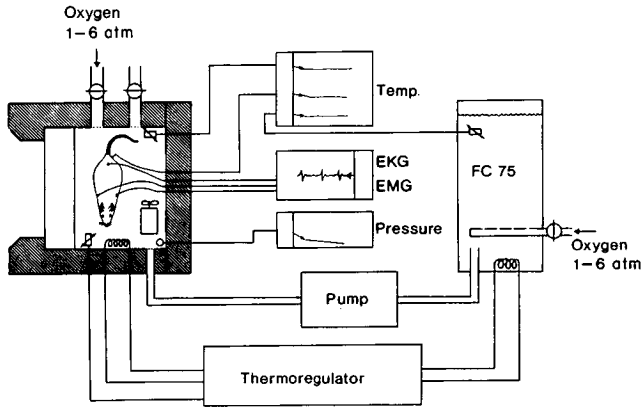


FIG. 1. Experimental setup for determination of hydrostatic pressure tolerance in liquid-breathing mice.

connected with an Esterline Angus writer.* The accuracy of the pressure recordings was ± 3 atm. Liquid and rectal temperatures were recorded (accuracy ± 0.1°C) with thermistors and Esterline Angus writers.

Oxygenation of the fluorocarbon liquid prior to an experiment was made in a special pressure vessel. The liquid was, in most experiments, oxygenated at 4 atm of oxygen pressure. In a few experiments an oxygen pressure of 2 or 6 atm was used. Before letting the fluid into the compression chamber it was flushed and pressurized with oxygen to the oxygenation pressure of the liquid.

Five different, essentially linear, compression rates were applied—i.e., 0.5, 1, 2, 4 and 6 atm/min. Each compression rate was tested in four animals. Special emphasis was not placed on the decompression procedure; the decompression rate was varied between 1 and 100 atm/sec. By adjusting the liquid temperature the animal's rectal temperature was kept at 17, 21, 27 or 31°C. Six animals at each temperature level were used.

Results

The pressure at which breathing and/or cardiac activity ceased was designated maximum tolerated pressure (MTP). When the oxygenation pressure of the liquid was 2 atm, the MTP was distinctly lower (110, 115 and 130 atm in three animals) than at an oxygen pressure of 4 atm (mean MTP 224 atm). Increasing the oxygenation pressure to 6 atm apparently had no advantage over 4 atm. The following results were all obtained with an oxygenation pressure of 4 atm. With a compression rate of 2 atm/min the mean MTP in a group of six mice at 17°C rectal temperature was 149 atm (Fig. 2). At 21°C the mean MTP was 216 atm and at 27°C, 216 atm. The highest individual MTP, namely 272 atm, was observed in the latter group. When the temperature was increased to 31°C the mean MTP was reduced to 130 atm.

*Labgraph Speed Servo S-601-S Esterline Angus, Indianapolis, Indiana.

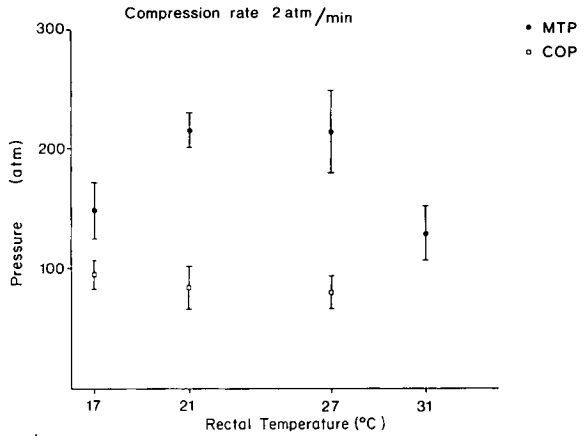


FIG. 2. Maximum tolerated pressure, MTP (dots) and convulsion onset pressure, COP (squares) in atm (mean ± 1 S.D., n = 6) versus rectal temperature in liquid-breathing mice. Compression rate 2 atm/min. Oxygen pressure in the liquid 4 atm. At 31°C rectal temperature no clear convulsions were seen on the EMG.

As shown in Fig. 3, compressing at a rate of 0.5 atm/min at a rectal temperature of 21°C gave a mean MTP of 140 atm. Doubling the compression rate (1 atm/min) gave a mean MTP of 165 atm. With 2 atm/min, the mean MTP was 216 atm and 4 atm/min it was 243 atm. The highest compression rate (6 atm/min) gave a MTP of 190 atm. In two experiments (4 atm/min, 21°C) the MTP was reproduced within 40 atm by decompressing the animals unil respiration started again and then repeating compression. Neuromuscular disturbances ranging from fine tremor to tonic convulsions were seen in most experiments. When

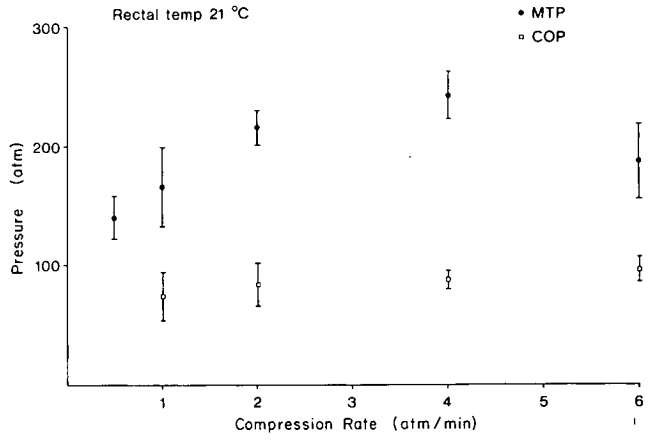


FIG. 3. Maximum tolerated pressure, MTP (dots) and convulsion onset pressure, COP (squares) in atm (mean ± 1 S.D., n = 4) versus compression rate in liquid-breathing mice. Rectal temperature 21°C. Oxygen pressure in the liquid 4 atm. At a compression rate of 0.5 atm/min no clear convulsions were seen on the EMG.

beginning, the tremor occurred in connection with the breathing movements and the onset pressure was thus difficult to establish. The tremor yielded no obvious EMG changes.

When tonic convulsions were seen, a typical EMG-recording was obtained (Figs. 4 and 5). The pressure at which this pattern first appeared is referred to as the convulsion onset pressure (COP). It is plotted versus temperature in Fig. 2 and versus compression rate in Fig. 3. The COP was 85 ± 15.0 (S.D.) atm in the whole material. There was no obvious change in COP with rectal temperature but there was a tendency to a higher COP the faster the compression rate. Thus, the COP (76 atm) at a compression rate of 1.0 atm/min was lower than the COP (100 atm) at 6 atm/min at the 5% significance level.

Low body temperatures (Fig. 4) and high compression rates (Fig. 5) appeared to exaggerate the severity of convulsions. At body temperatures of 27°C and above, the duration of the convulsions was less than 10 sec, whereas the duration in the 17°C and 21°C groups was

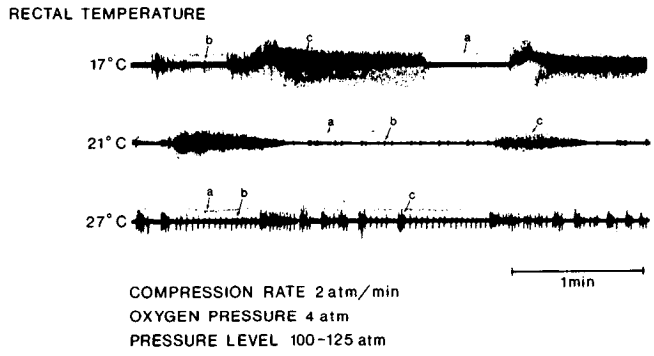


FIG. 4. Electromyographic registration (*a*, EKG; *b*, breathing EMG; *c*, convulsion EMG) in liquid-breathing mice at 100-125 atm hydrostatic pressure and different rectal temperatures. Compression rate 2 atm/min. Oxygen pressure in the liquid 4 atm.

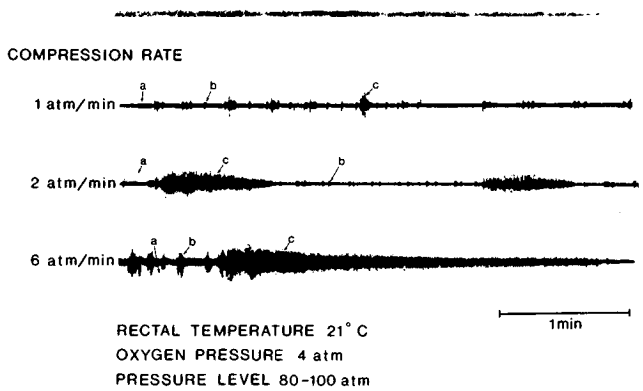
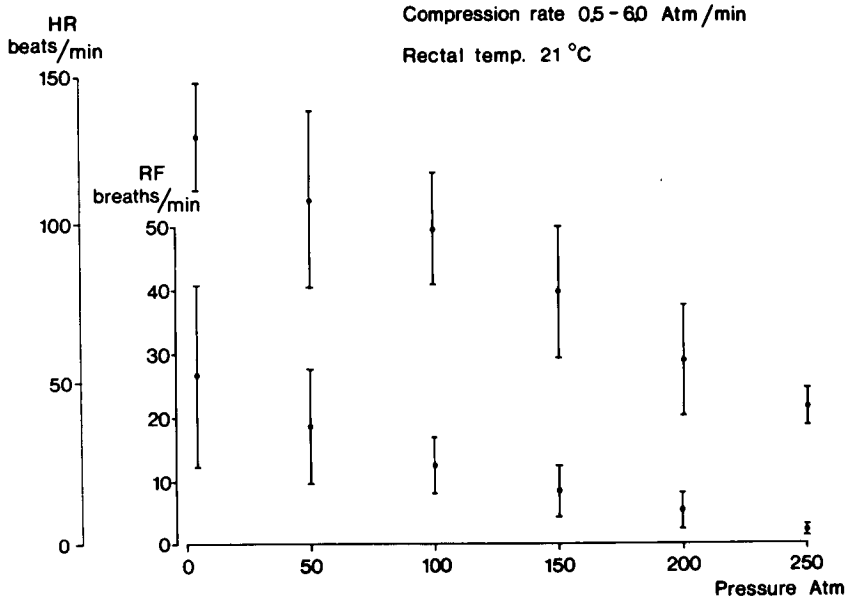


FIG. 5. Electromyographic registration (*a*, EKG; *b*, breathing EMG; *c*, convulsion EMG) in liquid-breathing mice at 80-100 atm hydrostatic pressure and different compression rates. Rectal temperature 21°C. Oxygen pressure in the liquid 4 atm.

1-3 min when using a compression rate of 2 atm/min. With the highest compression rate—i.e., 6 atm/min—and a rectal temperature of 21°C, the duration of the convulsions was up to 4 min. Convulsions were few and of short duration at a compression rate of 1 atm/min and almost absent at 0.5 atm/min. In three animals the reproducibility of the COP was tested (compression rate 2 atm/min). It was reproduced within ± 10 atm several times during the same experiment by reducing the pressure and compressing again. The tendency to convulse decreased with increasing pressure; convulsions were usually absent at pressures above 200 atm.

In all experiments heart rate and respiratory frequency decreased with increasing pressure. This observation is illustrated in Fig. 6 with results obtained at a rectal temperature of 21°C and different compression rates. The effects of pressure on heart rate and respiratory frequency were independent of the rate of compression and, consequently, also independent of the length of time spent in the liquid. Reducing the pressure before reaching MTP, which was tried in a few experiments, in some cases led to an increase in heart rate and respiratory frequency, although previous frequencies were not reached.



Compression rate Atm/min	n	n	n	n	n	n
0.5	4	4	4	2	-	-
1.0	4	4	4	3	2	-
2.0	6	6	6	6	6	-
4.0	4	4	4	4	4	3
6.0	4	4	4	4	2	-

FIG. 6. Heart rate (HR) and respiratory frequency (RF) in liquid-breathing mice plotted against hydrostatic pressure. Mean ± 1 S.D. is given. Number of observations (n) and compression rates at different pressures are given below the diagram.

Respiratory standstill usually preceded cardiac standstill. However in some cases at high pressure levels and temperature, a dramatic reduction in heart rate preceded the cessation of breathing. In animals surviving determination of MTP, the heart rate usually increased toward the end of the final decompression.

Although no special attention was paid to the electrophysiology of the heart, changes in the EKG were observed. In some mice total AV blocks were seen which apparently were not correlated to pressure. Prolongation of the P-Q interval and transitory disappearance of the P wave in the pressure interval between 50 and 150 atm was observed in some animals. The EKG amplitudes were usually unchanged during an experiment. The electromyographic tracings from respiratory activity usually declined shortly before MTP was reached. During the very fast decompressions (1-100 atm/sec) a pronounced bradycardia or even cardiac standstill lasting several seconds was seen. In some cases respiration was resumed before cardiac activity. Animals which retained a regular heart activity and which were decompressed within 1 min after the last breath at pressure usually survived. Three animals which were successfully decompressed from 250 atm are still alive and appear normal 7 months after the experiment.

A few preliminary experiments at 37°C have also been made (four animals). However, only a fast compression rate (4 atm/min) was used to ensure that presumably critical pressures should be reached within the period of time (60 min) that mice can sustain liquid-breathing at such a high body temperature. The spread in the results was considerable (MTP 30-130 atm).

Discussion

Most of the present experiments were made with fluorocarbon liquid oxygenated at 4 atm. From earlier studies it was concluded that this oxygen pressure would satisfy the oxygen uptake capacity of liquid-breathing mice having body temperatures up to 31°C (5). The rationale of applying 4 atm of oxygen pressure was further supported by the fact that increasing it to 6 atm did not influence MTP, whereas the MTP was clearly reduced when the liquid was oxygenated at 2 atm.

It is suggestive that the comparatively low oxygenation pressure (700 mm Hg) used by Kylstra et al. (3) might unfavorably have influenced the hydrostatic pressure tolerance (in terms of survival) in their liquid-breathing mice.

In the present experiments, body temperature appeared to influence MTP; there seemed to be an optimum between 21°C and 27°C (Fig. 2). The decline in MTP at lower temperatures might have been due to the higher convulsive activity in cooler animals. On the other hand, MTP declined at body temperatures above 27°C despite a reduced tendency to convulse. It is noteworthy that the highest pressure (300 atm) reached in helium-oxygen-breathing animals (mice) was obtained under pentobarbital anesthesia and conditions likely to have induced subnormal body temperatures (4). When, in the latter study, unanesthetized animals with a presumably more normal body temperature were used, their pressure tolerance seemed to have been decreased—i.e., it varied between 104 and 205 atm.

It should be stressed that the length of time spent in the liquid in the present experiment does not seem to have influenced the MTP. Thus, in separate experiments, animals with a body temperature of 21°C (not compressed above 4 atm) survived liquid breathing for more

than 5 hours and more than 2 hours with a body temperature of 31°C (and more than 1 hour at 37°C). Although liquid breathing obviously imposes a great workload on the respiratory muscles, simple exhaustion does not seem to be the explanation for an animal reaching a certain MTP, because breathing was usually resumed when a limited decompression was made.

The effects of varying the rate of compression seem to indicate that there was an optimum with regard to MTP (about 240 atm) near a rate of 4 atm/min (Fig. 3). As described, immersion per se does not seem to have been a critical factor. However, the time spent under pressure may have limited the MTP in experiments with low compression rates. The reduction in MTP (to about 190 atm) with the highest compression rate (6 atm/min) may have been due partly to the greatly increased convulsive activity. The observation by Kylstra et al. (3) of a comparatively low pressure tolerance (apparently about 100 atm) may have been due to the high compression rates (about 16 atm/min) which might be deduced from their report. The tremor and convulsions seen in the present experiments closely resembled those described by Kylstra et al. (3) and Brauer et al. (1). The onset also occurred in the same pressure range. The former investigators (3) predicted that convulsions would occur at higher pressures in normothermic animals than in hypothermic animals. To the extent that our results in the temperature range 17°-31°C allow extrapolation to 37°C, we have not been able to confirm this prediction. On the other hand, the warmer the animal, the shorter was the duration of convulsions.

It is not yet clear whether the COP is different in helium-oxygen-breathing from that in liquid-breathing mice. The onset pressure of convulsions ranged between 70 and 120 atm in the helium-oxygen breathing animals of Lever et al. (4); it was between 90 and 110 atm in the material of Brauer et al. (1); and it was 110 atm in that of Miller et al. (7), compared to a COP of 85 ± 15.0 (S.D.) atm in the present study and 50 to 80 atm in the liquid-breathing animals of Kylstra et al. (3).

No convulsions were seen in helium-oxygen-breathing animals compressed to 122 atm by MacInnis et al. (6). It has been suggested by Brauer et al. (1) that this absence of convulsions was due to the slow compression rate (0.5 atm/min). However, the latter authors (using a different strain of mice) did see convulsions when employing the same rate of compression. They suggest that different strains of mice may differ in susceptibility to convulsions. However, it is clear from the present results that convulsions in liquid-breathing mice are promoted by rapid compression. In contrast, the COP was only moderately influenced even by a tenfold change in compression rate.

Animals exposed to pressures capable of inducing convulsions have been observed to exhibit a certain adaptation with time if kept at a constant pressure so that convulsions became less prominent (3, 8). However, a further pressure increase by only a few atm induced new convulsions. In the present experiments, it was demonstrated that proceeding to very high pressures (above 200 atm) eliminated convulsions. It is an open question if this phenomenon is due to the same mechanism as the above-mentioned adaptation.

The reductions in heart rate and breathing frequency in the animals used in these experiments apparently were due to the pressure applied. Time obviously was not an important factor because the reductions were the same irrespective of compression rates. Furthermore, animals kept at a constant, low pressure (4 atm) in liquid remained stable in breathing and heart action for many hours.

ACKNOWLEDGMENT

This work was supported by the Delegation for Applied Medical Defense Research (Proj. no. U59/1971).

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LOCOMOTOR ACTIVITY AND OXYGEN CONSUMPTION IN SHALLOW AND DEEP SEA INVERTEBRATES EXPOSED TO HIGH HYDROSTATIC PRESSURES AND LOW TEMPERATURE

A. G. Macdonald

High hydrostatic pressure is an important factor in deep diving physiology. At pressures greater than about 20 atm many different kinds of experimental animals exhibit abnormal and increased motor activity. At higher pressures severe muscular spasms or convulsions are seen. The evidence indicates that pressure elicits these activities by a direct effect on excitable tissue. We may infer that at the molecular level equilibria and rate constants are shifted in accordance with molar volumes (11); in particular, slight changes in the association-dissociation equilibria between enzymes and ligands, in structural components of the cell and even in solutes probably contribute to the disordered functioning of excitable tissues.

To physiologists interested in animal life at great depths in the oceans the high pressure hyperexcitability phenomena are as important as they are to diving physiologists who seek to extend man's depth range for free diving. Study of the hyperexcitability phenomena in both marine animals and experimental mammals is only at the stage of description and definition. This paper attempts to contribute to this area of physiology in three ways. First, the locomotor changes observed when a marine crustacean is subjected to high hydrostatic pressure are described and new experimental data which provide quantitative measures of some of the phenomena are reported. The response of a deep sea crustacean to high pressure is then described and, finally, the similarity between the responses to pressure of marine invertebrates and experimental mammals, including primates, is noted.

A Shallow Water Animal at High Pressure—*Marinogammarus marinus*

The amphipod *Marinogammarus* is a convenient sea shore animal whose tolerance to high pressure may be compared to that of small deep sea crustacea, which are the only living deep sea animals currently available for experimental work (Fig. 1). *Marinogammarus* exhibits three levels of locomotor response when subjected to increments of 50 atmospheres hydrostatic pressure at 5-minute intervals (16). The summary given here applies over the

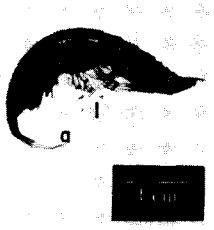

FIG. 1. Marine crustacean. *Marinogammarus marinus:* shallow water, colour grey-brown. *a*, antennae; *l*, crawling limbs; *p*, pleopods.

temperature range 3-13°C with the effects being manifest at lower pressures at the lower temperatures.

The type I response occurs over the range from 20 to 150 atm and involves increased normal and abnormal movements. At the lower pressures, crawling and swimming by means of pleopods are enhanced (Fig. 1). At intermediate pressures movement is fast and jerky; above 100 atm the body is thrown into periodic violent spasms in the dorsal-ventral plane.

The type II response occurs at 200 atm. The animals' movements are rapidly inhibited but recover in a few minutes after decompression.

The type III response follows rapid compression to 500 atm. The animals are immobilised and require much longer to recover from a 15-minute exposure to 500 atm than do animals exposed to 200 atm for many hours.

OXYGEN CONSUMPTION—TYPE I AND TYPE II RESPONSES

An increase or decrease in locomotion should be reflected in the animal's oxygen consumption which might, therefore, provide a measure of the type I and II responses. Comparable Q_{O_2} measurements may be carried out on deep sea animals at sea and, in fact, preliminary measurements have already been attempted.

Groups of *Marinogammarus* were placed in the high pressure respirometer shown in Fig. 2. The respirometer consists of a conventional oxygen electrode mounted in a Perspex chamber of 30 ml volume. This contained sea water which was stirred at constant speed by means of a paddle and shaft passing through the pressure vessel end closure. The stirring did not affect the animals at atmospheric pressure.

High pressure was applied by pumping liquid paraffin into the dead space of the apparatus and was measured by a calibrated Bourdon tube gauge. The results of typical experiments at atmospheric and high pressure are shown in Fig. 3. The results of experiments in which 100 atm was applied either rapidly or slowly and in which 200 atm was applied only rapidly are given in Table I. From these preliminary data a number of conclusions are clear.

At atmospheric pressure *Marinogammarus* respires oxygen at a fairly constant rate until a P_{O_2} of approximately 0.06 ata is reached. Rapid compression to 100 atm causes a 45% increase in respiration not seen when pressure is applied slowly. The increase in respiration fades after 1 hour at pressure. Rapid compression to 200 atm causes a sudden and severe reduction in respiration which is sustained for 4 hours or more (type II response). The variable but low rate of oxygen consumption at 200 atm cannot be distinguished from that

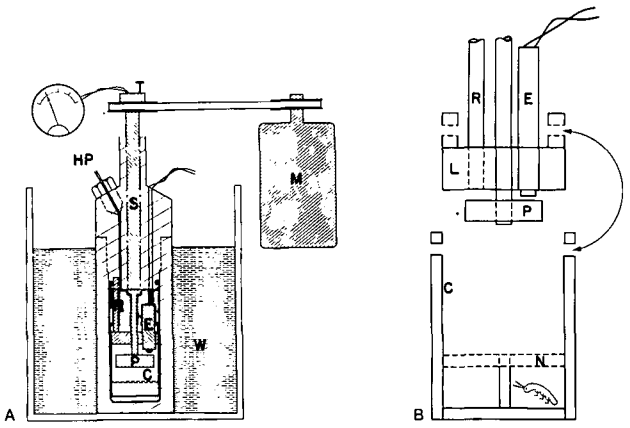

FIG. 2. High pressure respirometer.

A. *M*, variable speed motor and drive belt; *T*, tachometer, which allowed a constant rate of stirring to be maintained; *S*, rotating shaft, with details of the rotary seal omitted; *P*, paddle within the respirometer chamber *C* which is suspended on the rod *R*. *E*, oxygen electrode with electrical feed-throughs to the exterior of the vessel. *HP*, high pressure capillary connexion to the pressure vessel which is shown with wide-spaced cross hatching. Narrow-spaced cross hatching shades those components already listed. Fine stippling shows the interior dead space filled with liquid paraffin. Sea water fills the respirometer chamber *C*. The bore of the pressure vessel is 5 cm and it stands in a water bath *W*.

B. Details of the respirometer. A symbolic animal occupies the lower part of the chamber and is confined by nylon gauze, *N*. Arrows indicate the alignment of screw holes. The lid of the respirometer, *L*, seals the chamber with a sliding fit. Other labels the same as in A.

measured in spot tests with exoskeleton scraped free of tissue or with animals killed by exposure to 40°C for 2 minutes. This, together with the fact that *Marinogammarus* is able to survive in oxygen-free sea water for several hours, precludes the use of respiratory measurements as a criterion for life in long exposures to 200 atm.

The animal's anaerobic capacity also brings into question the use of oxygen consumption measurements to quantify the type I response. Accordingly attempts have been made to measure the type I response by other means.

LOCOMOTOR PERFORMANCE AT 100 ATM, 3°C (TYPE I RESPONSE)

Animals were mounted singly in a small observational pressure vessel and confined within a Perspex (plastic) cylinder. Movement of the animal during a 3-minute period of observation was recorded manually on a chart recorder. The sum of individual periods of movement is expressed as a percentage of the total 3 minutes of observation and referred to as "activity." This criterion is obviously limited. It ignores any bending movements of the animal which yield no progression and it takes no account of the force or speed of swimming or crawling. On the other hand the criterion is appropriate to the very obvious changes in activity which are induced by 100 atm and is a reasonable measure of motor output to the limb and dorsal muscles with which the crawling and swimming movements are performed.

After being placed in the pressure vessel an animal required several hours to settle down to a characteristically inactive state, after which pressure was raised to 100 atm in two 50-atm

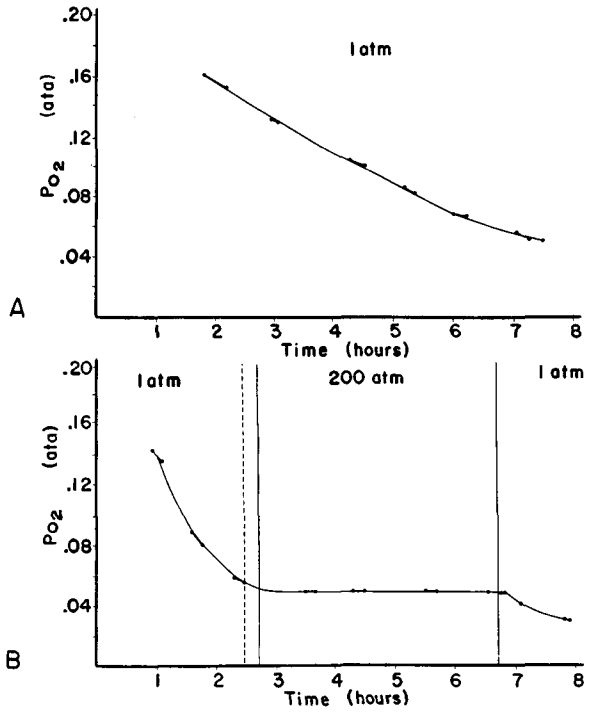

FIG. 3. Oxygen consumption of *Marinogammarus marinus* at **A**, 1 atm, and **B**, 200 atm. The partial pressure of oxygen in sea water is plotted on the vertical scale. Changes in the activity of oxygen caused by pressure (7) are ignored. The vertical lines denote pressure changes with the dashed line showing the start of stepwise compression. The oxygen electrode was continuously polarised but readings were normally taken at intervals by running the paddle motor for 10 minutes. The pairs of points correspond to the initial and final value obtained from each 10-minute reading. Five animals were used in **A** and 10 in **B**. Temperature 3°C.

steps with a 5-minute interval, or more slowly in 6-atm steps at 4-minute intervals. Figure 4A shows the change in activity following rapid compression to 100 atm. Pressure was held constant for 2 hours during which time activity subsided from an initial 60% to a normal level. In other experiments pressure was returned to 1 atm after only 20 minutes, causing an abrupt reduction in activity (Fig. 4A). In any particular individual the change in activity is closely linked with change in pressure; in favourable cases the animal's activity was seen to change simultaneously with the pressure decrease.

Slow compression causes a lesser rise in activity (to 30%) which then declines to near normal levels after 1 hour at 100 atm (Fig. 4B). Note that half the activity elicited during slow compression is apparent at a quarter of the final pressure.

The result of experiments in which a very slow rate of compression was used is given in Fig. 4C. In these experiments compression started soon after placing the animal in the pressure vessel and proceeded to increase smoothly to 100 atm over 5½ hours. The curve in Fig. 4C shows how the gradual decline in the animal's activity is only slightly disturbed by the increase in pressure. Spasms were seen at 85 atm under these conditions, whereas during compression to 100 atm over a 1-hour period they were typically seen at 50 atm.

TABLE I

MARINOGAMMARUS MARINUS: Q_{O_2} AT 3°C AT HIGH HYDROSTATIC PRESSURE[a]

		Pre-compression (over two-hour period)	At Pressure (first hour)	At Pressure (second hour)	After Decompression
Rapid compression to 100 atm (50-atm steps at 5-minute interval) 5 animals per experiment	1	0.059	0.077	0.042	—
	2	0.072	0.081	—	—
	3	0.050	0.105	0.045	—
	Mean relative rate	100 %	145 %	71.6 %	—
			During increase in pressure	First hour at 100 atm	
Slow compression to 100 atm (6-atm steps at 4-minute intervals taking 1 hour overall) 5 animals per experiment	1	0.093	0.093	0.050	—
	2	0.088	0.075	0.044	—
	3	0.045	0.043	0.035	—
	Mean relative rate	100 %	93.4%	57 %	—
			Over a 4-hour period at pressure		
Rapid compression to 200 atm (50 atm steps at 5-minute intervals) 9 or 10 animals per experiment	1	0.053	0.005		0.024
	2	0.040	0.013		—
	3	0.090	0.013		0.040
	4	0.070	0.002		0.015
	Mean relative rate	100 %	12 %		41%
Control experiments	1	0.018			—
Heat-killed animals	2	0.014	0.010		After 14 hours at 200 atm 0.008
Exoskeleton	1	0.016			
Sea water only	1	No consumption of oxygen detectable			
Animals at 1 atm 5 per experiment	1	(0.064) constant over first 3 hours then declining to 56%			
	2	(0.053) constant over first 3 hours then declining to 41%			

[a] The figures are ml O_2 at NTP/gm wet wt/hr. Relative rates of oxygen consumption, uncorrected for extraneous oxygen consumption, ignore errors due to changes in Q_{O_2} with declining P_{O_2}.

Although *Marinogammarus* responds to small increases in hydrostatic pressure as part of its natural behaviour, the activity shown in Fig. 4B may be distinguished from such behaviour. First, the threshold of the pressure sense in *Marinogammarus* is about 3 atm (4 ata). This pressure elicits antennal movements and some normal swimming or crawling, neither of which increases during slow compression in the 3-10 atm range. Second, the activity scored in Fig. 4B comprises jerky locomotion with spasms increasingly apparent at the higher pressures.

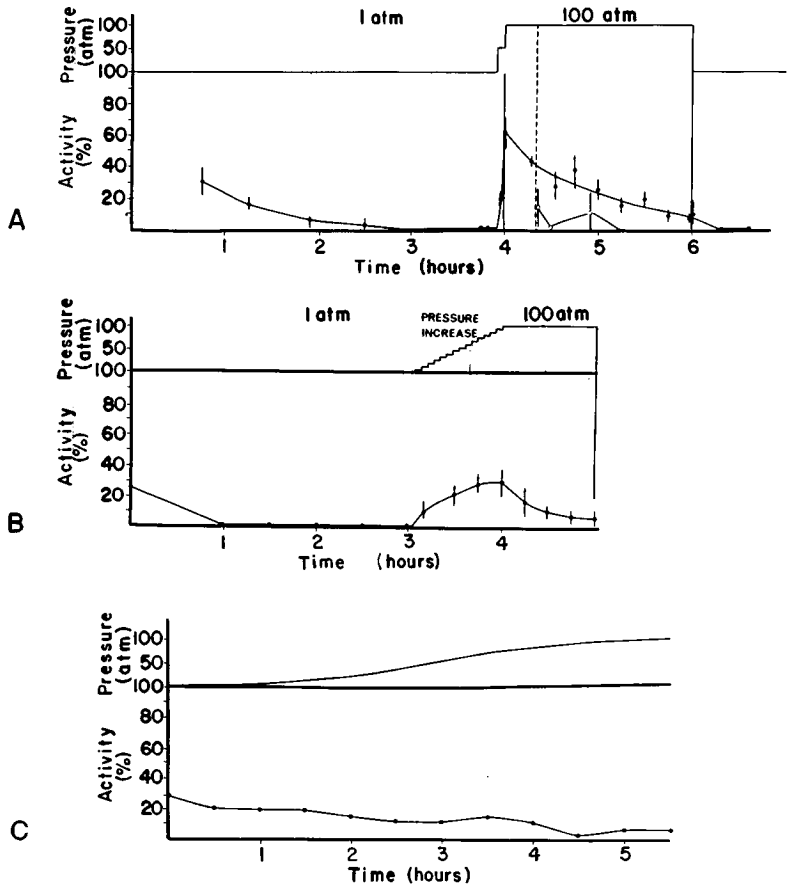

FIG. 4. Changes in the locomotor activity of *Marinogammarus marinus* at 3°C during sudden and gradual compression to 100 atm. Activity, defined in the text, is plotted on the vertical scale with hydrostatic pressure in atm plotted separately above it.

A. Upper curve, pressure increases in two steps and is held constant for 2 hours. Lower curve, activity declines after setting up the experiment but rises with the application of pressure. Solid points refer to animals held at pressure for 2 hours. Circles refer to animals which were decompressed after 20 minutes, as shown by the vertical dashed line. Each point is the mean of not less than six experiments, each involving one animal ± the standard errror. The decline in activity following decompression at 20 minutes is highly significant.

B. Upper curve, pressure increases in approximately 6-atm steps to 100 atm and is held constant for 1 hour. Lower curve, as in A.

C. Upper curve, pressure increases at the rate shown over a period of 5½ hours. Lower curve, as in A, but without a preliminary settling down period. The mean level of activity at 100 atm (5½ hours) is not significantly different from zero, but the activity at 3½ and 3 hours after the start of compression is significantly above zero. Smooth compression was achieved by heating a connected pressure vessel.

LOCOMOTOR PERFORMANCE AT 200 ATM, 3°C FOR LONG PERIODS (TYPE II RESPONSE)

Figure 5 shows the decline in the movement of the pleopods in *Marinogammarus* subjected to a pressure of 200 atm, reached by way of 50-atm steps at 5-minute intervals. Although inhibition of both the movement of the pleopods and the animal as a whole is severe, Fig. 5 shows that the degree of inhibition progresses little with time. Figure 6 shows

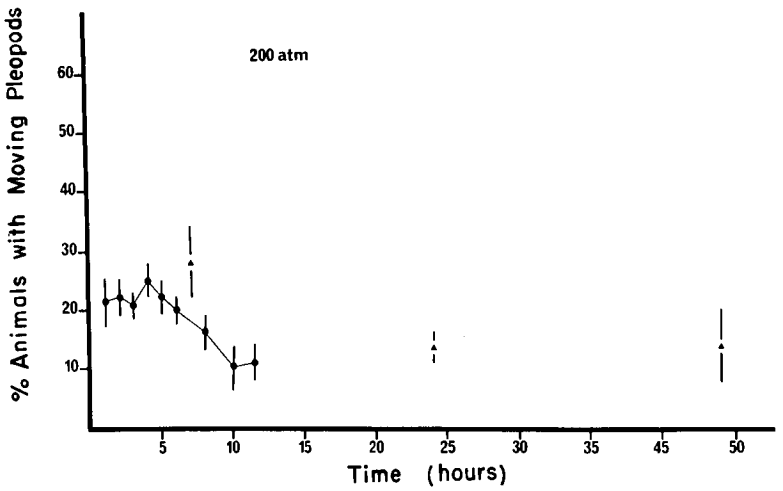

FIG. 5. The effect of prolonged exposure to 200 atm on movement in *Marinogammarus marinus* at 3°C. Groups of 10 animals were confined in a pressure vessel; the number with moving pleopods is plotted against the time of exposure to 200 atm. Solid points refer to animals confined in 100 ml sea water (16) and triangles to animals kept in flowing sea water. In the latter case each point refers to not less than 3 experiments ± the standard error.

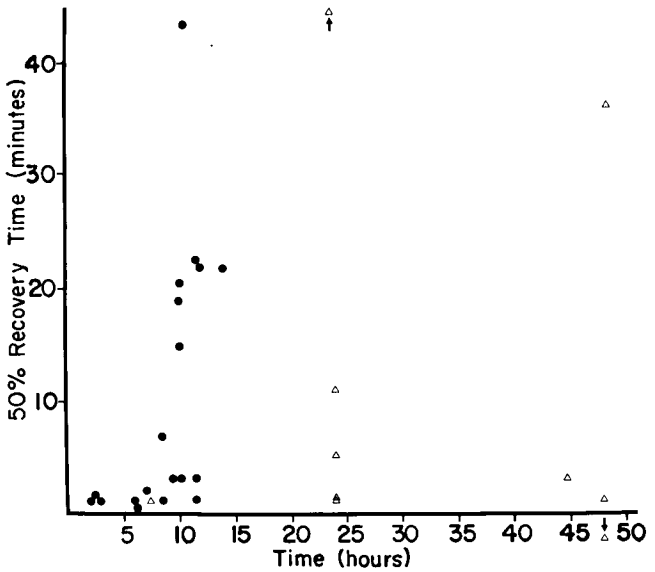

FIG. 6. Recovery of *Marinogammarus* from the inhibitory effects of 200 atm at 3°C. The time in minutes, for half a group of 10 animals to recover some movement of the pleopods at atmospheric pressure following decompression from 200 atm is plotted on the vertical scale. The duration of exposure to 200 atm is shown on the horizontal axis in hours. Solid points refer to animals which were confined in 100 ml sea water (16), triangles to animals which were maintained in flowing sea water. The arrow at the top of the figure points to a recovery time of 160 minutes. The arrow at the bottom of the figure refers to the failure of the animals to recover in one experiment.

the recovery of groups of 10 individuals which were held at 200 atm for various periods. The first set of points was obtained from animals confined in a limited volume of water (16), but more recently animals have been held in a high pressure aquarium with sea water flowing through the vessel, either continuously or discontinuously, at constant pressure. Even after 48 hours at 200 atm the pleopods of some animals recover rapidly in certain experiments. Typically, the recovery of the pleopods precedes a more general recovery. Thus 200 atm depresses activity in a highly reversible way; it remains to be seen if these phenomena are confined to *Marinogammarus* or are of more general occurrence. Low temperature may be important, in which case a comparable experiment with a hypothermic, liquid-breathing mammal would be an interesting study.

The activity data and the respiratory data show some agreement. At 200 atm the animals are immobilised and their Q_{O_2} is reduced to 12%, a maximal figure which ignores errors mentioned in Table I. The rate of oxygen consumption increases after decompression from 200 atm. After rapid compression to 100 atm both activity and oxygen consumption show a return to near normal levels over a period of 2 hours, and both are affected less by slow compression than by rapid compression. The most important discrepancy between activity and Qo_2 is that seen during slow compression to 100 atm.

To a first approximation the effect of pressure on respiration seems to be dominated by the locomotor activity of the animals, but pressure may also affect aerobic metabolism directly. Measurements of the oxygen consumption in a variety of intact cells, tissues and whole animals subjected to high pressure have been carried out separately by Fontaine (9), Macdonald (17), Teal (26), Kono (14), Ponat (22), and Fenn and Boschen (8). The general conclusion is that aerobic metabolism is not particularly sensitive to pressure, and in intact animals it is less sensitive than the animal as a whole. This area requires much more detailed study.

A Deep Sea Animal at High Pressure—*Gigantocypris mülleri*

The ostracod *Gigantocypris* has its centre of distribution at a depth of approximately 1500 m but may occur at a depth as shallow as 500 m. It therefore lives at pressures of between 50 and 150 atm. The temperature at such depths in the Atlantic Ocean is typically in the range of from 10°-2°C. The animal is spherical in shape, with adults attaining a diameter of 1.5 cm (Fig. 7). Apparently healthy individuals have been recovered from deep plankton tows and studied at sea.

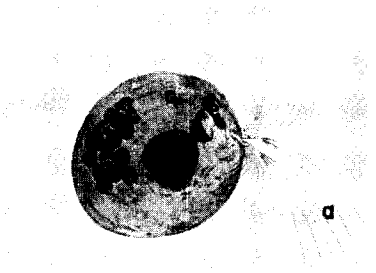

FIG. 7. Marine crustacean. *Gigantocypris mülleri*: deep sea, colour red. *a*, antennae; *c*, carapace; *e*, eye. (From 10.)

Locomotor Activity at High Pressure, at 3°C

Table II sets out the locomotor performance of *Gigantocypris* at selected pressures and 3°C (16). At atmospheric pressure the animals show vigorous swimming and retain a constant level of activity at 3°C for several days. Their failure to demonstrate pressure hyperexcitability and their sustained activity at 200 atm for 8 hours is, therefore, of considerable interest.

TABLE II

The Pressure Tolerance of *Gigantocypris mülleri* at 3°C[a]

Pressure (atm)	Number of Expts	Total Number of Animals	Change in Locomotor Activity During Compression	Change in Locomotor Activity at Pressure
200 (50-atm steps at 5-min intervals)	4	17	None	None. Longest experiment 8 1/4 hr; shortest experiment 1 hr
300 (as above)	4	16	None below 200 atm; 3 of 4 experiments showed decrease in activity at pressures higher than 200 atm	Reduced level of activity sustained for 4 hr. In one experiment activity declined thereafter to feeble paddling motions which were observed for a total of 7 1/4 hr
400 (as above)	3	11	None below 200 atm; activity reduced at pressures higher than 200 atm	Severe reduction in activity after 30 min
500 (68-atm steps at 1-min intervals)	5	12	None below 200 atm; activity reduced at pressures higher than 200 atm	11 out of 12 individuals stationary at 15 min

[a]From (16).

It appears that *Gigantocypris* is an animal adapted to life at a pressure of not more than 200 atm and whose excitable tissue is insensitive to the effects of lesser pressures which, in all other comparable shallow water animals, elicit hyperexcitability phenomena. Two qualifications must be added. The absence of pressure hyperexcitability in *Gigantocypris* may be due to the presence of light in the experiments (the animal normally lives at very low light intensities), or it may be related to adaptation to low temperature or to the decompression experienced during recovery. There is no evidence to support any of these possibilities and some observations run counter to them, but further work with this animal is obviously highly desirable.

Oxygen Consumption at High Pressure

Some measurements of the oxygen consumption of *Gigantocypris* at pressure have been carried out and described by Teal (18). Figure 8 shows that adults are not affected by

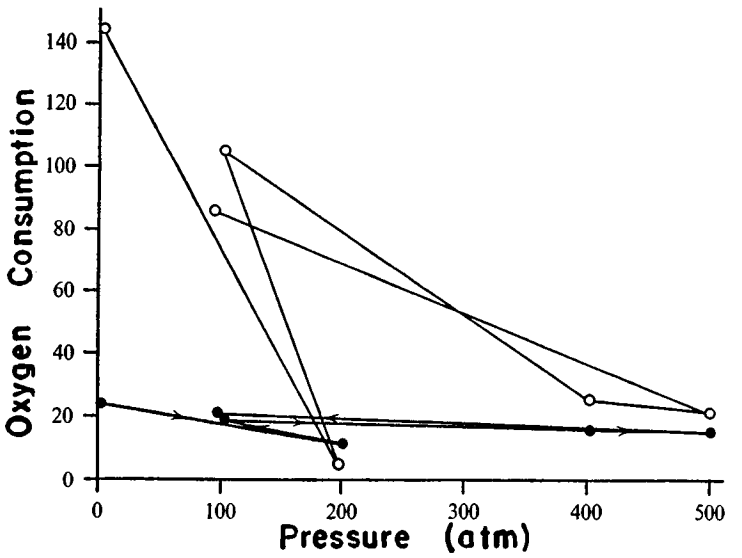

Fig. 8. Oxygen consumption of *Gigantocypris mülleri* at high pressure. (From 18.) Oxygen consumption in mm^3 O_2/gm/hr. See text.

pressure whereas the young are. Significantly, the young frequent shallower water than do adults.

The temperature and pressure coefficients of the heart rate have also been measured (16). A pressure of 200 atm decreases the heart rate to approximately 70% of the rate at atmospheric pressure. The hearts of five individuals have been observed, of which two showed arrhythmia at 1 atm. Both were restored to a steady beat at 100 atm. It would be premature to conclude that the heart of *Gigantocypris* has a special requirement for a moderate hydrostatic pressure although this possibility is a strong one.

Animals which live deeper in the sea must possess more extreme adaptations to pressure, and their physiological investigation presents an exciting challenge. The recovery of such animals under ambient conditions is a highly desirable prerequisite to many physiological experiments. Equipment which is capable of recovering planktonic animals (of a size only slightly smaller than *Gigantocypris* or *Marinogammarus*) from great depths at ambient pressures and temperatures has been developed and described (16, 20). Measurements of the oxygen consumption and the activity of animals from great depths should be possible with this equipment, but the availability of research ships capable of operating in the deep ocean is a most serious limitation to its use.

Prevalence of Pressure Hyperexcitability Phenomena

Regnard (23) was probably the first to observe the effect of pressure on intact animals in the 1880s. Table III summarises some selected observations from a variety of sources.

Moderate pressures disturb the coordination of unicellular and multicellular animals which normally live at atmospheric pressure. In aquatic animals the rapid application of

TABLE III

High Pressure Hyperexcitability Phenomena in Selected Animals

Animal	Neuromuscular System	Rate of Compression	Observations and Comments
I Protozoa *Paramecium, Tetrahymena* (freshwater)	None—cilia coordinated by unknown mechanisms	—	Pressure (30-400 atm) reduces activity but increases activity of other protozoa (13, 21)
II Coelenterata *Cyanea capillata* (marine, jelly fish)	Nerve-net	—	Excised sub-umbrella tissue, beating rhythm increased by pressures up to 200 atm, diminished at higher pressures (6)
III Arthropoda Crustacea (i) Amphipoda *Marinogammarus marinus* (sea shore)	Segmental ganglia; few efferent motor neurones; peripheral inhibition (27)	18 atm/hr to 50 atm/5 min intervals 3°-13°C	Movement increased when greatly compressed at 100 atm/hr but little at 18 atm/hr. Spasms at 50 atm (100 atm/hr) or 85 atm (18 atm/hr), at 3°C. 200 atm inhibitory (16, also this paper)
Parathemisto spp. (shallow water, open ocean)		50 atm/5 min 3°-13°C	Similar to above (18)
Cyphocaris anonyx (deep water, approximately 500 m)		50 atm/5 min 3°C	100 atm stimulates but 200 atm not inhibitory (19)
(ii) Ostracoda *Gigantocypris mülleri* (deep water, 1,500 m)		50 atm/5 min 3°C	No hyperexcitability at 100 atm; no inhibition at 200 atm (16, also this paper)
IV Vertebrata Acanthopterygii (i) Teleostei *Anguilla rostrata* (shallow water fish)	Typical vertebrate organization	24 atm/hr	Tremor threshold 57 atm; convulsion threshold 105 atm (2)
(ii) Mammalia Rodentia rat (Wistar)		188 atm/hr 18 atm/hr	Tremor threshold 37 atm Tremor threshold 107 atm (5)
Primates *Saimiri sciureus* (squirrel monkey)		24 atm/hr	Tremor threshold 33 atm; convulsion threshold 62 atm (3). Slower compression raises threshold slightly
Homo sapiens		Stepwise compression	Tremors in fingers paticularly marked after a compression step above 20 atm (1)

pressure may cause a simultaneous increase in activity and, in the case of *Marinogammarus*, rapid decompression can cause an equally rapid reduction in activity. The search for the neurophysiological basis of pressure hyperexcitability should be guided by this characteristic and by the accommodation demonstrated in Fig. 4. Pressures of less than 200 atm lower the threshold in the squid axon but not in the motor nerves of *Bufo marinus* (24, 25). Both types of nerve show modified action potentials at pressure, with the falling phase in particular being extended. Muscle is profoundly affected by pressure both at the level of excitability and in the contraction mechanism (4).

In animals which require a gaseous environment the link between the application of pressure and subsequent changes in activity is more tenuous. However, liquid-breathing mice demonstrate abnormal muscular activity at pressures which elicit similar activity in mice breathing gas (15). Carefully controlled experiments demonstrate hyperexcitability phenomena in a variety of mammals and leave little doubt that pressure per se is a major if not exclusive cause of the effects seen (3). The similarities between crustacea at 3°C and mammals at 37°C are surprising. Differences in temperature may, in part, account for this. Table III is but a brief summary of our superficial knowledge. It suggests that the excitable tissues in those animals which undertake significant vertical migrations or which inhabit the deep sea have undergone adaptative changes, and if humans wish to follow suit they have a comparable difficulty to overcome.

Summary

The pressure hyperexcitability phenomena seen in the shallow sea crustacean *Marinogammarus marinus* are detectable at 25 atm and distinguishable from its behavioural response to 3 atm. Compression to 100 atm causes an increase in activity, but slow compression has less effect than rapid compression. Spasms are seen at about 50 atm when pressure is raised gradually to 100 atm over an hour. Compression to 100 atm over 5½ hours increases activity very slightly and causes body spasms at 85 atm. The increased locomotor activity following rapid compression to 100 atm is associated with an increased Q_{O_2} which subsequently declines. Activity also declines at pressure. Inhibition of locomotion at 200 atm is associated with a large decrease in Q_{O_2} and is rapidly reversed after decompression.

Gigantocypris, a deep sea crustacean which lives at 150 atm, fails to show hyperexcitability when exposed to rapid compression. At 200 atm its activity is unaffected but at higher pressures it is diminished. The Q_{O_2} of adults is also unaffected by short-term exposure to 100 and 200 atm.

The similarity between the pressure hyperexcitability phenomena seen in crustacea and vertebrates, including primates, is briefly considered.

Appendix

Practical Notes on the Use of Oxygen Electrodes to Measure the P_{O_2} of Oxygen Dissolved in Water and Subjected to High Hydrostatic Pressure

Both membrane-covered electrodes (12) and naked "high-speed" oxygen electrodes (17) have been used at high pressure, the latter at pressures up to 1,000 atm.

Membrane electrodes show diminished current output at pressure, probably because the permeability of the plastic membrane through which oxygen diffuses is reduced. There is no evidence that pressures below 1,000 atm exert a significant effect on the electrode reaction. Spot tests, carried out in collaboration with Dr. J. Teal on an oxygen electrode of the type described in (12) but used without its plastic membrane, showed no effect when 500 atm was applied or removed and a surprisingly stable current output was found (Fig. 9).

In the present study a Beckman oxygen electrode was found to give an adequate performance. Figure 10 shows the effect of 200 atm on the current from an electrode at 3°C measuring a P_{O_2} of approximately 0.2 ata in sea water. Note the slight overshoot after

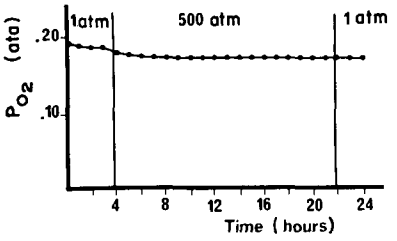

FIG. 9. The effect of 500 atm on the polarographic current obtained from an oxygen electrode lacking a plastic membrane and immersed in sea water. The partial pressure of oxygen in sea water at 25°C is shown on the vertical scale, ignoring changes in activity due to pressure. The decline in the P_{O_2} is an artefact of electrode poisoning. The electrode is described in (12) and was polarised 0.75 V relative to the reference anode, in the absence of a plastic membrane or electrolytes other than those in sea water. Constant stirring was provided by a magnetic device and the calibration was satisfactory.

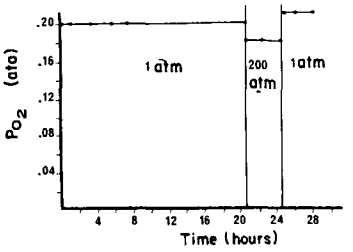

FIG. 10. The effect of 200 atm on the current obtained from an oxygen electrode employing a plastic membrane and immersed in sea water. Details as in Fig. 3.

decompression. The reduction in current output at high pressure is related to the P_{O_2} in the manner illustrated in Fig. 11. Long-term stability is good (Fig. 10).

The high pressure respirometer illustrated in Fig. 2 was shown to be proof against gas exchange with ambient liquids and free from serious temperature changes during stepwise compression or rapid decompression.

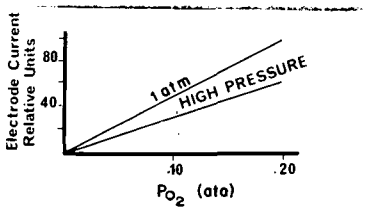

FIG. 11. Effect of high hydrostatic pressure on the calibration of an oxygen electrode immersed in water, diagrammatic.

ACKNOWLEDGMENT

This work was supported by a grant from the Natural Environment Research Council. I am grateful to the authors of Figs. 7 and 8 for permission to reproduce their figures.

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PRESSURE REVERSAL OF NITROUS OXIDE-INDUCED CONDUCTION FAILURE IN PERIPHERAL NERVE

S. H. Roth, R. A. Smith and W. D. M. Paton

The antagonism or reversal of anaesthesia was originally reported by Johnson and Flagler in 1950 (4). They observed that the depressive effects of anaesthetics on both luminescent bacteria and tadpoles could be reversed by hydrostatic pressure. The spontaneous swimming motion of tadpoles, depressed by anaesthetics, was restored to normal activity when the tadpoles were exposed to pressures of 150 to 350 atmospheres. This effect of pressure reversing the effects of certain anaesthetic agents has been observed by others on whole animals (7), luminescent bacteria (2, 3), model membrane systems (5), ion transport (5) and nerve fibers (18, 19). Recently a quantitative study on whole animals demonstrated that the anaesthetic effects of many gases could be reversed with pressure (11). Pressures required for complete reversal were in the range of 100 to 150 atmospheres. The hypothesis put forward from these findings suggested that anaesthetics expand the lipid component of membrane, thereby modifying the dimensions of these regions, and thus producing anaesthesia. This hypothesis has been proposed by earlier workers (12, 17); recently it has been shown that anaesthetics at surgical concentrations expand erythrocyte membranes by 0.4% (16), in excellent agreement with the calculated expansion of 0.5% (7).

The interpretation of the results of whole animal experiments is difficult due to the complexity of the central nervous system (CNS). It was desirable, therefore, to examine the effects of an anaesthetic and the reversal or antagonism of these effects by pressure on a simpler system, such as an isolated peripheral nerve. It was previously shown that various anaesthetic gases could produce conduction block in peripheral nerves. The purpose of this study was to examine the dose-related effects of nitrous oxide on the conduction of an electrically evoked response of a frog sciatic nerve, and the ability to reverse or antagonize these effects by pressure. For comparison, experiments were performed on the whole animal.

The present results indicate that nitrous oxide produces dose-related conduction failure in peripheral nerve at a higher concentration than required for anaesthesia in the whole animal, but that the effects both in nerve and animal are reversed with the application of pressure.

Methods

The sciatic nerves from pithed frogs (*Rana temporaria*) were dissected from the point of

connection to the vertebrae to a point past the knee including both peroneal and tibial nerves. Side branches were trimmed to a length of approximately 4 mm from the main trunk. All adherent loose connective tissue was carefully removed and, in most cases, the perineural sheath was left intact. A length of silk suture was tied to each end of the nerve, and the nerve was immediately placed in frog Ringer's solution previously oxygenated with a gas mixture of 95% O_2 and 5% CO_2.* The nerves were kept in continuously aerated Ringer's solution for a minimum of 1 hour prior to testing. The Ringer's solution was buffered at pH 7.0 with tris buffer. A nerve was suspended on platinum electrodes mounted in a specially designed "Perspex" chamber. The ends of the nerve were fixed to miniature clamps by short lengths of suture with little if any tension applied to the nerve. Sufficient humidity was maintained by placing absorbent cotton wool soaked in Ringer's solution along the bottom of the chamber.

Continuous monitoring of temperature at close proximity to the nerve was possible utilizing a miniature glass bead thermistor fitted approximately 10 mm above the nerve. This nerve chamber was then placed into a cylindrical stainless steel pressure vessel (capacity 300 ml). One end of the pressure vessel or "bomb" was fitted with a "Perspex" disk through which brass studs were threaded and sealed. These studs permitted electrical connections to be made from the nerve chamber inside the pressure vessel. The interior of the bomb was maintained at near room temperature (21 ± 1°C) with the aid of a "jacket" through which water circulated from a thermostatically controlled water bath.

Square-wave impulses from a stimulator via an isolation transformer were transmitted to the spinal end of the nerve using two platinum electrodes spaced less than 5 mm apart, cathode being distal to avoid anode block. A stimulus of 0.05 msec duration was delivered at a frequency of 1 per second throughout the experiment. Supramaximal stimulation was approximately three times greater than the voltage required for a maximum response. The evoked-action potential was recorded using platinum electrodes connected directly to the input of a dual-beam Tektronix 502 oscilloscope, set at a sensitivity of 2 to 5 mv/cm and at a sweep rate of 1 msec/cm, triggered by the stimulator. Tracings of action potentials were photographed on film using a Grass Kymograph camera. Utilizing two pairs of recording electrodes spaced about 10 mm apart, measurements were made between the peak heights of the two action potentials. Knowing the inter-electrode distance, conduction velocity was calculated. There appeared to be no significant difference in the results of experiments measuring the response from extracellularly recorded compound action potentials or monophasic action potentials (distal electrode placed on crushed portion of nerve).

Prior to each experiment the bomb was flushed with oxygen for 1 minute and then sealed. Control measurements were made after several minutes had passed before the addition of the anaesthetic gas. The nitrous oxide was slowly introduced into the bomb to the desired pressure, during which temperature change was negligible (less than 1°C). An interval of 15 minutes was allowed for equilibration (1).

Measurements were made on threshold, height of action potential, and conduction time. After block was established, helium was added to increase the total pressure. All measurements following the addition of helium were restricted to periods when transient changes in

*The nitrous oxide, oxygen, and oxygen-carbon dioxide mixture were obtained from British Oxygen Company, England. Helium (99.9%) was supplied as mineral helium by Air Products, England. The gauges used were cross-calibrated within 1% of each other. The following were used in this study: 0-150 p.s.i., Siebe Gorman Company Limited, Chessington, Surrey; 0-5,000 p.s.i., Barnet Instruments Limited, Barnet, Herts.

temperature (up to 5°C), due to increasing pressure, had subsided and temperature had returned to control level.

Experiments to demonstrate the effects of pressure alone were performed in a similar manner. After flushing the pressure vessel with oxygen, helium was added to the desired pressure and measurements were made after 15 minutes. Measurements were also carried out after prolonged exposure to pressure (i.e., 3 hours), and there appeared to be no difference.

The method employed for determining effective doses of nitrous oxide on whole animals (frogs) and the reversal by pressure was similar to that reported by Lever et al. (7) using the rolling response as a test for the righting reflex.

Results

Whole Animal (Frog) Studies

The righting reflex as measured by the rolling response as a function of nitrous oxide pressure, i.e., doses of anaesthetic, is shown in Fig. 1. The rolling response of several frogs at each concentration of anaesthetic is expressed as a percentage. The dose of nitrous oxide required to decrease the ability of the righting reflex in frogs by 50% (ED_{50}) was 0.84 ± 0.06 atm, determined by probit analysis. This value compares well with that reported for newts of 0.69 ± 0.10 atm (11); however, it was slightly lower than the dose required for mice of 1.5 atm (10).

Figure 2 shows the rolling response of frogs anaesthetized with 1.22 atm of nitrous oxide as a function of pressure. This dose of anaesthetic decreased the rolling response to 10%, and with helium to a total pressure of 80 atm, the response was raised to about 80%.

Studies on Isolated Nerve Preparation

The action potential amplitude was chosen as a measure of the response of the nerve to electrical stimulation. These measurements expressed as a percentage of control were made

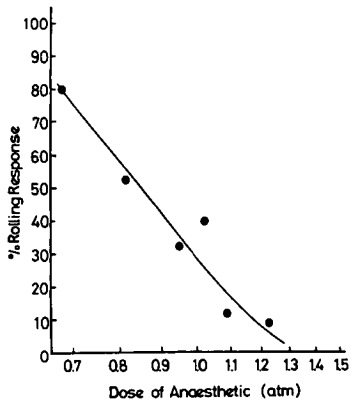

Fig. 1. Dose-response curve for nitrous oxide on frogs measured at 20°C by the rolling response.

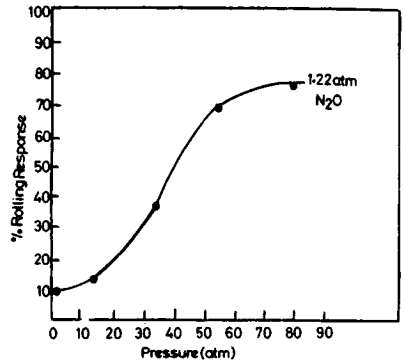

FIG. 2. Rolling response of frogs at 20°C anaesthetized with 1.22 atm nitrous oxide as a function of pressure.

at supramaximal stimulation at various doses of anaesthetic to obtain a dose-response curve. The results are shown in Fig. 3. The ED_{50} for nitrous oxide, the dose required to depress the amplitude of the action potential by 50%, on the frog sciatic nerve was calculated to be 4.86 ± 0.40 atm by probit analysis. Complete conduction block was obtained at approximately 10 atm, in agreement with the reported value for rat sciatic nerve by Carpenter (1).

Examples of compound action potentials of a frog sciatic nerve evoked by a supramaximal stimulation at control, two doses of anaesthetic (4.8 and 6.8 atm), and finally at a total pressure of 68 atm which included the anaesthetic at 6.8 atm, are shown in Fig. 4. The addition of nitrous oxide resulted in a decrease in the amplitude and a broadening of the action potential. At 6.8 atm of nitrous oxide almost complete failure in conduction was observed. An increase of pressure to 68 atm by helium resulted in both the height and the duration of the action potential being returned almost to control levels.

A stimulus-response curve for frog sciatic nerve was obtained by measuring the height of the electrically evoked action potential at increasing stimulus voltages. The results of this

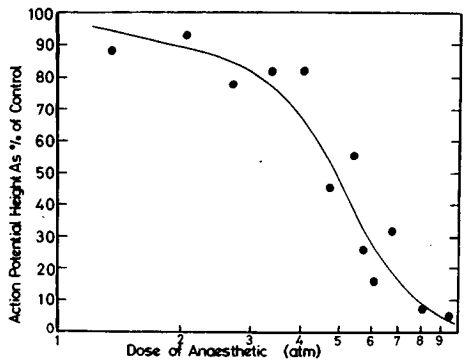

FIG. 3. Dose-response curve for nitrous oxide on frog sciatic nerve at 20°C measured as height of action potential expressed as % of control.

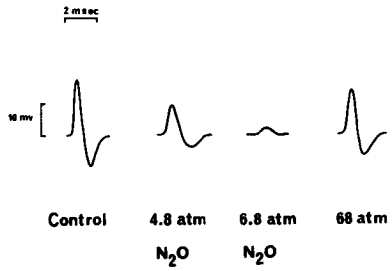

FIG. 4. Evoked-action potentials at supramaximal stimulation of frog sciatic nerve (20°C) at control, 4.8 and 6.8 atm nitrous oxide and at a total pressure of 68 atm which includes 6.8 atm of anaesthetic.

type of experiment are shown in Fig. 5. Stimulus-response curves for a single preparation at control (1 atm oxygen) and three doses of nitrous oxide (2.7, 4.1 and 5.4 atm) show the dose-related shift in threshold to electrical stimulation, and the depression of the maximal response as a function of anaesthetic dose. At a dose of 5.4 atm nitrous oxide, the pressure was increased to 68 atm total with helium. This resulted in the return of the response, at maximal and greater stimulation, to the control level. However, the threshold to electrical stimulation was shifted only slightly to a lower voltage.

The effect of pressure alone on the evoked-action potential at supramaximal stimulation is shown in Fig. 6. No apparent alteration in either amplitude or duration was observed to be of significance. The results of pressure per se on stimulus-response curves are shown in Fig. 7. Both threshold to electrical stimulation and maximal height of action potential are unaffected at a pressure of 68 atm. This was shown to be the case in earlier studies (8).

Figure 8 demonstrates the effect of pressure on the evoked response of an anaesthetized frog sciatic nerve. At a dose of 5.4 atm nitrous oxide, the action potential height was depressed by approximately 50%. When pressure was applied, the response was enhanced

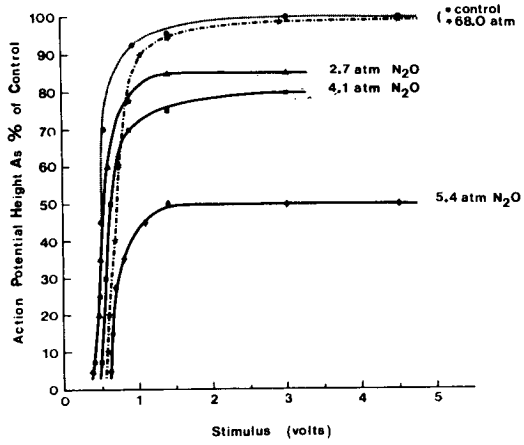

FIG. 5. Stimulus-response curve for frog sciatic nerve at 20°C at control, three doses of nitrous oxide (2.7, 4.1 and 5.4 atm) and at a total pressure of 68 atm which includes 5.4 atm of anaesthetic.

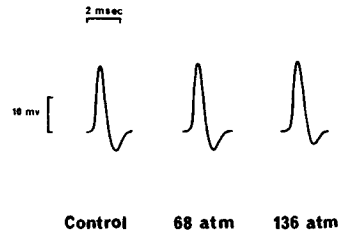

FIG. 6. Evoked-action potentials at supramaximal stimulation of frog sciatic nerve (20°C) at control, 68 and 136 atm pressure of helium.

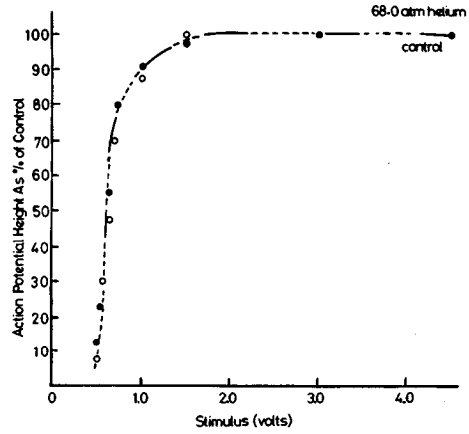

FIG. 7. Stimulus-response curve for frog sciatic nerve at 20°C at control and 68 atm pressure of helium.

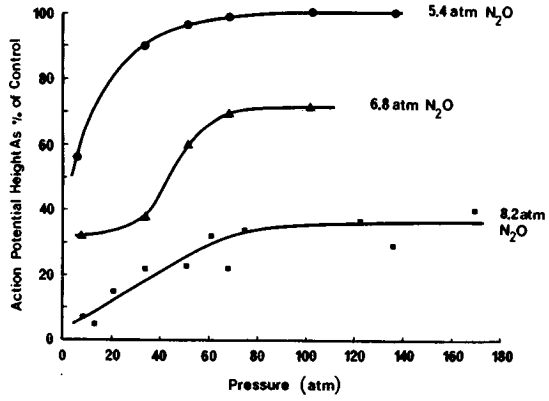

FIG. 8. Height of action potentials at supramaximal stimulation as % of control of frog sciatic nerve anaesthetized with 5.4, 6.8 and 8.2 atm nitrous oxide measured at 20°C each as a function of pressure.

as a function of pressure, and the effect of the anaesthetic was completely removed by approximately 100 atm. At a larger dose of anaesthetic, i.e. 6.8 atm of nitrous oxide, the response was about 35% of control, but in this case pressure increased the response to 70% of control. A dose of 8.2 atm of N_2O resulted in a depression of the height of the action potential to 10% of the control, and pressure increased the response to only 30% of control. The conduction velocity of the control was calculated to be about 20 m/sec, which is within the range for the preparation (9). At a dose of 5.4 atm of nitrous oxide, the conduction velocity decreased to 70% of the control. An increase of pressure to a total of 34 atm with helium augmented the velocity to 76%. A further increase in pressure to a total of 51 atm caused a further increase to 91% of the control. At higher doses of nitrous oxide, i.e. 6.8 and 8.2 atm, the decreased conduction velocity was never brought back to more than 80% of the control.

Discussion

Comparison of Effective Doses

That a general anaesthetic such as nitrous oxide can induce conduction failure in a frog sciatic nerve lends further evidence to the observed nonspecificity of action of anaesthetics (14). The data in this study support earlier findings that the dose of anaesthetic required for the whole animal is far less than the dose necessary to induce conduction failure in an isolated nerve preparation (1). The explanations that have been suggested for this difference in concentration are based on the observations that concentrations of various anaesthetics that block transmission in synaptic pathways are ineffective in depressing transmission in axons (6); the difference in the effect of a given dose of anaesthetic is dependent on fiber size and myelination (13). Although the two responses are apparently different, i.e. righting reflex in whole animal and electrically evoked conduction in peripheral nerve, they are related: both are dependent on neural transmission, and the effect of an anaesthetic is probably a result of a perturbation of membrane components of the excitable tissues.

Pressure Reversal of Anaesthesia

It is well established that the active site of anaesthetics is hydrophobic in nature (10). An extension of the Meyer-Overton lipid solubility theory of anaesthesia was first proposed by Mullins (12) to include the volume occupied by the anaesthetic molecules. An increase in volume of the hydrophobic site has been postulated as a mechanism of action of anaesthesia, and referred to as the Critical Volume Model (11). This hypothesis assumes that anaesthesia occurs when a critical volume is reached in the membrane as a result of the absorption of a lipid-soluble anaesthetic agent. At this critical volume, sufficient perturbation exists to interfere with the normal membrane function. That pressure can antagonize the effects of an anaesthetic implies the involvement of volume expansion in anaesthesia.

If the critical volume model is valid, the expansion for each system can be calculated by using the methods of Miller et al. (11). The calculated expansion is dependent upon the physical properties of the anaesthetic agent and of the active site. This includes the partial molar volume, solubility and dose of the anaesthetic agent in the membrane. However, these data are unknown; therefore, a model to represent the active site (membrane) is necessary for these calculations to be made. One model that has proved to be successful experi-

mentally is benzene (11). If we assume that benzene has characteristics similar to the active site, then a calculation of the volume increase in benzene by the absorption of nitrous oxide at the effective doses would approximate the expansion in the membrane. The calculated expansions at the ED_{50} dose, and at a dose of nitrous oxide that reduces each response to 10% of control are shown in Table I.

The expansion calculated for the active site in whole animal (CNS) was 0.56% at the ED_{50} dose. This agrees with the value for newts, 0.46%, and also is in agreement with the 0.4% expansion of red blood cell membranes by general anaesthetics at surgical concentrations (16). The expansion calculated for the peripheral nerve was 3.2% which also agrees with the values reported for the 2-6% expansion by local anaesthetics measured on red blood cell membranes (15). The anaesthetic effects of an ED_{50} on both the whole animal and the nerve preparation were completely reversed by similar pressures of 50-70 atm. However, the reversal from a 10% response in both systems was not of the same order when pressure, to a total of 68 atm, was admitted. This difference in response level is shown in Table I (column 4). The expansions at 10% effects and at the responses at 68 atm of pressure are shown in columns 3 and 5 of Table I. Although the pressure of the system was limited to 170 atm, it can be seen from Fig. 8 that the maximum amount of reversal appeared to have been attained.

The compressibilities in the two systems were calculated (column 6), and compared to the isothermal compressibility of benzene (20). The value for the nerve fiber is unrealistic and suggests that benzene is either not a good model for the nerve, or that reversal by pressure on the two systems is a result of different mechanisms. The sites of action of anaesthetics may not be similar in the two systems, and pressure may affect one more readily than the other.

We may conclude that although pressure reverses nitrous oxide-induced conduction failure in peripheral nerve, it appears not to be comparable to the reversal of anaesthesia as observed on newts (7) and tadpoles (4).

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TABLE I

RESPONSE, EXPANSION AND COMPRESSIBILITIES OF FROGS, ISOLATED NERVE PREPARATION AND NEWTS IN THE PRESENCE OF N_2O AND PRESSURE.

	ED_{50} ± S.E. (atm)	Calculated Expansion at ED_{50} (%)	Calculated Expansion at 10% Effect (%)	Response Level from a 10% Effect at 68 atm Total Pressure (%)	Calculated Expansion at Level of 10% Effect at 68 atm Total Pressure (%)	Calculated Compressibility β (atm^{-1})	Isothermal Compressibility for Benzene β (atm^{-1})
Frog rolling response	0.84 ± 0.06	0.56	0.78	75	0.47	7.3×10^{-5}	9×10^{-5}
Frog sciatic conduction blockade	4.86 ± 0.40	3.23	6.65	34	4.11	40×10^{-5}	9×10^{-5}
Newt rolling response	0.69 ± 0.10	0.46	0.72	95	0.19	10.5×10^{-5}	9×10^{-5}

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AN OXYGEN- AND PRESSURE-SENSITIVE ENZYME: Na-K ADENOSINETRIPHOSPHATASE

S. F. Gottlieb, G. J. Koehler and L. V. G. Rhodes

Possible disturbances of cellular physiology are of major concern to those involved in high pressure environmental (HPE) activities. Oxygen toxicity, inert gas narcosis, dysbarism and pressure are the prime restrictions hindering man in his explorations and exploitations of the earth's hydrosphere as well as in the therapeutic applications of hyperbaric medicine (3, 15). In HPE, physiological and biochemical changes (PBC) occur which exert a continuum of effects. These range from subtle and possibly negligible effects to which man may acclimate, to those which are profound and debilitating, thereby prohibiting useful function and threatening permanent injury (3-6, 15, 18, 20, 21, 23, 26, 31, 39, 41).

Several techniques are used to prevent the emergence of the more overt, untoward changes related to work at high pressures. In diving operations oxygen toxicity is mitigated or avoided by the use of artificial gas mixtures with appropriate low oxygen tensions, whereas in hyperbaric medicine O_2 toxicity is essentially avoided by controlling exposure time (15, 18). Inert gas narcosis and dysbarism effects may be minimized, if not avoided, by using appropriate inert gases as oxygen diluents (15, 17), as well as by adhering to appropriate compression and decompression schedules, alone or in combination with drugs. The subtle chemical and physiological changes occurring in high pressure exposures stress homeostatic mechanisms and thereby partially diminish the organism's ability to acclimate to further internal or external environmental stressors. The effects noted may be due to pressure per se, or to the inhalation of specific gases used as oxygen diluents, or to a combination thereof. It therefore becomes imperative to explore the influences due to O_2, potential oxygen diluents and pressure to help discover and delineate possible limiting effects of exposure and to explore resultant organismal acclimatization to PBC resulting from HPE. Studies of subtle changes resulting from pressure exposure have important practical and heuristic implications in the biomedical sciences.

In exploring mechanisms of oxygen toxicity initial efforts in the present study were directed to the effects of oxygen on membrane phenomena. It was postulated that toxic effects of oxygen may be explained by a fundamental effect of O_2 on cell membranes, and that the differences in P_{O_2} required to produce the effects may be due to differences in structure and composition of various membranes (11).

While testing the above hypothesis, it was found that the time for oxygen inhibition of two membrane-associated phenomena—sciatic nerve conduction and sodium active transport across frog skin—can be depicted by an exponential equation (11, 16):

$$P = bT^{-K}$$

where,

P is the P_{O_2} at which the phenomenon is observed;
b is the y intercept;
T is the time for the response to occur.

Many phenomena concerning oxygen toxicity can be represented by an exponential function which describes the inverse relationship between P_{O_2} and exposure time before the symptoms of O_2 toxicity become manifest. Dickens (12) calculated equations for predicting O_2 toxicity based on data of others. Rearrangement of his equation results in the same general equation derived here from nerve and frog skin studies.

Although O_2 adversely affected these two membrane-associated phenomena—nerve conduction and sodium active transport—it was not determined if the observed effects were due to a direct action of O_2 on cell membranes or to an indirect effect resulting from O_2 disruption of cellular energy-supplying mechanisms. Since O_2 had been shown by others to inhibit energy-supplying mechanisms in a variety of tissues (15, 18, 20), these studies were shifted from the organ and cellular level of biological organization to the molecular level by studying the effects of O_2 on the functioning of a specific component of membranes; it was hoped that the inhibition could be described by the same general equation derived from nerve and frog skin studies (11, 16).

Two theories concerning molecular mechanisms of O_2 action are oxidation of sulfhydryl groups (SH) and lipid peroxidation; the SH oxidation theory is predominant (20). These theories help delineate specific membrane components that should be studied to distinguish between direct and indirect effects of O_2 on membrane function.

The Na^+-K^+ activated ATPase is thought to be a membrane-bound enzyme involved in sodium active transport; it has been reported to be a lipoprotein which requires an SH group for functioning (37). Thus this enzyme lends itself to a "double-barreled" attack by O_2: on the lipid moiety and on the SH moiety of the enzyme. Therefore it was of interest to learn the effect of different O_2 tensions on Na^+-K^+ activated ATPases. For these studies enzymes isolated from rat intestine and beef heart were used.

Method

Intestinal ATPase was obtained from the mucosal lining of fasted, Sprague-Dawley rats according to the technique of Cinti (10). DNA of the intestinal preparation was determined by Burton's (9) modification of the Schmidt-Tannhauser method. Total organic nitrogen of the intestinal preparation was determined by micro-Kjeldahl: 10.0 mg of lyophilized tissue contained 1.92 mg DNA and 1.5 mg total organic nitrogen. Total intestinal ATPase and Na-K-Mg ATPase activity and phosphate were assayed using the methods of Bonting et al. (7). Statistical analyses of the data obtained with the intestinal preparation were performed

on an IBM Systems 360 computer using GDAS language (25). The "intestinal" data were graphed according to the computer analysis as to which curve best fit the data. Best fit was determined by best accountability of total variance, with the additional constraint that all transformed variables selected for the fit were statistically significant ($P \geqslant 0.95$). Transforms were chosen according to the criteria of Beale (2, 19). Best fit was found using the techniques of Garside (14). Generalized analysis of variance was used to determine overall effects of treatments. Individual effects were statistically investigated using Duncan's multiple range test.

Cardiac Na-K-ATPase was prepared according to the method of Matsui and Schwartz (29). Phosphate and protein were analyzed for by the methods of Martin and Doty (28) and Lowry et al. (27), respectively.

Enzymatic activity was carried out in a volume of 1.6 ml (intestinal ATPase) or 1.1 ml (cardiac ATPase) in 10-ml beakers to provide a large surface area to volume ratio, thereby minimizing the diffusion barrier. Exposure to experimental gases was done using two pressure chambers simultaneously. They were pressurized either with the same gas at two different pressures, or with different gases at the same total pressure. Simultaneous with the pressure experiments, a 1 atmosphere absolute (ata: 0 pounds per square inch gauge [p.s.i.g.]) control was run. Individual experiments were performed in quadruplicate which, according to preliminary studies, was needed to ensure a beta error of no greater than 10%. Each pressure studied was repeated two to four times. Prior to pressurization, the pressure chambers were flushed with the experimental gas at a rate of 5-7 L/min for 3 minutes. Pressurization was controlled at the rate of 2.7 lbs/sec. U.S.P. grade O_2, N_2 or He was supplied from commercially available high pressure cylinders.

Rapid decompression was employed to help cool the reaction vessels and to permit rapid addition of cold 0.5 ml of 10% trichloracetic acid (TCA) to the intestinal ATPase reaction vessels (RV) and 1.0 ml 10% TCA to the cardiac ATPase RV.

Constant temperature was maintained by circulating water from a constant temperature reservoir through 1/4 inch soft copper tubing wrapped around the outside of the chambers; temperature difference among the three chambers did not vary by more than 0.5°C. The copper coil-encircled pressure chambers and 1 atm incubator were wrapped with 2 inch-thick fiberglass insulation and an outer layer of aluminum foil. Temperature in all chambers was monitored by inserting thermistors in dummy reaction vessels containing 1.6 ml H_2O and read on a Telethermometer.* Reaction media attained 37°C within 5-10 minutes, starting from room temperature.

Results

The data depicted in Fig. 1 indicate that at 25°C activation of intestinal Na-K ATPase (iATPase) occurs in the pressure range of 15-60 p.s.i.a. O_2 (1-4 ata) with the peak at 45 p.s.i.a. O_2 and also in the range of 200-1,000 p.s.i.a. O_2; in the pressure range of 90-200 p.s.i.a. O_2 there is a marked inhibition. At 37°C (Fig. 2), the effects of O_2 on the enzyme activity noted at 25°C are not as marked, at least in the lower pressure ranges. The slight activation observed in the range of 45-70 p.s.i.a. O_2 is not significantly different from the 100% control; inhibitory effects observed at 15 and 90-135 p.s.i.a. O_2 are statistically significant. To resolve the question as to whether the observed effects at two different

* Yellow Springs Instrument Company.

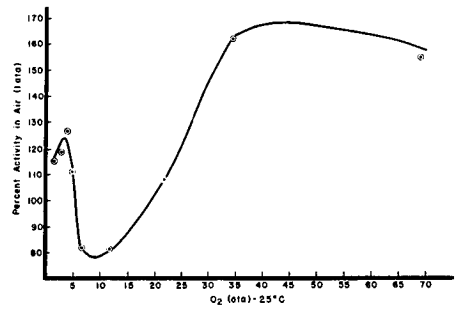

FIG. 1. Ata oxygen at 25°C versus intestinal Na-K-Mg ATPase activity. Percent activity calculated relative to 1 ata air control.

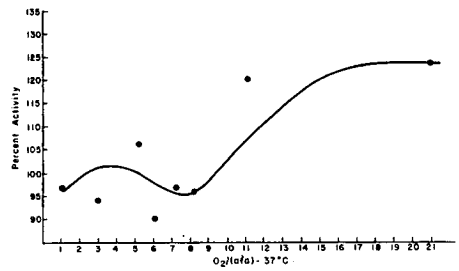

FIG. 2. Ata oxygen at 37°C versus intestinal Na-K-Mg ATPase activity. Percent activity calculated relative to 1 ata air control.

temperatures were due to O_2 or to pressure per se, similar experiments were performed using N_2, a diatomic gas having a molecular volume similar to O_2. Data in Figs. 3 and 4 show that qualitatively similar changes occur under N_2 as under O_2 when the incubation temperature is increased from 25° to 37°C. At 25°C the effects of N_2 resembled those of O_2; in all cases of activation and inhibition the effects of N_2 were less intense than those of O_2 at

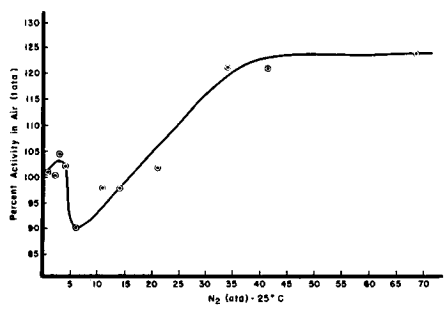

FIG. 3. Ata nitrogen at 25°C versus intestinal Na-K-Mg ATPase activity. Percent activity calculated relative to 1 ata air control.

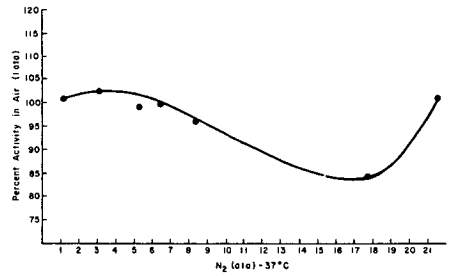

FIG. 4. Ata nitrogen at 37°C versus intestinal Na-K-Mg ATPase activity. Percent activity calculated relative to 1 ata air control.

25°C. In contrast to O_2, N_2 at 37°C in the range of 15-105 p.s.i.a. does not significantly inhibit iATPase activity. Activation of iATPase in the range of 30-80 p.s.i.a. is barely significant. A progressive increase in the degree of inhibition of iATPase is noted in the P_{N_2} range of 120-285 p.s.i.a. Nitrogen pressures greater than 285 p.s.i.a. tend to activate iATPase.

Qualitative and quantitative differences in effects of O_2 and N_2 on iATPase activity at equal gas pressures raised the question of pressure controls and also suggested that the effects of O_2 on iATPase involved factors other than those due to pressure, molecular volume or diatomic structure.

The question of a proper pressure control was partially resolved by repeating the above experiments using helium, a monoatomic, chemically inert gas to increase ambient pressure. Except for two differences, equal pressures of He mimic the effects of N_2 at 37°C (Fig. 5): 1) a greater stimulation of iATPase was observed with He in the range of 15-90 p.s.i.a. than with N_2 in the range of 45-60 p.s.i.a.; and 2) the increase of inhibition noted in the range of 105-260 p.s.i.a. was greater with He as compared to N_2.

The question as to whether the effects of O_2 were specific for iATPase or resulted from the crude homogenate preparation was answered, in part, by repeating some of the above experiments using a more purified Na-K ATPase obtained from beef heart (cATPase). The data (Fig. 6), obtained at 37°C using cATPase, are reminiscent of the data obtained at

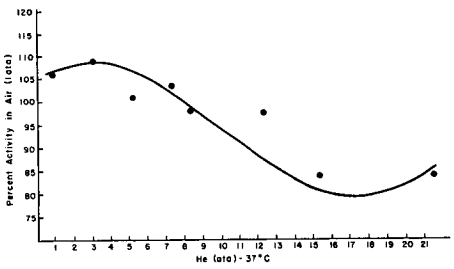

FIG. 5. Ata helium at 37°C versus intestinal Na-K-Mg ATPase activity. Percent activity calculated relative to 1 ata air control.

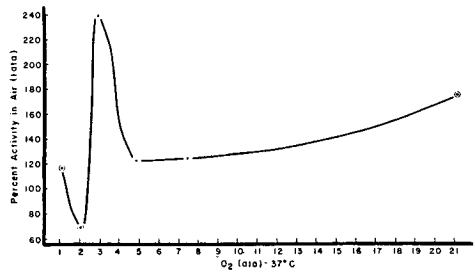

FIG. 6. Ata oxygen at 37°C versus cardiac Na-K-Mg ATPase activity. Percent activity calculated relative to 1 ata air control.

25°C with iATPase. Oxygen appeared to exert inhibitory or activating effects on cATPase depending on P_{O_2}. At 15 p.s.i.a. there was, approximately, a 10% increase in activity which at 30 p.s.i.a. O_2 declined to about a 30% inhibition compared to 15 p.s.i.a. air controls. At 45 p.s.i.a. O_2 there was a marked increase in enzyme activity which returned to normal 1 ata air control at 75 p.s.i.a. O_2; a gradual rise in activity occurred as the P_{O_2} increased from 75 to 315 p.s.i.a. The data obtained with He on cATPase are depicted in Fig. 7. Enzyme activity in the range of 15-115 p.s.i.a. He increased; in the range of 115-315 p.s.i.a. He, cATPase activity decreased, although it still was greater than the corresponding 15 p.s.i.a. air controls.

The role of substrate protection in failing to find O_2 inhibition of either iATPase or cATPase was investigated by exposing iATPase to various O_2 tensions in substrate-free medium for 2 hours, followed by incubation in the presence of ATP for 2 hours at 15 p.s.i.a. air. The results obtained (Fig. 8) were qualitatively similar to those obtained when iATPase was exposed to different pressures of oxygen in the presence of ATP.

Discussion

There are several aspects of the data that require discussion: the effects of oxygen, the responsitivity to O_2 of iATPase and cATPase, the effects of pressure, and the effects of the

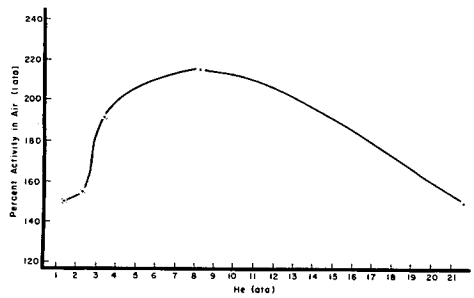

FIG. 7. Ata helium versus cardiac Na-K-Mg ATPase activity. Percent activity calculated relative to 1 ata air control; 37° C.

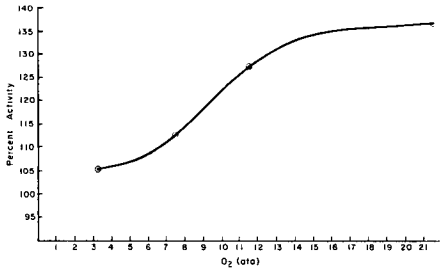

Fig. 8. Substrate protection: ata oxygen versus intestinal Na-K-Mg ATPase activity. Percent activity calculated relative to 1 ata air control; 37°C.

inert gases. Because of their interrelationships, the last two items are best discussed together.

Oxygen

An intriguing observation was that, contrary to what theory predicted, O_2 did not inhibit iATPase or cATPase as an overall effect, even though this enzyme has an SH group and a lipid moiety, both of which should be susceptible to O_2 inactivation. Oxygen did not inhibit enzyme function even in the absence of ATP and therefore substrate protection was not a factor. It may be concluded that O_2 did not cause significant lipid peroxidation, for, had lipid peroxides been formed, the enzyme should have been inactivated (38).

Of particular interest is the activation of ATPase by O_2 and pressure. The activation of ATPase by O_2 in the low pressure ranges is reminiscent of the activation of myosin ATPase when one-half the SH groups had been tied up with an appropriate blocking agent (24, 34, 35). The differences in iATPase responsitivity to O_2 in the range of 90-150 p.s.i.a. in the presence and absence of substrate (Fig. 2 vs. Fig. 8) may be due to substrate-induced conformational changes in iATPase around the active site (35). Whereas an excess of SH-blocking agents results in complete enzyme inactivation (24, 34, 35), excess O_2 and/or pressure yields enzyme activation.

The difference in effects on iATPase between O_2 and SH inhibitors may be explained by Skou's suggestion "that the effect of *N*-ethylmaleimide (NEM) and 2,4-dinitrofluorobenzene (DNFB) on Na-K-Mg ATPase activity is not due to their effect on SH groups as such, but to a change in the steric configuration, which is secondary to the effect on the sulfhydryl groups" (36). If O_2 is oxidizing the SH groups but not secondarily drastically affecting ATPase steric configuration, the implications arise that NEM and DNFB are exerting inhibitory effects on ATPase in two ways: 1) SH blocking; and 2) the size, shape, and charge of their respective molecules contributing to marked secondary changes in ATPase structure. This idea is supported by the observation that NEM inhibits by reacting with ATPase at two sites (1).

Our data are consistent with the idea that pressure and/or the particular physical properties of the gases induce ATPase conformational changes and/or alter volume relationships of reactants and products which function to alter enzyme kinetics but not completely inhibit ATPase function. The above concept should be kept in mind when a comparison is made of the effects of the different gases on ATPase activity.

Responsiveness of iATPase and cATPase to O_2

The responsiveness of the two ATPase preparations to O_2 and He is deserving of comment. The iATPase studies were done using a mucosal homogenate whereas the cATPase was a more refined preparation. Whether the qualitative and quantitative differences in responsiveness to O_2 and He of the ATPases (cATPase data in the lower pressure ranges were much more pronounced than iATPase) are due to the degree of purity of the preparation, organ difference (heart vs. intestine) or species difference (beef vs. rat) or a combination of these factors is unknown at present.

Effects of Pressure and Inert Gases

Helium, a monoatomic gaseous element having a small molecular volume and low molecular polarizability is generally used as a pressure control, in moderate pressure ranges, in experiments involving gases and where hydrostatic control experiments are difficult to perform. If He is a true pressure control, then cognizance must be taken of the observations that He exerted a more pronounced inhibition or activation of iATPase than O_2 or N_2 at equal pressures and, except for the range of 45-60 p.s.i.a. O_2 and above 255 p.s.i.a., He exerted a greater activating effect on cATPase than did O_2. Furthermore, any deviation in effects obtained with other gases in comparison to He must be due to the specific physical and chemical properties of these gases. In an attempt to separate direct and indirect effects of pressure on ATPase activity, the percent relative enzyme activities observed under O_2 or N_2 were divided by the percent relative enzyme activity obtained under equal pressures of He. The data, now compensated for pressure, are depicted in Figs. 9 and 10. With iATPase, 15-135 p.s.i.a., O_2 slightly inhibits activity whereas oxygen tensions greater than 135 p.s.i.a. activate iATPase. Pressure-compensated analysis of cATPase (Fig. 10) is qualitatively similar to the uncompensated data (Fig. 6). In contrast to O_2, 15-200 p.s.i.a. N_2 resulted in a nonsignificant inhibition of iATPase; at pressures greater than 200 p.s.i.a., N_2 activated iATPase (Fig. 9). The same form of analysis indicates O_2 causes a greater activation of iATPase than N_2. These data suggest that a polar diatomic molecule (O_2) exerts a greater protective effect than a nonpolar diatomic molecule (N_2) against the iATPase inhibiting effects of pressure per se.

The concept of equal thermodynamic activity (8, 13, 30) was used in an attempt to separate the effects of pressure per se from those attributable to dissolved gases. The data in

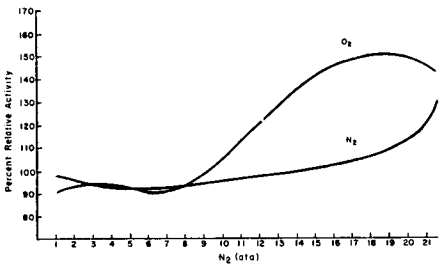

Fig. 9. Pressure-compensated nitrogen and oxygen versus intestinal ATPase activity. Percent activity calculated relative to 1 ata air control.

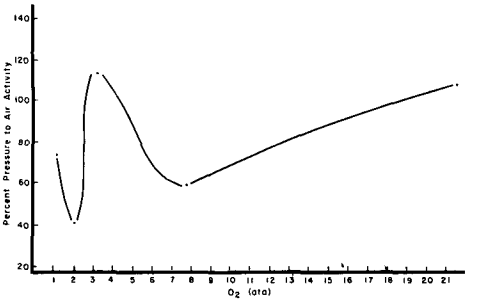

FIG. 10. Pressure-compensated oxygen tensions versus cardiac Na-K ATPase activity. Percent activity calculated relative to 1 ata air control.

Fig. 9 were replotted—i.e., the quotient obtained by dividing the percent relative activity under O_2 or N_2 by the percent relative activity obtained under an equal pressure of He was plotted against the thermodynamic equivalent for O_2 or N_2 for those pressures. The data in Fig. 11 indicate that at equal thermodynamic activities O_2 and N_2, when compensated for pressure, behave in a similiar manner. The apparent greater inhibition by O_2 in the range of 5-10 mM/L is not statistically significant. Based on this thermodynamic analysis, it appears that the difference in effects of O_2 and N_2 at equal pressures (Fig. 9) is probably due to the greater solubility of O_2 than N_2 in the reaction medium.

A second way of analyzing the effects of gases on ATPase is to obtain the ratio of the percent relative activity under O_2 or N_2 to the percent relative activity under an equivalent thermodynamic activity of He. The data in Fig. 12 indicate that the O_2 and N_2 curves are qualitatively similar. In the range of 0.8-1.6 mM/L only the O_2 inhibition is statistically significant. In the range of 3.2-8.0 mM/L both gases activate iATPase with peak activation occurring at 6.0 mM/L. The O_2 activation is significantly greater than the activating effect of N_2. This additional analysis supports the concept that a polar diatomic molecule exerts a greater protection against inhibiting effects of pressure per se when compared to a nonpolar diatomic molecule.

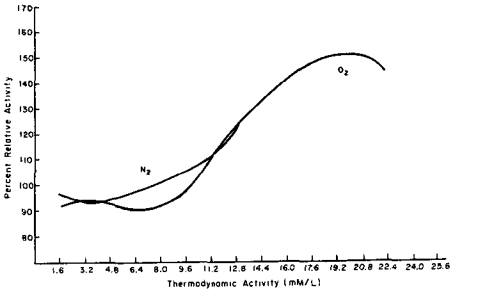

FIG. 11. Thermodynamic activity of pressure-adjusted nitrogen and oxygen versus intestinal ATPase activity. Percent activity calculated relative to 1 ata air control.

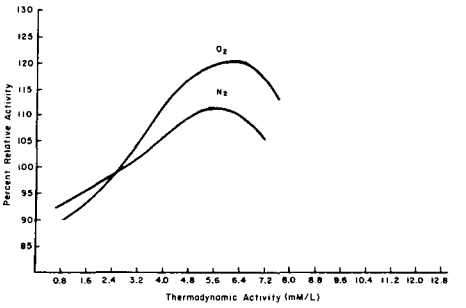

FIG. 12. Thermodynamic activities of nitrogen and oxygen versus intestinal ATPase activity under helium at the same thermodynamic activity.

SIGNIFICANCE

The original intent of this work was to study mechanisms of O_2 toxicity. Instead of the anticipated O_2 inhibitory effects that hopefully could have been described by the previously derived general equation (11, 16) both inhibitory and stimulatory effects, not only of O_2 but also of N_2 and pressure, were found. Although the ATPase data suggest that the O_2 inhibitory effects seen in nerve conduction (11) or Na transport (16) cannot be explained by direct O_2 inhibitory effects on the membrane, unless, of course, the right membrane component(s) was not examined, these data may support the suggestion we made earlier (15) that in searching for mechanisms of O_2 toxicity, one should not just be looking for enzyme inhibitory effects but perhaps one should also be looking for alterations in enzyme kinetics due to enzymatic activations. Along these lines it should be noted that Sanders and Hall (33) found an increased respiration with increased P_{O_2} in liver and cerebral hemisphere homogenates using succinate and alpha-ketoglutarate as substrates. Sanders and Hall (33) were unable to find any correlation between their previously observed (32) depressions in rat cerebral hemisphere, liver, and kidney cortex ATP concentrations with increasing P_{O_2} and irreversible changes in respiration and oxidative phosphorylation of succinate and alpha-ketoglutarate. Although there appeared to be a correlation between decreased ATP concentration of the cerebral hemisphere, liver and kidney and a reduction in succinic dehydrogenase activity at 5 ata O_2, no such correlation was seen at 1 and 3 ata O_2 for 2 hours. Perhaps the findings of increased ATPase activity at 3 ata O_2 could explain their findings of lowered ATP concentration (32) at 3 ata O_2 during a similar 2-hour time period.

The long-lasting activation of Na-K ATPase or other enzymes by O_2 may provide a biochemical basis, with all its theoretical and therapeutic ramifications, for the long-lasting improvement in cognitive functions seen in aged persons exposed to oxygen under pressure (22). Furthermore, these data may suggest that the marked improvement seen in coronary patients, treated by increased O_2 tensions in pressure chambers, may have a biochemical basis in addition to just the well-known alleviation of hypoxemia (15). Also our data may provide a biochemical explanation for the positive effects of increased O_2 tensions on intestinal paralysis (40).

The significance of the observation that the ATPases used in these experiments are pressure-sensitive in relation to understanding problems concerning compression, decompression, inert gas narcosis, etc. awaits further study.

ACKNOWLEDGMENTS

These studies were supported in part by an NIH grant GM 16761 and a grant from the Indiana Heart Association, both awarded to Sheldon F. Gottlieb.

Figures 2, 4, 5, 8, 9, 11, 12 are reprinted from *Aerospace Medicine* **43**: 269-273, 1972 with permission of the editor.

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HYDROSTATIC PRESSURE AND HEMOGLOBIN OXYGENATION

J. M. Wells

The magnitude and direction of the change in the equilibrium constant of a reaction, as a function of hydrostatic pressure, are determined by the difference in volume of the reactants and products. This may be expressed quantitatively by the equation:

$$Kp = Koe^{-\Delta VP/RT}$$

where

Kp and K_O are the equilibrium constants at pressure P and at 1 atmosphere, respectively;
ΔV is the difference in volume of reactants and products;
R is the gas constant;
T is the absolute temperature.

It is apparent that any oxygenation-linked property of the hemoglobin molecule which brings about a change in volume would render hemoglobin oxygenation-pressure-sensitive.

Circumstantial evidence for such a volume change has been available for some time. Haurowitz (7) observed through the microscope that the structure of hemoglobin crystals changed when they were exposed to oxygen, indicating a molecular reorganization during oxygenation. Likewise, both the x-ray crystallographic studies of Perutz and co-workers (13) and the dielectric studies of Takashima and Lumry (14) indicate conformational changes of the hemoglobin molecule accompanying oxygenation. Wyman (16) suggests that oxygenation leads to conformational changes involving the whole quartenary structure of protein, and that such changes alter the environment of the dissociable groups responsible for the Bohr effect in such a way as to produce the observed changes in their pKs. The same conformational change is assumed to account for the homotropic (heme-heme) interactions of the four oxygen sites.

Both the osmotic pressure measurements of Guidotti (6) and the molecular weight determinations of Benesch and Benesch (2) indicate a dissociation of the tetrameric hemoglobin molecule into subunits on oxygenation. Guidotti's model for hemoglobin oxygenation allows both the tetrameric molecules and the $\alpha\beta$ dimers to combine with oxygen, each with a different affinity for the ligand. Taylor and Hastings (15) have shown that at a given oxygen pressure, pH 6.6 and 37°C, hemoglobin in 4-molar urea solution combines with more oxygen than in the absence of urea. It has been well established that urea solutions of this

concentration dissociate hemoglobin into subunits (3, 10) Antonini et al. (1) have shown that isolated α and β chains of human hemoglobin have a much greater affinity for oxygen than the intact tetrameric molecule. Pressure-induced changes in the degree of aggregation of sickle cell hemoglobin (12) and other subunit proteins have also been observed (9, 11).

A primary step in this study was determination of the oxyhemoglobin dissociation curves of solutions of washed, lysed and filtered human red blood cells at pH 6.8 and 25°C. This low pH was used because it shifted the dissociation curve to the right. This made the determination easier, but the hemoglobin was still above the pH at which a negative Bohr effect occurs.

The dissociation curves at 100 atm were determined on solutions of 15% hemoglobin by a technique identical in principle to that used by Enns et al. (4) to measure changes in gas solubilities in water as a function of hydrostatic pressure. It involves the measurement of the equilibrium gas pressure developed across a small diameter Teflon tube immersed in the test solution at different pressures. A specially constructed and calibrated oxygen electrode was also used to measure changes in P_{O_2} in the hemoglobin solution on pressurization. Dissociation curves at 1 atm were determined by the techniques of Hemmingsen (8). Curves at 100 atm were constructed by displacing the 1 atm curve along the P_{O_2} axis to correspond to the measured change in P_{O_2} on the application of pressure. The change in percent saturation accompanying the measured change in Po_2 can be calculated from the solubility coefficient of O_2 at the respective pressures and the O_2 capacity of the solution. In this case, it turned out to be a maximum of 0.05% and was thus neglected in the construction of the curve.

Figure 1 shows the data plotted as percent change in P_{O_2} on the application of 100 atm pressure as a function of the P_{O_2} at 1 atm. Figure 2 was calculated on the basis of these data.

The dissociation curves shown in Fig. 3 (1, 340, 680, and 1,020 atm) were determined on 0.15% hemoglobin solutions in a high pressure optical cell with a spectrophotometric technique. From these curves, the Po_2 at 25, 50 and 75% saturation (p^{25}, p^{50} and p^{75}) were measured graphically and are shown in Fig. 4. The ΔV for the reaction at half-saturation was calculated over the measured pressure intervals using the equation above and was found

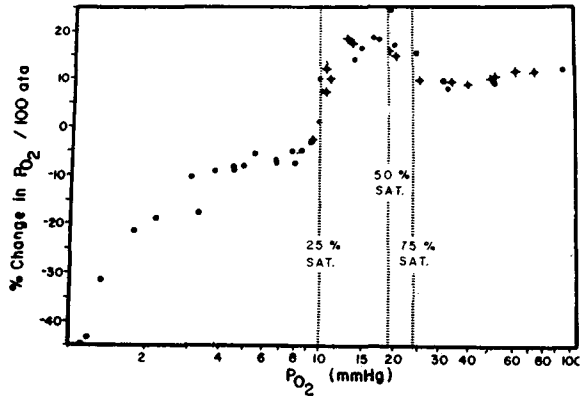

FIG. 1. Percent changes in P_{O_2} in hemoglobin solutions on the application of 100 ata pressure as a function of P_{O_2} at 1 ata.

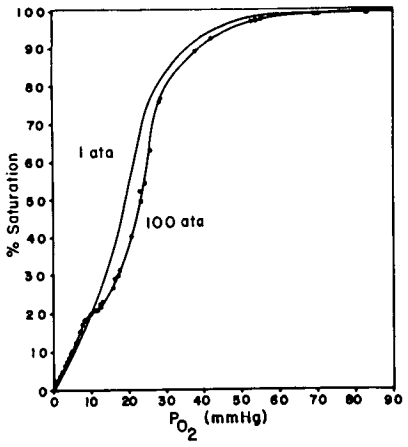

FIG. 2. Oxyhemoglobin dissociation curves of hemoglobin solutions, pH 6.8, 25°C at 1 and 100 ata hydrostatic pressure.

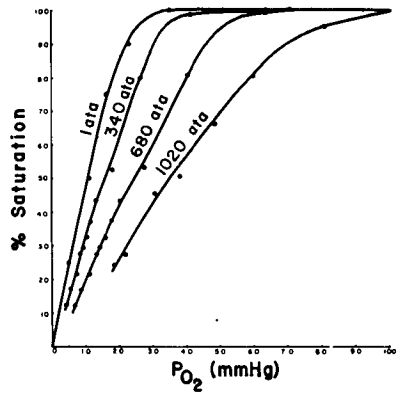

FIG. 3. Oxyhemoglobin dissociation curves of hemoglobin solutions, pH 6.8, 25°C at 1, 340, 680 and 1,020 ata hydrostatic pressure.

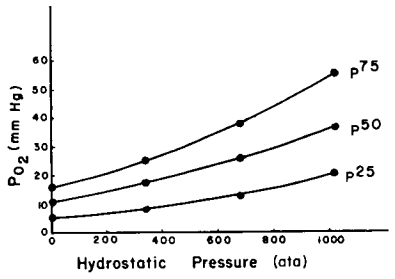

FIG. 4. Partial pressures of oxygen at 25, 50 and 75% hemoglobin saturation (p^{25}, p^{50}, p^{75}) as a function of hydrostatic pressure.

to decrease from 33.4 ml/mole between 1 and 340 atm to 26.7 ml/mole between 680 and 1,020 atm. This decrease in ΔV with increasing pressure means that one cannot calculate the effect of pressure on oxygenation equilibria on the basis of the ΔV measured at atmospheric pressure unless the compression of the components of both sides of the reaction is known at the respective fractional saturation.

Fenn (5) measured the ΔV for whole blood oxygenation at 1 atm and found no significant ΔV. The compression of whole blood was measured in this study at a high and a low P_{O_2} over a temperature range of 30°C (Fig. 5) and was found to be quite different with different degrees of saturation. This differential compression helps to explain two difficult points. The asymmetric shift of the dissociation curve may be explained on the basis of differential compression of intermediate stages of oxygenation. Likewise, on the basis of Fenn's data and the compression data derived in the present study, one would predict the observed shift to the left of the dissociation curve at low P_{O_2}. More data are needed to calculate the position of the dissociation curve at higher degrees of saturation by this method.

The observed changes in oxygenation equilibium are relatively small to be of physiological significance at the pressures to which men and gas-breathing experimental animals are presently exposed. Also, the possibility of other factors operating in vivo made it desirable to obtain arterial blood samples for analysis of oxygen content.

Several years ago, a technique for obtaining meaningful information from decompressed and foaming blood was developed at the Physiological Research Laboratory of the University of California, San Diego. Blood samples were withdrawn from catheterized animals inside pressure chambers through a very small valve into a preweighed syringe. The syringe and contents were then weighed again to determine the quantity of blood actually drawn. The contents of the syringe (gas bubbles and blood) were then injected into a Van Slyke apparatus for analysis.

This procedure was used at the University of North Carolina on Rhesus monkeys breathing helium-oxygen mixtures at pressures up to 100 atmospheres.

The helium content of blood was used as a check on the method, assuming that it follows Henry's law to these pressures. A quite linear relationship was obtained when He content was plotted against pressure. Figure 6 shows typical data obtained for a monkey breathing 0.5 atm O_2 in helium. It is apparent that whatever factors are operating in vivo, including possible respiratory resistance, they do not cause a decrease in the oxygen content of arterial blood.

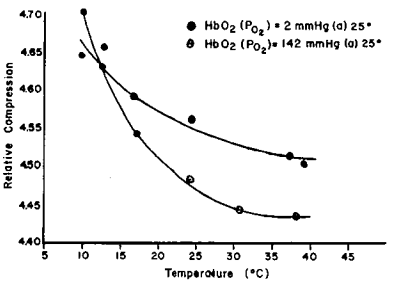

FIG. 5. Relative compression of oxyhemoglobin at two different degrees of oxygenation.

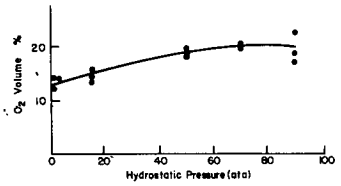

FIG. 6. Oxygen content of the blood of a Rhesus monkey breathing 0.5 ata O_2 in He as a function of pressure.

Clearly, more information is needed on the effects of hydrostatic and inert gas pressures and possible interactions of the two on hemoglobin oxygen transport at oxygen pressures commonly used during diving operations. Work presently underway suggests that a pressure-nitrogen-oxygenation interaction does in fact exist and that, at elevated nitrogen pressures, hemoglobin-oxygenation equilibrium is quite different from that at the same hydrostatic or helium pressures. Such a relationship may be of significance in the use of N_2-He-O_2 mixtures for diving or in the use of N_2-O_2 saturation diving systems.

ACKNOWLEDGMENTS

This work was supported by Research Grant GM-10521 from the U.S. Department of Health, Education and Welfare, and a Faculty Grant at the School of Medicine, University of North Carolina from the Medical Research Foundation of North Carolina.

The author gratefully acknowledges the assistance and advice of Drs. P. F. Scholander, T. Enns, and A. B. Hastings.

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PART VI. HYDROSTATIC PRESSURE*

DISCUSSION

E. B. Smith, Chairman

Dr. Smith: There are three areas for discussion, in the order of the papers presented. The first is concerned with the effect of pressure per se, or hydrostatic pressure, on the whole animal. The second is concerned with how the effects of pressure can be ameliorated by anesthetics and other pharmacological agents. Thirdly, the fundamental biochemical and biophysiological processes which might ultimately determine the pressure limit should be considered. We will conduct the discussion along these three lines.

Dr. Doff: Dr. Zimmerman, for the beautiful scanning electron microscopic photograph which you had of a cell, was your microscope arranged in such a way that you took this photograph at high pressure or was it really a decompressed cell?

Dr. Zimmerman: Actually we fixed the cells under compression in a chamber, and we do not decompress until the cells are fixed.

Dr. Gottlieb: Dr. Örnhagen, how did you measure convulsions in your preparation? Was it just a visual observation? Also, how did you distinguish between convulsions and shivering?

Dr. Örnhagen: We measured them with electromyographic recordings. We did not get any real registration with tremors or shivering, so it was tonic convulsions that registered.

Dr. Gottlieb: You pointed out the anomaly that as the body temperature increased the convulsion onset pressure increased, which is contrary to what one would predict. What is your explanation for that?

Dr. Örnhagen: We did not get any change in convulsion onset pressure with rising body temperature. We got a different type of convulsion. It was much shorter with higher body temperature. At temperatures above 31°C we did not get any real tonic convulsions at all.

Dr. Miller: This question concerns the hydraulic compression of mice. You did not actually describe what your mice were dying of.

Dr. Örnhagen: They died of cessation of breathing or cessation of cardiac activity. Primarily cessation of breathing was our end point. We stopped the compression then, and at 31°C and very high pressures we sometimes got cessation in cardiac activity or rather a drop in cardiac activity before the cessation of breathing—but mainly it was cessation of breathing with cardiac activity. If you then decompress, the respiration usually started again.

During decompression, which was usually very fast, up to 100 atmospheres per second, we found near cardiac standstill or extreme bradycardia during the decompression procedure, but when we reached pressures of about 50 atmospheres, the cardiac activity speeded up.

Dr. Smith: I want to highlight the remarkable parallel in the results obtained by pressurization during liquid breathing and in exposure to oxyhelium mixtures—about 130 atmospheres at 30°C, slightly higher pressures at lower temperatures. At the same compression rate these fit together beautifully. I think the liquid-breathing experiments indicate that oxyhelium mixtures do act on animals almost entirely through the transmission of hydrostatic pressure.

Dr. Macdonald: With certain marine organisms you can have survival in the absence of oxygen for hours, and indeed days. These animals also can survive the pressure paralysis. Those animals which are unable to sustain anaerobic metabolism die rapidly and do not show the pressure paralysis. I would like to know how the mammal tissues, which can go anaerobic, survive at the higher pressures?

Dr. Örnhagen: I have no information. I cannot tell you anything about that.

**Panelists*: S. F. Gottlieb, A. G. Macdonald, H. C. Örnhagen, S. H. Roth, J. M. Wells, A. M. Zimmerman

Dr. Lambertsen: Dr. Smith, could you elaborate further upon the comparison you just made by telling us what you personally think is any observable effect of helium, different from the effects of hydrostatic pressure, in mice at about 100 atmospheres?

Dr. Smith: The point I made was that there was, fundamentally, at a coarse level of observation, no difference between our results and your results. Within the variability that might be expected in liquid-breathing or in looking at the results overall, one sees that hydrostatic pressure and pressure transmitted through oxyhelium mixtures produced essentially the same effects.

Dr. Lambertsen: I did hear that and, having said that, would you now go on to state whether there was any detectable effect of the inert gas separate from the effect of pressure?

Dr. Smith: I would say that in the case of helium there is not a detectable effect. I would also say that was true of neon. It may be true of hydrogen, although I think there is some room for debate; with the densely heavier—more potent, if we like—other inert gases, effects are obvious.

Dr. Lambertsen: To what pressure would you be willing to make that statement—if you were personally pressurized—now?

Dr. Smith: I would be reluctant to make any statement beyond 150 and 200 atmospheres where we know the answer; it may well be that one can go to higher pressures although I think it would be unwise to forecast beyond the range where oxyhelium has been used on mammals at the present time.

Dr. Macdonald: Can we discontinue the oversimplification talking about pressure limits? The effect depends upon duration of exposure. There is plenty of evidence that minute metabolic leakages or dislocations can occur at quite moderate pressures, so you have to put some sort of time restriction on these pressure limits.

Dr. Brauer: We have plotted the rate of compression versus the convulsion thresholds for gas-compressed animals over a fairly large range of compression rates, going from about 2 atmospheres per hour to well over 2,000 atmospheres per hour. We could have done this for other indicators. This family of curves is quite continuous and, indeed, coherent. For other thresholds the slopes of these things are different. For instance, for the tremor thresholds in mice there is virtually no slope. But there is an implication in this which is pertinent to some of the things we have been discussing: namely, that if you go rapidly to some particular point where convulsions begin and then you stay at that pressure and wait—in effect what you are looking at is a gradual progression down toward slower and slower compression rates. If you were to extrapolate this logic you would predict that you would get into a region where you should not have convulsions.

This, I think, is highly compatible with the kinds of events we have seen and I think probably with some of the things that were shown for the liquid-breathing animals: that is, if you carry the animals to their first seizure and if you stop at that point, then you may never see another one again. You may see a brief episode or an episode that may last 5 or 10 minutes, with the animal progressively normalizing thereafter. If you raise the animal slightly above this, then you will see the pattern we have described before—where a second, a third and a fourth seizure may occur; but as you pluck those out they space farther and farther apart.

I am coming increasingly to the conviction that the phenomena that we are looking at are, in fact, related not to the absolute pressure but, at least in important part, to the transition from one pressure to another. I think this is compatible with the various data we have seen here and now provides, in part, an answer to the question of the differences between inert gas effects and hydrostatic effects in hydraulic compressions—in that the effects tend to be dominated by two events: first, by the compression rate (which, as you go over a wide range of compression rates, becomes quite important) and secondly, by the previous history of the animal.

We have a large amount of data now which show that monkeys that have had one convulsion, then require slightly higher and eventually quite significantly higher pressures to have another convulsion.

Dr. Dow: One could easily get the impression from this session's direction that hydrostatic pressure effects from 0 to 100 atmospheres are not going to be too worrisome to us. Perhaps this is justified, but I thought it would be appropriate to mention some work by Dr. Enright that indicates that hydrostatic pressures as low as 5 centimeters of water apparently can be detected by some organisms which have no gas phase in them and a compressibility less than sea water.

The other thing missing from this session's direction was any consideration of energy metabolism under hydrostatic pressure: whether one could really exclude the effects of oxygen. And this points up one area that Dr. Fenn has worked on; Marquis and Fenn apparently have evidence that glycolysis itself is sensitive to hydrostatic pressure.

Dr. Macdonald: First of all, the question of the acute sensitivity of some of these marine animals to such low pressures—this is an extraordinary area of sensory physiology which needs looking at carefully. The current hypothesis, which I do not believe, is that you have a potential across the pressure sensor of the animals, and this is

rather a kind of special potential. It generates, so the hypothesis states, a layer of nascent hydrogen which is the compressible phase which is the starting point of the pressure transduction going on in the animal.

The fact that Enright could not detect any great compressibility in the animal is not pertinent: only a tiny pocket of this nascent hydrogen is necessary. The author of the hypothesis, Digby, has postulated it in such a way that you cannot possibly get at it. It is on the order of a few angstroms in depth and it occurs on the outside surface of an animal like a crab. The question is, how do you identify this layer?

Dr. Kent: I think both Dr. Halsey and I would take exception to Dr. Smith's comment that liquid-breathing mice are not much different from the gas-breathing mice in both helium and neon breathing atmospheres. The study had mice at relatively low temperatures in comparison with their normal body temperatures. Also, with build-up of CO_2 there is an anesthetic effect with elevated P_{CO_2}s—the range certainly in humans of 300 or 400 millimeters of mercury (less than half an atmosphere)—and I think we have some studies which also bear some relationship to this.

Therefore it is very difficult to state that liquid-breathing mice are essentially not much different from mice breathing a gas phase. The differences in physiology that have to take place, at least as studies have been described, must be considered.

Dr. Örnhagen: I am not ready yet to say that there are no differences between helium/oxygen-breathing and liquid-breathing mice until liquid-breathing at 37° has been accomplished.

Dr. Hempleman: When you go through the literature the two main physical variables that seem to be important in compression effects are the pressure and rate of rise of pressure which is dependent upon the pressure itself. But it is aggravating when you do look at the work on small animals to notice the unrealistic rates of compression which they tend to apply; then they will say some particular event is independent of the rate of rise of pressure, when really the rates of rise of pressure that have been used on the mammals are very, very rapid compared with the rates of rise of pressure which are seen to be necessary in man.

Dr. Brauer: We have used a procedure for evaluating the anticonvulsant potency of various inert gases against the high pressure neurological syndrome. The interesting aspect is that, as you go to higher nitrogen concentrations the convulsion thresholds climb and the slope of that curve lends itself quite well to assessing potency, so a comparison can be made.

The slope you construe for tremors (which really represent the early phases of motor disturbances) is much flatter. Now when you compare relative anticonvulsant, relative anti-tremor potency against relative narcotic potency you get straight lines for helium, nitrogen, nitrous oxide and some other gases. The lines are parallel—this means the exponent is identical—for the tremor and for the convulsion phase, but the tremor potencies are about one-third as large as the others.

One of the things that intrigues me is that this correlation of the anticonvulsant with the narcotic potencies confirms the point that Dr. Roth was making: that is, that these effects appear to be intimately and causally related with the narcotic potency. I call your attention to the fact that indeed this reversal of high pressure effects is true not only for the general anesthetics and inert gas anesthetics but also holds for a quite wide range of other central nervous system depressants.

Dr. Gottlieb: I would like to know how does one distinguish between a true protective effect of these gases and a possible masking effect of these pharmacological agents?

Dr. Smith: If one goes to quite fundamental processes—such as the inhibition of the light intensity of luminous bacteria—one almost invariably observes that general anesthetic inhibition can be restored, at least partially, by hydrostatic pressure. And I think this gives some evidence that this is a rather fundamental mechanism of the mode of action of anesthetics and not just an irritant that removes the observed response.

Dr. Macdonald: Emphasis has been put so far on antagonism between these inert gases and pressure. At Aberdeen we have found a very clear-cut synergism. The physiological preparation we have used is a dividing cell. If you take a dose of halothane—which is so low it does not affect the cell's division—and combine that with a pressure of 100 atmospheres—which is also in itself so low that it does not affect division—together there is a total inhibition of division.

We cannot explain this effect. Under slightly higher doses of halothane we get the now classical antagonism.

Dr. Miller: I would like to touch on two points under discussion, with respect to old work on newts.

When a newt is exposed to increased hydrostatic pressure, you see paralysis. When the control response is 100% the newt follows the chain when it rotates and walks well; when response is zero the newt is completely paralyzed: the legs and the tail curl up. That is what happens in water. On decompression the animal survives. If you compress with helium he does a little bit better; the paralysis is postponed a little. If you compress with neon the paralysis is postponed a bit more. Hydrogen postpones the paralysis even further. The paralysis is also postponed by adding nitrogen.

Another point I would like to make is that if you first anesthetize the animal with nitrogen so that he has lost his righting reflex, and then you start adding helium, a very spectacular reversal of the anesthesia with pressure is seen.

If you rationalize the reversal of anesthesia as being a function of the volume in the membrane—or assume that you need to achieve a certain membrane volume expansion to achieve anesthesia—and the reason that more anesthetic is needed at greater pressure is that you need more expansion to offset the compression—then a straight line graph should be the result if the theory is right. This is a test for nitrous oxide/helium mixtures, and both our results with newts at 20 and 30°C and Halsey's results with mice seem to fall pretty well within this simplistic theory.

Dr. Smith: We will now discuss the last two papers and seek comments on the molecular and biochemical consequences of exposure to high pressure.

Dr. Orsi: Dr. Zimmerman, did you find any evidence that there was misreading of the message RNA or some abnormal coupling to the ribosomes?

Dr. Zimmerman: Actually the high pressure prevents the synthesis of what we call Q-1 and Q-2 RNA. Q-1 is the ribosomal precursor RNA. Q-2 is the so-called DNA-like RNA, the message RNA. In high pressure, depending upon the magnitude of pressure and the duration, one can inhibit the synthesis of both the ribosomal precursor as well as the message material. That is one effect of the pressure.

So there is less total ribosomal and less total message RNA. In addition, the ribosomal RNA that is there, that is in the cell prior to compression and during compression, is functional, since we were able to show that by removing the ribosomes from compressed cells and adding a synthetic message, these ribosomes were able to synthesize polyphenylalanine as effectively as ribosomes isolated from control cells.

Dr. Raymond: I would like to ask Dr. Wells if he would extrapolate his data to tissue oxygenation in vivo. In asking the question I take the liberty of mentioning some results obtained by Dr. Keithow of the Naval Medical Research Institute. He has used whole blood and, at pressures of 700 p.s.i. and higher, has shown a striking leftward shift of the oxyhemoglobin dissociation curve. This is at variance with the data you presented.

Dr. Wells: Is this hydrostatic pressure alone you are talking about or inert gas pressure with a shift of the dissociation curve?

Dr. Raymond: This is helium inert gas pressure. No effect was found with argon, if I am paraphrasing his findings correctly, nor was any effect found with old—that is, banked—blood.

Dr. Wells: I might comment briefly that there is some binding of hemoglobin with nitrogen; for instance, and when one gets to about 600 feet of sea water, it does affect the oxyhemoglobin dissociation curve. Right now I really cannot reconcile any differences that might occur between our respective curves, except that mine was at a rather peculiar pH: I had reduced the pH to 6.8 to move the curve as far to the right as I could before starting. This made the measurements a lot simpler. This was both for the 100 atmosphere and for the 1,000 atmosphere curves.

I am not sure what the shape of Dr. Keithow's curve was, but if you extrapolate mine to the tissue level—we are talking about loading in the lungs now and we are certainly down on the dissociation curve—from the asymmetric shape, especially the lower portion around 20%, you would suspect some change in the rate processes governing tissue oxygenation.

Dr. Wells: Actually in the calculation of those results I did take the change in the solubility coefficient into account to calculate the P_{O_2} at 1,000 atmospheres.

Dr. Sanders: This is addressed to one of the questions Dr. Gottlieb raised as to tissue specificity on ATPase activity with temperature changes. If you compare this activity in liver, kidney and brain of the rat you will find there is a wide difference in variation in moving from 25° to 37°C, so that I would anticipate not only tissue specificity but possibly species specificity.

Dr. Behnke: Do high pressures protect against the effects of ionizing radiation on DNA and ATPase activity in these chemical changes?

Dr. Smith: Since no one can answer, there is still some work to be done.

Part VII. OXYGEN

OXYGEN AND BRAIN METABOLISM

J. D. Wood

The breathing of oxygen at high pressure (OHP) results in damage not only to the central nervous system but also to other tissues such as the lungs and the red blood cells. The present paper, however, will be confined to the effect of OHP on the metabolism and function of the brain. A review presentation such as this often consists of a brief summary of earlier data followed by a more detailed account of recent results. However, a somewhat different approach will be taken here since the findings in the field of oxygen poisoning have already been well-documented in various review articles and in the proceedings of several symposia and congresses (2, 4, 10, 12, 16-20, 26, 29, 31). Instead of an emphasis on the past, current hypotheses regarding the etiology of OHP-induced convulsions will be evaluated, and different approaches that might be taken to elucidate the basic mechanisms involved in the seizure process will be delineated.

The fundamental question which must be asked is, "What is the cause of OHP-induced convulsions?" Many research workers in the field would answer that the cause is unknown; this is only partially true. In view of the well-established ionic basis of nerve transmission, it is fairly safe to predict that changes in the membrane permeability to ions such as sodium and potassium are the direct cause of the seizures. Although this aspect of the problem has not been studied in very great detail, there is supporting evidence for such a postulation. For example, exposure of brain cortex slices to high pressure oxygen significantly affects the concentration of the electrolytes in the tissue (Table I).

The next question that must be posed is, "What is producing the derangement in ionic permeability?" It is to this question that one must answer, "We do not know." The present paper will therefore be devoted to ways and means of obtaining an answer, together with a discussion of the results already available which may have some bearing on the situation.

At least three possible mechanisms may be involved in the OHP-induced derangement of ionic permeability: (*a*) the membrane itself may be damaged or its function altered; (*b*) the oxidative metabolism required for supplying energy to the ionic pumps may be impaired; and (*c*) neurotransmitter metabolism may be changed.

Membrane Damage

The membranes in the central nervous system may be expected to exert very fine control over the diffusion and/or transport of substances. One must, therefore, consider what

TABLE I

EFFECT OF OHP ON SODIUM AND POTASSIUM LEVELS IN SLICES FROM GUINEA PIG CORTEX INCUBATED AT 37°C[a]

Incubation		Concentration (nmoles/kg)	
Pressure	Time (minutes)	Na^+	K^+
1 ata O_2	90		69.2
6 ata O_2	90		46.5
1 ata O_2	120	60.9	98.6
6 ata O_2	120	118.0	33.0

[a] Data from Kaplan and Stein (14) and Joanny et al. (13).

components of the membrane essential for this control are likely to be susceptible to attack by OHP. The major components of membranes are lipids and proteins, and both may be susceptible to OHP-induced changes, the lipids by oxidation of the unsaturated fatty acids, and the proteins by oxidation of the sulfhydryl (SH) groups. Such changes could (*a*) alter the physical structure of the membrane, or (*b*) inhibit the groups actively involved in the transport of substances through the membrane. The end result in either case would be changes in permeability.

There is good evidence that exposure of animals to OHP induces the formation of lipid peroxides (30, 35). The oxidation of lipids would not, however, be rapidly reversible. In view of the known rapid reversibility with respect to OHP seizures (15), it seems unlikely that such oxidative changes are the cause of the seizures. For this reason, the view is here favored that, if OHP-induced seizures are a result of direct damage to the membranes, the cause is likely to be changes of a reversible nature in the protein moiety, involving the oxidation of SH groups or perhaps the direct binding of oxygen to the active site. Detailed studies on this aspect are lacking, probably due to the great technical difficulties involved, but a major effort in overcoming these difficulties may pay considerable dividends and thus seems warranted.

Changes in Oxidative Metabolism

A vigorous oxidative metabolism is necessary to supply the energy for the extremely active sodium and potassium pumps that operate in brain tissue. An impairment of this metabolism will obviously interfere with nerve transmission. This aspect has seen considerable research effort, but the results are rather inconclusive. Both an inhibitory and a stimulatory effect of OHP on the respiration of brain preparations have been reported (21, 24, 27). Moreover, while Sanders and Hall (24) have shown that exposure of animals to OHP significantly decreases the levels of the high energy compound ATP, Gershenovich et al. (9) report no change in the rate of oxidative phosphorylation in vivo. Clearly, further studies are required in this area to clarify the situation.

Alteration in Neurotransmitter Metabolism

This aspect is dealt with in more detail than the others because, in my opinion, it seems to be a very likely cause of OHP-induced seizures. Neurotransmitters are chemical substances which are responsible for the transmission of nerve impulses from one cell to another. They are released from the nerve endings into the synaptic cleft where they bind to the post-synaptic membrane, thereby causing changes in ionic permeability which influence the excitability of the second (post-synaptic) cell. A schematic presentation of a synaptic area and associated metabolism is shown in Fig. 1. In this context, the term metabolism is used to denote not only the synthesis and degradation of the transmitter substances but also their release from, uptake into, and storage in brain cells. It must be stressed that the events depicted in Fig. 1 represent a general overview of neurotransmitter metabolism. All events do not exist for all transmitters, and the relative importance of the individual phenomena will vary from one transmitter to another. The concentration of the transmitter which is critical with respect to its effect on the post-synaptic cell is that in the synaptic cleft. Changes in the level of the substance in whole brain do not necessarily reflect changes in the concentration of the compound in the cleft, and this complication must always be borne in mind when evaluating the functional significance of observed changes in whole brain levels. It should also be apparent that the concentration of a transmitter in the synaptic cleft can be altered by aberrations in a variety of phenomena. It is just this situation which makes it so difficult to delineate the detailed changes in transmitter metabolism brought about by OHP and to evaluate the functional significance of these changes. These restrictions must be kept in mind as one evaluates the available data regarding OHP and the neurotransmitters acetyl choline, norepinephrine (NE), dopamine (DA), 5-hydroxytryptamine (serotonin; 5 HT) and γ-aminobutyric acid (GABA).

Is the metabolism of any one of these substances particularly sensitive to OHP? A partial answer may be found in work carried out by Dr. Tunnicliff and myself (28), where chick brain homogenates were exposed to OHP under standardized conditions, and then the activities of the various enzyme systems were compared to that in a portion of the homogenate which

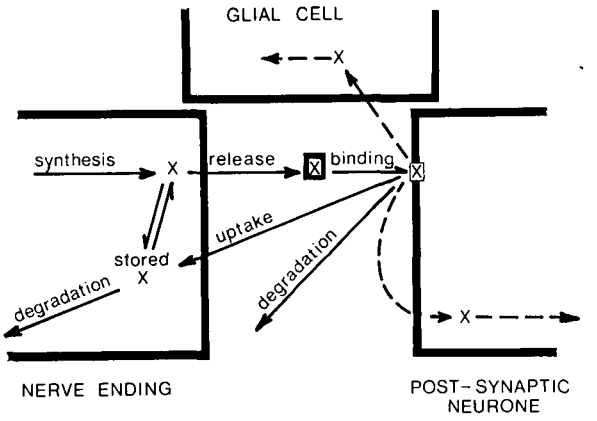

FIG. 1. Schematic presentation of a synaptic area and associated neurotransmitter metabolism. "X" indicates the neurotransmitter.

had not been thus exposed. The enzymes responsible for degrading the neurotransmitters (GABA-α-ketoglutarate transaminase, acetylcholinesterase, catechol-*O*-methyl transferase, and monoamine oxidase) were not significantly affected by the OHP. On the other hand, the enzymes responsible for synthesis of the transmitters (glutamic acid decarboxylase, choline acetylase, 5-hydroxytryptophan decarboxylase and dopa decarboxylase) were all significantly inhibited (Fig. 2). Among this group, glutamic acid decarboxylase, the enzyme which synthesizes GABA, was clearly the most sensitive. In view of these results, it seems necessary to examine the metabolism of all transmitters with respect to the etiology of the seizures, with perhaps particular attention being paid to GABA.

Biogenic Amines

First to be considered will be the group of compounds often termed the biogenic amines, i.e., NE, DA and 5 HT. OHP-induced decreases in the brain concentration of these three compounds have been reported, although some investigators failed to find any effect (Table II). The cause of the discrepancy is uncertain but may be due to species differences, the mouse being considerably more susceptible to OHP-induced seizures than the rat. If changes in the level of the amines are a cause of the seizures, it might be expected that alterations in the amine levels brought about by various drugs would alter the susceptibility of the animals to the convulsions. However, Blenkarn and coworkers (3) drastically altered the amine levels without causing any significant effect on the time to onset of OHP-induced seizures (Fig. 3). There is, therefore, no correlation between amine levels and the onset of seizures. This conclusion must, however, be tempered with the recognition of the above-mentioned possibility that overall amine levels do not necessarily reflect the concentrations of the compounds in the synaptic cleft. Moreover, consideration must be given to the possibility that it is the

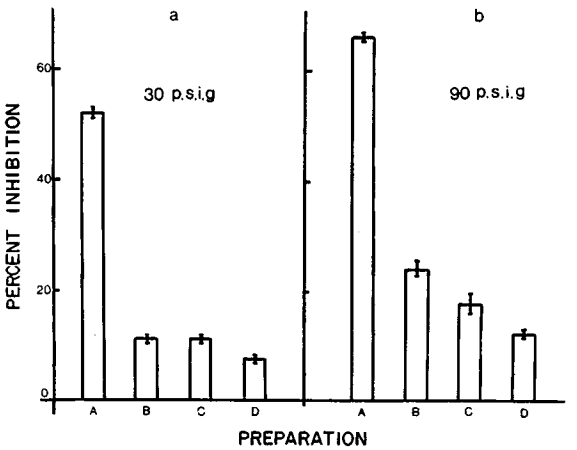

FIG. 2. Effect of OHP on the activity of enzymes involved in the synthesis of neurotransmitters. Homogenates prepared from chick brains were incubated for 20 minutes at 25° under either nitrogen or OHP (30 or 90 p.s.i.g. O_2) and the individual enzyme activities then determined. The nitrogen-exposed preparations were used as controls and the OHP-induced inhibition of activity was calculated. Each value in the figure represents the mean ±SE for five preparations. *A*. glutamic acid decarboxylase; *B*. 5-hydroxytryptophan decarboxylase; *C*. DOPA decarboxylase; *D*. choline acetylase.

TABLE II

EFFECT OF OHP ON BRAIN AMINE LEVELS[a]

Species	Exposure		State of Animals	% of Normal Value		
	Duration (min)	Pressure (ata)		NE	5-HT	DA
Mouse	28.5	2	Nonconvulsed	78	78	
	43.5	4		63	41	
	7.5	6		44	36	
Rat	60.0	6	Most had convulsed	60		70
Rat	60.0	4.95	Nonconvulsed	98	106	

[a] Data from Faiman et al. (7), Häggendal (11) and Blenkarn et al. (3).

turnover rate of the amines rather than their concentration per se which causes the seizures, particularly since it has been reported by Neff and Costa (22) and Diaz et al. (6) that the turnover rate of norepinephrine and serotonin is increased in animals breathing 100% oxygen in place of air at ambient pressure. In summary, there is no direct evidence indicating the involvement of the biogenic amines in the etiology of OHP-induced seizures; yet this possibility cannot be completely discarded. In the circumstances, further research on this aspect is warranted with particular stress being placed on studies at the subcellular level.

ACETYL CHOLINE

Another neurotransmitter substance which must be considered when studying the mechanisms involved in OHP-induced convulsions is acetyl choline. Although this compound is well-established as a transmitter agent, comparatively little work has been carried out on the effect of OHP on its metabolism and function. Additional studies in this area therefore are necessary.

γ-AMINOBUTYRIC ACID

The best evidence for the involvement of a neurotransmitter in the seizure process has been obtained with GABA. The great sensitivity of its synthesizing enzyme to OHP has already been indicated. In addition, my coworkers at the Defense Research Medical Laboratories and the Department of Biochemistry, University of Saskatchewan, have accumulated considerable evidence for a role for GABA. The results of these studies were presented at a previous Underwater Physiology Symposium and may be found in the Proceedings (19). Therefore, this will be only a brief summary. The susceptibility of animals to seizures was expressed by the use of CT_{50} values where this value represented the time to onset of seizures in 50% of the animals. The CT_{50} values varied considerably among species; for any one species, the CT_{50} value could be varied by altering the pressure of oxygen, the

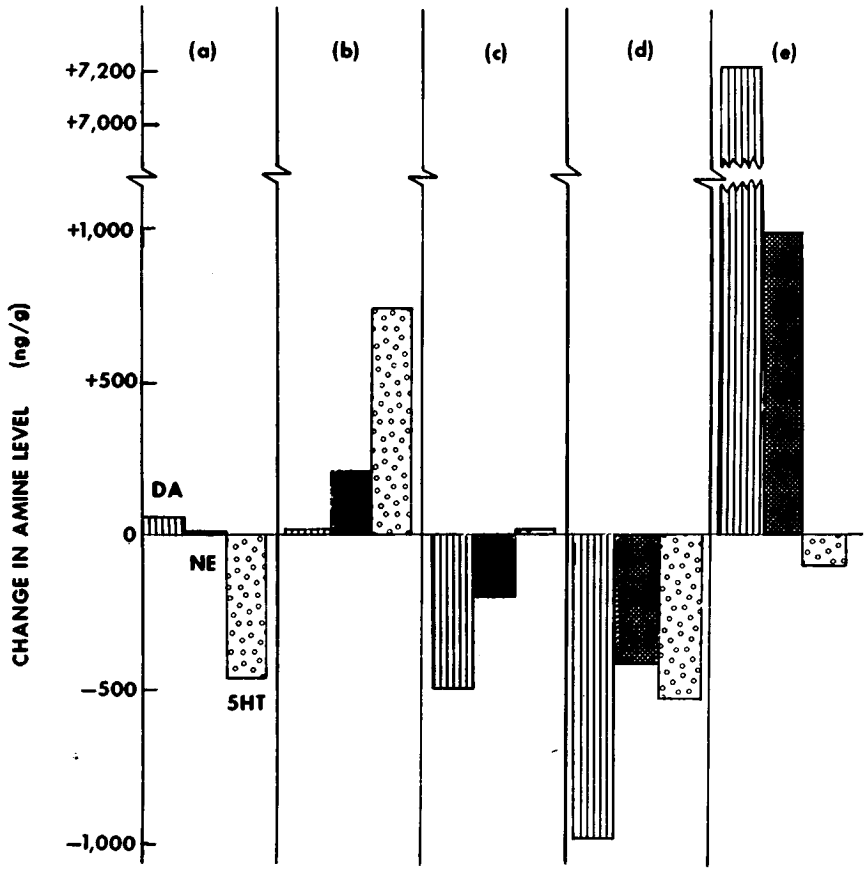

FIG. 3. Changes in biogenic amines which were without effect on the susceptibility of mice to OHP seizures. Dopamine, norepinephrine and 5-hydroxytryptamine are represented by DA, NE and 5-HT respectively. The pretreatments were as follows: (*a*) *p*-chlorophenyl-alanine (360 mg/kg), 96 hours prior to OHP exposure; (*b*) pargyline (100 mg/kg), 5 hours; (*c*) *a*-methyl-*p*-tyrosine (200 mg/kg), 2.5 hours; (*d*) reserpine (5 mg/kg), 5 hours; (*e*) DL-dopa (400 mg/kg), 0.25 hours. Levels of the amines in brains of untreated animals were 1011, 443 and 634 ng/g for DA, NE and 5-HT respectively. (Adapted from selected data of Faiman et al., 1971.)

CO_2 content of the breathing mixture, or the age of the animals. By manipulating the CT_{50} values in this way, an excellent correlation was demonstrated between the rate of decrease in the concentration of GABA and the susceptibility to seizures (Fig. 4). This finding together with other data presented previously (19) suggests a cause and effect relationship between changes in GABA levels and the onset of OHP-induced seizures.

However, the correlation between changes in whole brain GABA levels and susceptibility to OHP-induced seizures may be fortuitous. Studies with other convulsant and anticonvulsant agents indicate that the inhibition of the GABA synthesizing enzyme (GAD) is the primary factor in altering the excitability of the brain and that changes in whole brain GABA levels have only a secondary influence (33). This concept was expressed mathematically using the formula $E_S = \Delta\text{GAD} + 0.17\ \Delta(\log \text{GABA})$ where E_S represents the state of

excitability, and ΔGAD and Δ(log GABA) represent, respectively, the percentage change in GAD activity and the percentage change in the GABA concentration expressed logarithmically (33). A negative value of E_S indicates a hyperexcitable state. Conversely, a positive value indicates hypoexcitability.

A consideration of the OHP results in the light of this equation leads to the conclusions which follow: The level of GABA in brain is determined by the balance in activities of GAD and the GABA-degrading enzyme (GABA-T). Since the latter enzyme is unaffected by OHP (see previously), the observed OHP-induced changes in GABA level will be proportional to the degree of inhibition of GAD. It is clear from the equation that such a situation will result in an apparent correlation between changes in whole brain GABA levels and the excitability of the brain. Unfortunately, it has not been possible to determine OHP-induced changes in GAD activity in vivo due to technical problems, and this has precluded a direct

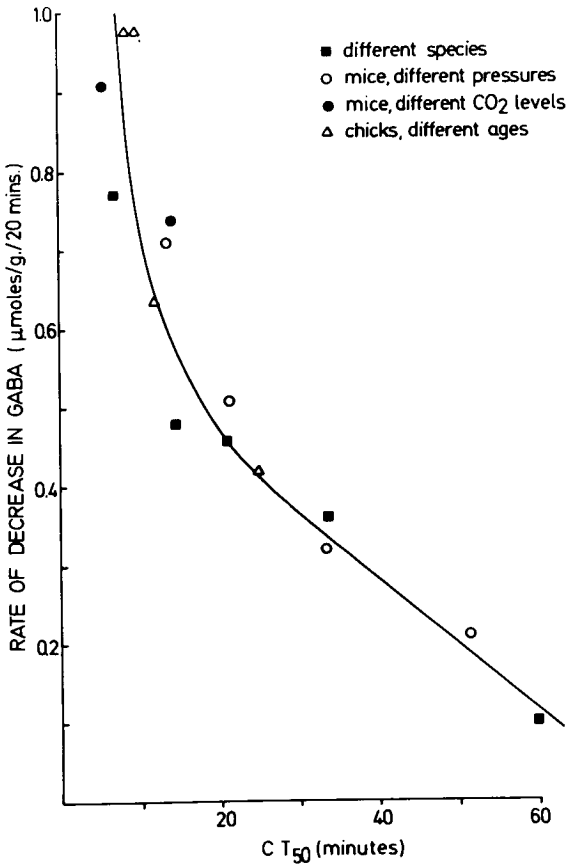

FIG. 4. Correlation between susceptibility to OHP seizures and rate of decrease in brain GABA levels. The CT_{50} value is the length of exposure to OHP which is required to convulse 50% of the animals. For details regarding type of species, pressures and CO_2 levels see Wood (32). The ages of the chicks were 2 days, 1 week, 2 weeks, and 3 weeks respectively, the CT_{50} values decreasing with age.

testing of the equation with respect to oxygen poisoning. Nevertheless, if the equation holds true for OHP it should be possible to develop, from theoretical considerations, an agent with anticonvulsant action against OHP-induced convulsions. The success of such an approach and an evaluation of the mode of action of previously reported anticonvulsant agents in the light of this new knowledge will now be described.

Protection Against OHP-Induced Seizures

From the equation, it may be reasoned that to obtain protection against OHP it will be necessary to develop agents which elevate GABA levels but which do not inhibit GAD activity, i.e., the agents must specifically inhibit GABA-T. In the search for a suitable compound we found that isonicotinic acid hydrazide (INH) inhibited both GAD and GABA-T, but that the simultaneous administration of pyridoxine with the INH prevented the inhibition of GAD without affecting the inhibition of GABA-T. The efficacy of this mixture was tested and indeed found to be excellent protection against OHP-induced seizures in chicks (Table III). In view of these developments, it now seems appropriate to examine some of the other agents which protect against OHP and assess whether or not they may be exerting this effect via the "GABA metabolism system".

Blenkarn et al. (3) report that the monoamine oxidase inhibitors, pargyline and iproniazid, possess anticonvulsant properties with respect to oxygen poisoning but that the effect is not due to their action on the monoamine oxidase. Since both agents elevate GABA levels (25), it is possible that their anticonvulsant action is indeed mediated through the GABA system. Supporting this possibility are the results of Schatz and Lal (25) which indicate a correlation between the pargyline-induced changes in GABA levels and the anticonvulsant action of the drug (Fig. 5).

Another anticonvulsant agent which may operate via its effects on GABA metabolism is lithium. This compound has been shown by Radomski, Rowe and Watson* to prevent OHP-induced decreases in GABA levels and to protect against the OHP-induced seizures.

TABLE III

EFFECT OF ISONICOTINIC ACID HYDRAZIDE AND PYRIDOXINE ON OHP-INDUCED SEIZURES IN CHICKS[a]

Pretreatment[b]	Percent Convulsed	Time to Onset of Generalized Seizures[c] (minutes)
None	100	12.8 ± 1.5
INH	100	23.8 ± 2.2
Pyridoxine	100	19.3 ± 3.3
Pyridoxine + INH	20	(40, 44)

[a] Data from Wood et al. (34).

[b] Three-week-old chicks were injected intramuscularly with 2.2 mmoles/kg INH and/or 4.4 mmoles pyridoxine 16 hours prior to exposure to 45 p.s.i.g. O_2 for a period of 60 minutes. Ten chicks were used per group.

[c] Zero time was taken as the point at which the required pressure (45 p.s.i.g.) was reached.

*See p. 517.

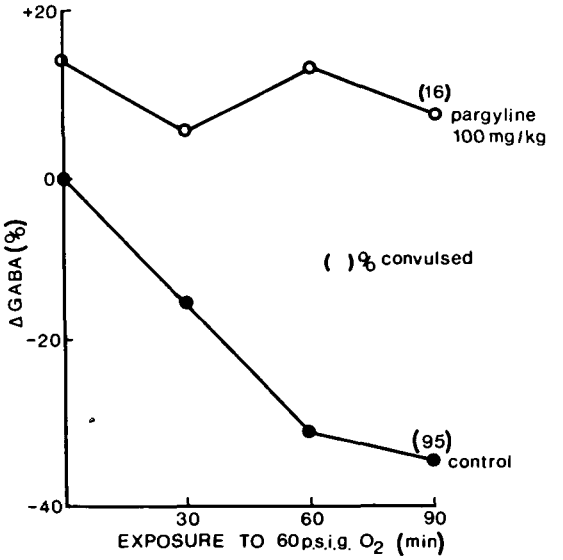

FIG. 5. Effect of pargyline on OHP seizures and OHP-induced changes in brain GABA levels. The pargyline was administered 30 minutes prior to the exposure to OHP. (From Schatz and Lal, 1971.)

One of the more effective agents which protect against OHP-induced seizures is succinate (5). Sanders and coworkers (23), who have performed most of the studies on this aspect, conclude that the protective action arises from the ability of succinate to support oxidative phosphorylation. While this hypothesis has merit, the possibility that the effect is mediated via a feedback inhibition in the glutamate-GABA-succinate pathway cannot be ignored.

Conclusions

An overview of the results summarized here indicates that considerable but not unequivocal evidence has been obtained in support of a role for GABA metabolism in the etiology of OHP-induced seizures. With regard to future research on this aspect, the greatest information return may well come from detailed investigations on the effect of "OHP protective agents" on GABA metabolism. Although most evidence supports a role for GABA metabolism in the seizure process, a role for the other transmitters is not excluded, particularly with respect to secondary effects. Since many compounds found in brain tissue are susceptible to oxidation, it would not be altogether surprising to find that OHP-induced convulsions are the net effect of several metabolic and structural defects.

This review points out possible approaches to future research in the area of hyperbaric oxygen and brain metabolism. In summary, they are (*a*) further studies on the effect of OHP on neurotransmitter metabolism, particularly at the subcellular level, (*b*) elucidation of the mechanism of action of various anticonvulsant agents with particular attention being paid to their effect on GABA metabolism, (*c*) studies on the direct action of OHP on membrane structure and function, and (*d*) clarification of the effects of OHP on brain respiration and oxidative phosphorylation.

An abundance of data now exists on the topic of OHP-induced convulsions and the results seem to be falling into place, albeit slowly. However, much remains to be done before the basic mechanisms causing the seizures are elucidated unequivocally.

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VENTILATORY RESPONSES TO HYPERCAPNIA AND HYPOXIA AT 1 AND 4 ATA

D. Doell, M. Zutter and N. R. Anthonisen

Added external airway resistances have been shown to depress the ventilatory response to CO_2 (4). However, when the CO_2 response was expressed in terms of inspiratory power, it was found to be independent of external resistances in resting subjects (9). Internal pulmonary resistance is augmented by increased gas density. Wood and Bryan (14), working in hyperbaric environments, extended the above findings and found that CO_2 responses were depressed when expressed as ventilation but unaffected when expressed as inspiratory power. Less is known about the effects of mechanical loading on the ventilatory response to hypoxia, probably because this response is difficult to reproduce and quantitate. The authors are aware of only two studies, both involving external resistances, and these indicated that the hypoxic response differs from the CO_2 response. Barnett and Rasmussen (2) found normocapneic hypoxic ventilatory responses were less depressed by external resistance than were ventilatory responses to CO_2, and Levison and Cherniack (7) were unable to demonstrate any resistance-induced depression of ventilation due to hypoxia. Ventilatory responses to CO_2 and hypoxia at 1 and 4 ata have been examined; under the latter condition pulmonary resistance should be approximately doubled (1, 13).

Material and Method

The method used was a rebreathing technique described by Tenney et al. (12). Subjects rebreathed from a 13.5 L spirometer with mixing motor but without CO_2 absorber. The mouthpiece and spirometer tubing were 7.5 cm in diameter and the total circuit volume was about 25 L. By charging the spirometer with different gases and conducting multiple rebreathing runs, a variety of alveolar gas tensions and their corresponding ventilations were obtained. During rebreathing runs, end tidal gas was drawn by hand into syringes and was immediately analyzed for P_{O_2} and P_{CO_2} on appropriately calibrated electrodes (IL Model 113). Samples were taken at 1- or 2-minute intervals during each rebreathing run, the syringes being flushed and aliquots taken from five consecutive breaths. Ventilation was recorded on the spirometer kymograph, running at 160 mm/min throughout rebreathing, which allowed measurement of the tidal volume and duration of the five sampled breaths.

Hyperoxic CO_2 response curves were evaluated by having subjects rebreathe 100% O_2 at 1 ata and room air at 4 ata. During these runs, end tidal P_{O_2} was always in excess of 300 mm Hg. Hypoxic responses were evaluated at 1 ata by having subjects rebreathe mixtures of 14.5% O_2, and 1.5% CO_2, 10% O_2 and 2%-3% CO_2, and occasionally 10% O_2. At 4 ata, subjects rebreathed mixtures of comparable P_{O_2} and P_{CO_2}; before these runs they breathed at 5% O_2 mixture so that the initial alveolar P_{O_2} would be comparable to that at 1 ata. Hypoxic rebreathing runs were terminated when extreme hyperventilation or cyanosis became apparent; we tried to avoid an end tidal P_{O_2} of less than 40 mm Hg. No subject had subjective or objective symptoms other than the urge to breathe.

The subjects were six men, aged 21-30, only one of whom was experienced with respiratory experiments; three others were experienced SCUBA divers. All studies were carried out in the seated position in a hyperbaric chamber under similar conditions of temperature and lighting. No samples were taken during the first minute of rebreathing. The experiments took 18 months to complete. In each subject rebreathing runs were scattered over a 3- to 4-month period; only on rare occasions did one subject complete two rebreathing runs on the same day. Decompression from 4 ata exposures was accomplished according to U.S. Navy Tables (15). When subjects were exposed to hypoxia, they were decompressed as though the exposure had been to 4.33 ata for 5-10 minutes longer than the actual exposure time. No subject or investigator exhibited signs of decompression sickness.

Critique

The rebreathing technique used in these studies did not have the theoretical advantage of the technique described by Read (10); with his approach, $P_{A_{CO_2}}$ is virtually the same as that in chemosensitive areas, so that simultaneous measurements of $P_{A_{CO_2}}$ and ventilation yield a valid representation of stimulus and response (11). With the present technique, $P_{A_{CO_2}}$ lagged behind medullary arterial P_{CO_2} by a time interval dependent on the cerebral circulation. Tenney et al. (12) compensated for this by measuring ventilation 25-30 sec after alveolar gas sampling but pointed out that with their circuit, which was the same as that used in this study, such a modification did not materially change the results. The Read technique was not used by the authors because rapid-response CO_2 analyzers are not suited to hyperbaric conditions and because an examination of hypoxic responses at low P_{CO_2} was of particular interest. In fact, the hyperoxic CO_2 response curves at 1 ata were found to be very similar to those generated by the Read technique in the same subjects. Use of a 25-sec delay between measurements of $P_{A_{CO_2}}$ and ventilation changed the hyperoxic CO_2 response curves very little. A delay of 25 sec would have influenced hypoxic response since ventilation changed rapidly during these runs, but a delay of such magnitude is almost certainly not physiological under hypoxic conditions. In two subjects, hypoxic ventilatory responses were calculated using both a 6-sec and a 12-sec delay between the end tidal sample and the corresponding ventilation. These modifications had the effect of increasing the ventilatory response by less than 5%, which in no way affected the interpretation of the data. It was, therefore, chosen to record in all instances the ventilation which was measured at the same time as end tidal gas was sampled. Although the absolute validity of this procedure was questionable, it was felt important to adopt a uniform procedure for comparing data gathered at 1 and 4 ata. Since initial gas tensions and circuit volumes were the same at surface and depth, and since there was no reason for metabolic rate to change with barometric

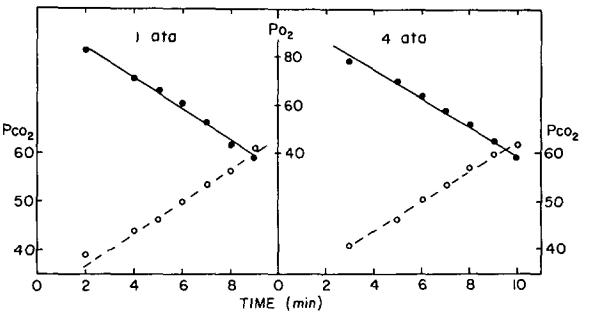

FIG. 1. Time course of changes of $P_{A_{O_2}}$ (closed circles) and $P_{A_{CO_2}}$ (open circles) at 1 ata and 4 ata. In both instances initial $P_{A_{O_2}}$ = 100 mm Hg and initial spirometer P_{O_2} = 100 and P_{CO_2} = 10 mm Hg.

pressure, spirometer gas tensions should have changed at the same rate under the two conditions. This was, in fact, the case (Fig. 1); the length of the rebreathing run varied from 5 to 20 min according to the initial P_{O_2} but did not differ systematically with barometric pressure; the rate of change of P_{O_2} and P_{CO_2} varied little in a given subject.

Results

Figure 2 shows hyperoxic CO_2 response curves in all six subjects. Data from two rebreathing runs at each barometric pressure are shown. With the exception of BH, all subjects showed a brisk CO_2 response at 1 ata. At 4 ata all subjects showed a depressed response curve; this was more evident in some subjects than in others, but in most the curves were clearly different at ventilations of 50 L/min or $P_{A_{CO_2}}$ of 50-55 mm Hg.

Figure 3 shows hypoxic response curves at normal $P_{A_{CO_2}}$ in five subjects and at $P_{A_{CO_2}}$ 44-46 mm Hg in subject WM. Two subjects (BH and TW) demonstrated very small increases in ventilation although $P_{A_{O_2}}$ approached 40 mm Hg. A third (DD) showed a rather variable response, but the other three subjects demonstrated distinct responses of 40-60 L/min. In no subject was the response at 4 ata separable from that at 1 ata. At higher levels of $P_{A_{CO_2}}$, the hypoxic ventilatory response was depressed in most subjects (Fig. 4). Mathematical quantitation of hypoxic response curves was carried out by relating $\dot{V}_E$ to $P_{A_{O_2}}$ by the equation $\dot{V}_E = \dot{V}_{E_O} + A/P_{A_{O_2}} - 32$ (3), in which the $P_{A_{O_2}} - \dot{V}_E$ relationship is assumed to be hyperbolic with $\dot{V}_{E_O}$, the ventilation without hypoxic stimulation, and A, the shape constant directly related to the stimulatory effect of hypoxia. At low $P_{A_{CO_2}}$, A at 1 and 4 ata did not differ but at higher $P_{A_{CO_2}}$ at 1 ata it exceeded those at 4 ata. In one subject (BH), A did not differ up to $P_{A_{CO_2}}$ = 54 mm Hg.

Discussion

The hypoxic response curves of Figs. 3 and 4 represent data gathered over a 2-3 mm Hg range of $P_{A_{CO_2}}$. Since in hypoxia small differences of $P_{A_{CO_2}}$ are associated with large differences in minute ventilation, it would obviously have been more exact to have used smaller ranges of $P_{A_{CO_2}}$. However, only measured, not interpolated, values of alveolar gas tensions and ventilation were used, which necessitated accepting a range of $P_{A_{CO_2}}$. In any event, $P_{A_{CO_2}}$ as measured had an accuracy of ± 1 mm Hg and restricting the range of

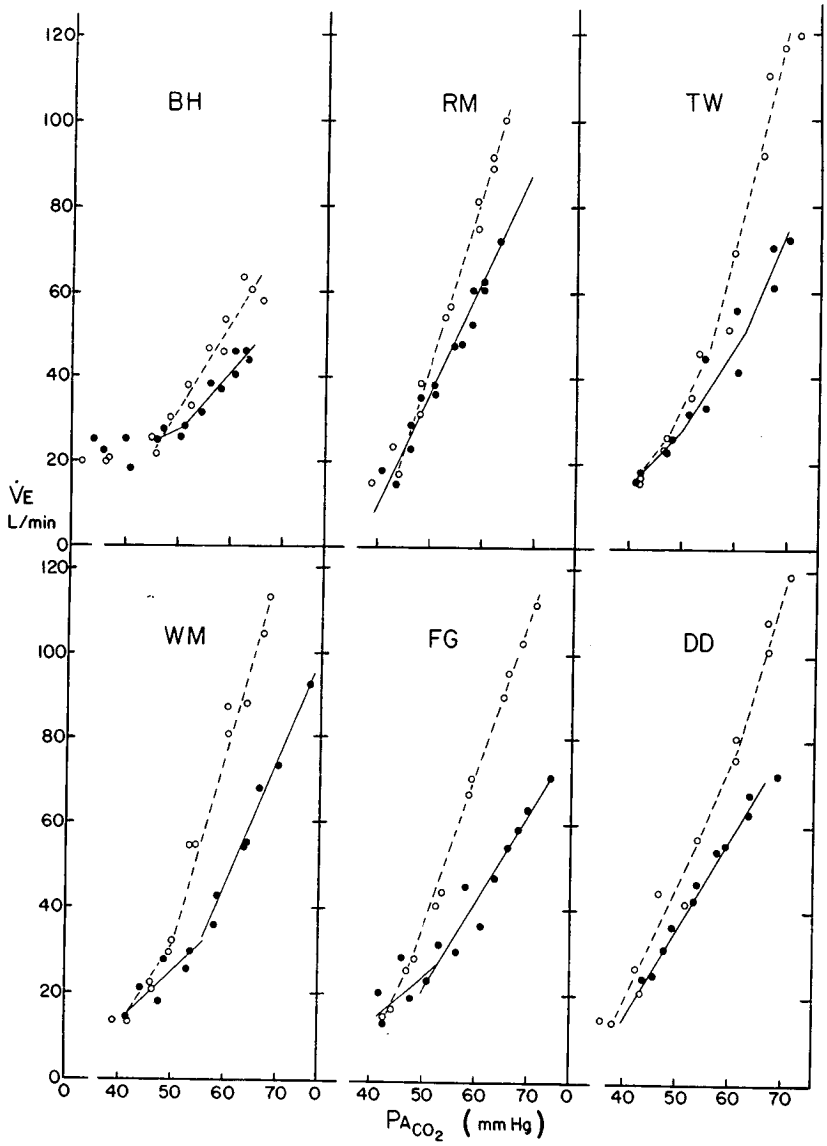

FIG. 2. Hyperoxic CO_2 response curves in all six subjects. Ordinates: ventilation in L/min. Abscissae: $P_{A_{CO_2}}$ in mm Hg. Shown for each subject are data from two rebreathing runs at 1 ata (open circles, dashed line) and two rebreathing runs at 4 ata (solid circles, solid lines). The curves were fitted by eye.

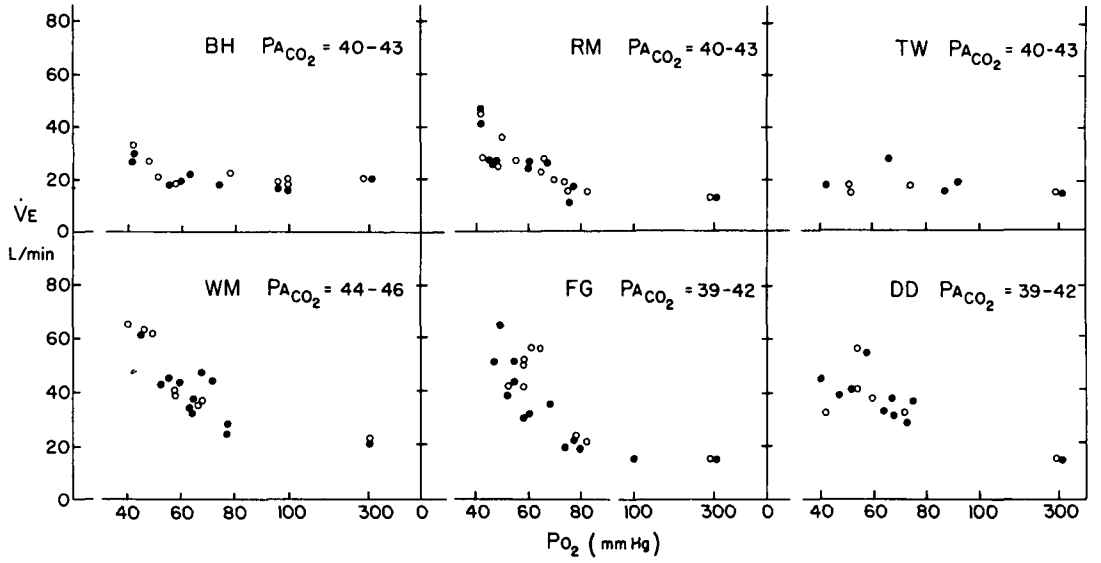

FIG. 3. Hypoxic ventilatory responses at physiological PA_{CO_2} in all six subjects. Ordinates: ventilation in L/min. Abscissae: PA_{O_2} in mm Hg. Open circles represent data gathered at 1 ata, closed circles represent data gathered at 4 ata. The PA_{CO_2} which applied to these data are indicated.

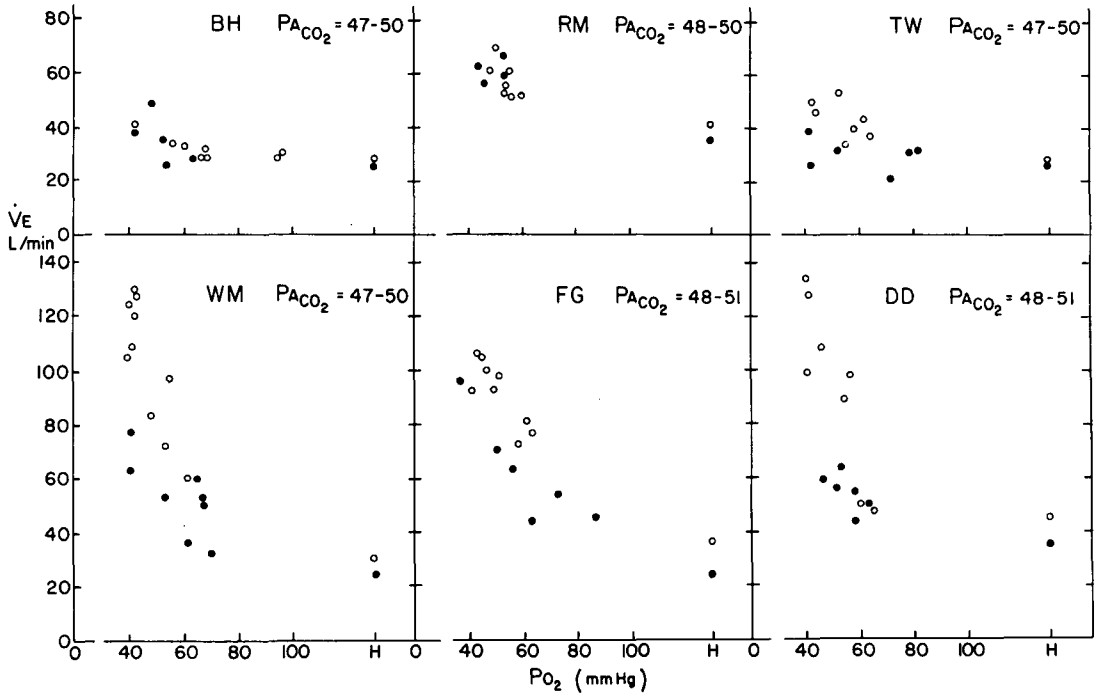

FIG. 4. Hypoxic ventilatory responses at high PA_{CO_2}. Ordinates: ventilation in L/min. Abscissae: PA_{O_2} in mm Hg. The PA_{CO_2} which applied to these data are indicated. Open circles represent data gathered at 1 ata, closed circles represent data gathered at 4 ata.

acceptable $P_{A_{CO_2}}$ did not reduce the scatter of the ventilation-$P_{A_{O_2}}$ curves. In no subject did the mean $P_{A_{CO_2}}$ used for a given pair of hypoxic response curves (Figs. 3 and 4) differ with barometric pressure.

These hyperoxic CO_2 response curves are similar to those of Wood and Bryan (14), who found that the inspiratory power-$P_{A_{CO_2}}$ relationship was independent of gas density. It is therefore likely that the depression of ventilatory response observed by the authors was due to the increased work per breath imposed by quadrupling gas density. However, increased airway resistance failed to depress the ventilatory response to hypoxia at normal $P_{A_{CO_2}}$. This finding seemed genuine since all of the subjects demonstrated it. It could be argued, in the case of subjects TW and BH, that 1 ata hypoxia alone was an insufficient stimulus to substantially increase inspiratory power; therefore, a resistance-dependent decrement in ventilation would not have been observed. Furthermore, the data of subject DD showed so much variation that resistance-induced effects could have been present without detection. However, at 1 ata the other three subjects regularly attained ventilations of 40-60 L/min in response to hypoxia at physiologic $P_{A_{CO_2}}$. At these levels of response, inspiratory power must have been affected by a fourfold change in gas density; CO_2 responses were depressed, hypoxic responses were not. Furthermore, subject BH, who showed little hypoxic response at low $P_{A_{CO_2}}$, showed no density-induced blunting of hypoxic response even at high $P_{A_{CO_2}}$ when ventilation was substantially increased. These results are quantitatively similar to those of Levison and Cherniack (7) who were unable to reduce the ventilatory response to hypoxia by application of external resistances. On the other hand, Barnett and Rasmussen (2) found that at normal $P_{A_{CO_2}}$ the ventilatory response to hypoxia was depressed by external resistances but to a smaller degree than the ventilatory response to hypercapnia. These workers used resistances of the order of 20-30 cm H_2O/L/sec which caused a 20%-40% decrement in hypoxic response as studied by a steady state technique. The resistive loads imposed in the present study were much smaller, and changes of ventilatory response of less than 20% would probably not have been detected; therefore, the two sets of results were not necessarily contradictory. It was of interest that Barnett and Rasmussen (2) were unable to detect a resistance-induced depression of ventilation in response to hypoxia at lowered $P_{A_{CO_2}}$ (ca. 30-35 mm Hg).

It would appear, therefore, that there is general agreement among the three studies dealing with this problem: while the ventilatory response to CO_2 is readily depressed by resistive loading, the ventilatory response to hypoxia is much less sensitive to this intervention and, with modest loads at normal $P_{A_{CO_2}}$ may be unaffected. This implies that the effector arm of the ventilatory control system differed according to the stimulus; the ventilatory stimuli of hypoxia and hypercapnia were in some way differentiated by the central nervous system. Given a CO_2 stimulus, the ventilatory control system responded with a fixed augmentation of inspiratory power while, given a hypoxic stimulus, the system responded with an increment of ventilation independent of inspiratory power. Although this type of differentiation may exist and is the only conclusion which can be drawn from these experiments, it is perhaps more rational to hypothesize that stimuli are differentiated according to receptors rather than the specific gas tensions which elicit them. This hypothesis would propose that the output of central chemoceptors, as illustrated by the hyperoxic CO_2 response, is in terms of inspiratory work, while the output of peripheral chemoceptors, as typified by the normocapneic hypoxic response, is in terms of ventilation. It is conceivable that stimulus differentiation would be expressed in a different "style" of breathing, i.e., the same increments in ventilation attained by different increments of frequency and tidal

volume, but the data of this study gave no evidence that such was the case, nor have others found such evidence (5).

When both hypoxia and hypercapnia were present, the ventilatory response was usually less at 4 ata than at 1 ata (Fig. 4). It was not clear whether this was entirely due to depression of the CO_2 response per se or whether the hypoxic potentiation of the CO_2 response was also affected.

The results of these experiments may have some relevance to the problem of ventilatory control in chronic obstructive lung disease. It is well known that such patients have reduced CO_2 sensitivity, and this is probably due in part to ventilatory obstruction. Although studies are few, it would appear (6) that hypoxic sensitivity in such patients is not greatly different from normal, unless there is severe CO_2 retention. This is, of course, what the present results would predict.

ACKNOWLEDGMENT

These experiments were conducted in the hyperbaric chamber of the Department of Surgery of the Royal Victoria Hospital, with the permission and cooperation of the chief surgeon, Dr. L. D. MacLean. Mr. Bob Wigmore ran many of the hyperbaric exposures.

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ADIPOSE TISSUE BLOOD FLOW AT HIGH AND LOW OXYGEN TENSIONS

M. Hansen and J. Madsen

It is well known that breathing oxygen reduces cardiac output without any appreciable change in arterial blood pressure. Consequently, an increase in peripheral vascular resistance must take place under the influence of oxygen (1, 6, 23, 24).

The vasoconstrictive effect of oxygen breathing at 1 atm has been demonstrated in a number of vascular beds. Plethysmographic studies on extremity segments have shown an 11% reduction in blood flow rate (4, 19). Myocardial blood flow has been found to be reduced by 20% (7, 22, 25), while cerebral flow is decreased by 13-15% (12, 13). Increased vascular resistance during O_2-breathing has also been demonstrated in the kidney (10, 16), in lung segments (3), and in retinal arterioles (20).

No investigation of a possible effect of oxygen on adipose tissue blood flow (ATBF) has been reported so far. A dependence of ATBF on oxygen tension would be of particular interest for the diving physiologist because the oxygen tension of the inspired gas varies considerably during most pressure exposures, and because adipose tissue with its great solubility for nitrogen is an important "slow tissue" with respect to inert gas exchange.

The present paper reports on variations in ATBF with oxygen concentrations between 10 and 100% in the inspired air at sea level.

Methods

Experimental Subjects

The experimental subjects were fasting normal male volunteers, 20-41 years old.

Adipose Tissue Blood Flow

ATBF was determined in the para-umbilical subcutaneous fat by the ^{133}Xe washout method as worked out by Larsen et al. (14). Approximately 0.05 mCi ^{133}Xe, dissolved in 0.1 ml sterile saline, was slowly injected into the middle of the adipose layer. The activity of the ^{133}Xe depot was registered continuously by a scintillation detector fixed to the abdominal wall over the depot.

After an initial period of distribution, a monoexponential elimination curve is obtained, from the slope of which $(-k)$ the local perfusion rate can be calculated, assuming gas equilibrium between blood and tissue and homogeneity of the tissue examined (11, 14). The calculation is as follows:

$$\text{ATBF} = (k)\left(\frac{\alpha\text{Xe adipose tissue}}{\alpha\text{Xe blood}}\right)(100\ [(\text{ml})\ (100\ \text{g}^{-1})\ (\text{min}^{-1})])$$

A ratio of 10/1 for the partition of Xe between adipose tissue and blood was used (2, 14).

Experimental Procedure

At least 1/2 hour elapsed from the injection of ^{133}Xe to the beginning of measurements. During the experiments, the subjects lay immobile and supine, breathing humidified gas mixtures through a low-resistance SCUBA mouth piece. By means of a manifold-stopcock arrangement, the breathing gas could be changed without the subject noticing it. In some experiments, expired air was continuously sampled for O_2 and CO_2 analysis. During the experiments the subjects were entertained by music or a slide series in order to distract them from the experimental procedures. Each breathing mixture was administered for 15 to 30 minutes, according to the type of experiment.

In experiments with controlled hyperventilation the respiratory frequency was kept constant. A pointer showed the subject whether to change or to maintain his respiratory depth, according to the alveolar CO_2 tension desired.

Figure 1 illustrates the findings from a typical experiment and shows how the effect of a change in breathing gas was calculated.

Results and Discussion

The average ATBF during air breathing was 6.4 ml · 100 g^{-1} · min^{-1} (S.D. 4.5). In Table I this figure is compared to other ATBF measurements using the same technique. It is well known that ATBF decreases with increasing thickness of the subcutaneous layer (14, 15). This may explain the difference between the results of Häggendal et al. (9) and of the present paper on the one hand and those of Larsen et al. (14) and Nielsen et al. (18) on the other, since the subjects of the present study—like those of Häggendal—were lean medical students, while those of Larsen and Nielsen were more or less obese patients. The figures are of the same order as mean ATBFs estimated from whole body nitrogen elimination or ^{85}Kr uptake curves. Table I also gives nitrogen half-times calculated from ATBF rates. These confirm that adipose tissue is a quantitatively important "slow tissue" with respect to rate of inert gas exchange. The considerable variation in ATBF from person to person in all papers referred to should be noted.

Table II shows the changes in ATBF when the subjects inhaled breathing mediums with different oxygen contents. Alveolar oxygen tensions calculated from continuous oxygen analyses are shown in the table.

When the oxygen content of the inspired air is reduced to 10%, the ATBF increases at an average of 54%. If the oxygen content is increased, ATBF is reduced as shown, the reduction with pure oxygen amounting to an average of 26%. The effect of a change in P_{O_2}

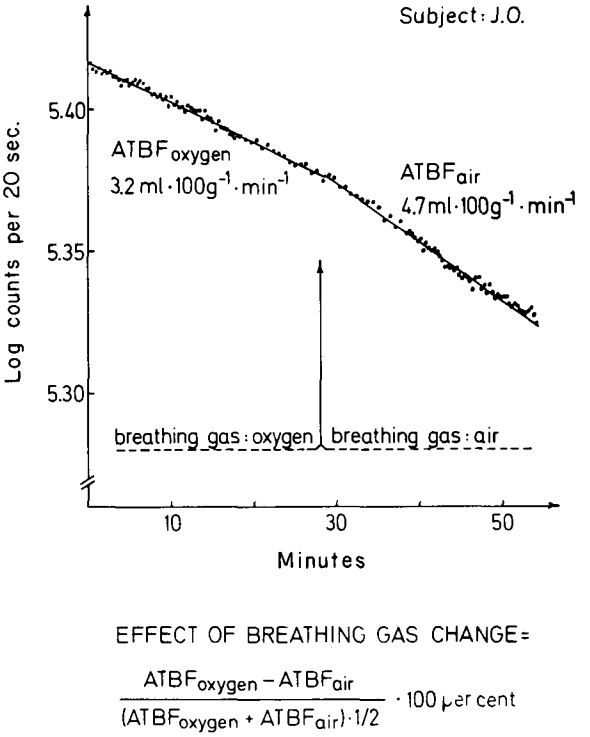

FIG. 1. ^{133}Xe elimination curve from a typical experiment.

TABLE I

ATBF DETERMINATIONS IN MAN

Reference	Tissue	ATBF (± S.D.) (ml · 100 g^{-1} · min^{-1})	N_2-Half time (minutes)
^{133}Xe washout			
Present experiments	Abdominal subcutaneous fat	6.4 (4.5)	30-180 [a]
Häggendal et al. (9)	Abdominal subcutaneous fat	6.7 (3.3)	35-100 [a]
Larsen et al. (14)	Abdominal subcutaneous fat	2.6 (1.7)	80-380 [a]
Nielsen et al. (18)	Abdominal subcutaneous fat	2.1 (0.9)	115-290 [a]
Sejrsen (21)	Crural subcutaneous fat	3.0 (1.5)	75-230 [a]
Whole body N_2 elimination			
Lundin (17)	Whole body fat	2.4-4.0	90-150 [b]
Whole body ^{85}Kr uptake			
Lesser and Deutsch (15)	Whole body fat	1.3-3.2	110-265

[a] The values given are calculated from mean ATBF + 1 S.D. and mean ATBF — 1 S.D., assuming a fat/blood partition ratio for N_2 of 5:1.

[b] Determined during breathing of 100% oxygen.

TABLE II

AVERAGE CHANGES OF ATBF DURING BREATHING OF VARIOUS O_2-N_2 MIXTURES (EXPRESSED AS CHANGE FROM CONTROL STATE BREATHING AIR)

$F_{I_{O_2}}$	percent	10	30	63	100
$P_{A_{O_2}}$	mm Hg	40	190	400	650
Average change in ATBF	percent	+54	−5	−14	−26
S.D.		26.7	16.5	25.7	23.3
Number of changes [a]		22	12	12	68
Significance of average change	*P*	<0.001	>0.2	>0.05	<0.001

[a] Experiments with 10% oxygen were made on 11 subjects, experiments with 30 and 63% oxygen on 12 subjects, while experiments with 100% oxygen were carried out on 20 subjects.

varies considerably from experiment to experiment as seen from the standard deviations. However, the mean effects were highly significant both during hypoxia and during pure oxygen breathing.

In two subjects the individual variation in oxygen effect was examined by repeating the same experiment seven times (Table III). It is seen that the effect may vary considerably even in the same person.

When the breathing medium is shifted from air to pure oxygen or vice versa, the change in ATBF occurs without any appreciable lag time. In the hypoxia experiments, however, up to 10 minutes could elapse before ATBF reached a constant rate. This may be explained by the time course of the alveolar oxygen tension, which, in the 10% oxygen experiments, falls off slowly into a still more pronounced hypoxia (Fig. 2), while in the 100% oxygen experiments, the alveolar oxygen tension reaches a level of 600 mm Hg in 2 minutes. The final increase to about 650 mm Hg seems to be unimportant.

The primary purpose of the present experiments was to examine the changes in ATBF when the P_{O_2} of the inspired air is changed. It is, however, quite possible that the effects observed are triggered by something other than the altered P_{O_2}. As first shown by Dautrebande and Haldane [8], breathing of pure oxygen at 1 atm causes a slight hyperventilation

TABLE III

EFFECT OF OXYGEN BREATHING AT 1 ATM ON ATBF IN TWO NORMAL SUBJECTS [a] (% CHANGE FROM AIR BREATHING CONTROL)

Subject	Mean Effect (percent)	(± S.D.)
J. B.	−22	(11.0)
M. W.	−25	(28.4)

[a] Seven air → oxygen → air experiments were done on each subject.

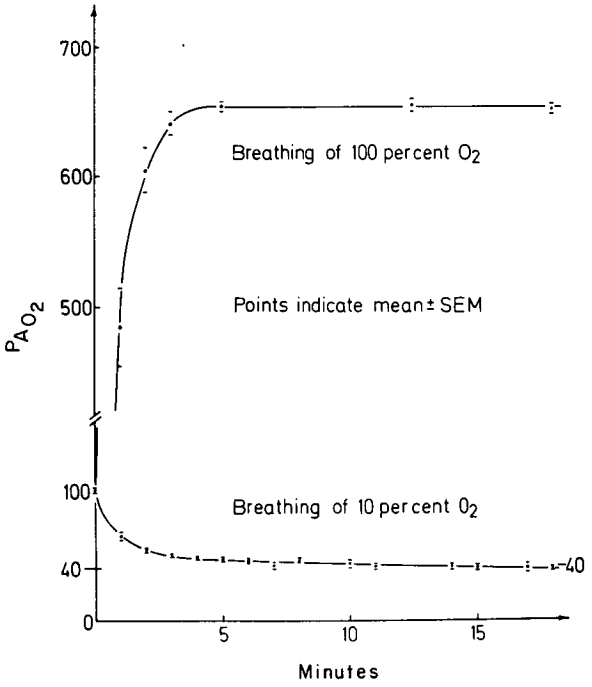

FIG. 2. Alveolar oxygen tensions during breathing of 10 and 100% oxygen. The figures were obtained from end-expiratory air, the expiratory air being continuously monitored by a Rapox oxygen analyzer.

with a fall in the alveolar and arterial CO_2 tensions. ~~The~~ These experiments ~~summarized in Table IV~~ confirm this. Since CO_2 has a local vasodilating effect, a fall in the arterial P_{CO_2} might be a factor leading to a reduced ATBF. ~~Table IV shows that~~ The reduction in ATBF during oxygen breathing does not depend on the simultaneous change in arterial CO_2 tension.

During oxygen breathing a slight increase in cerebral P_{CO_2} occurs (13). Conceivably it could contribute to the peripheral vasoconstriction. If such a mechanism were important, however, ATBF would be expected to increase during hyperventilation, when both arterial and cerebral P_{CO_2} are lowered. Such an increase was not found in the hyperventilation experiments, ~~of Table V.~~

In these experiments ~~(Table V)~~ the alveolar P_{CO_2} was lowered to the same extent as in the hypoxia experiments. Obviously hyperventilation per se does not contribute to the increased ATBF observed during hypoxia.

An important question is whether the effect of oxygen on ATBF has any practical implications. Conceivably, faster decompression schedules could be constructed if general changes in tissue perfusion rates could be foreseen and taken into account. Increased oxygen tension reduces the perfusion rate of all tissues examined so far. However, the great variation in the effect of oxygen on ATBF makes it unlikely that allowances could practically be made for this effect.

At the Third Underwater Physiology Symposium, Bornmann (5) reported a case of bends following oxygen breathing during an otherwise uncomplicated decompression from a saturation dive. Such a case may have been caused by a reduced ATBF.

TABLE IV

ATBF AND ALVEOLAR CO_2 CONTENT DURING BREATHING OF VARIOUS GAS MIXTURES AT 1 ATM[a]

Breathing Mixture	$F_{A_{CO_2}}$ (percent)	ATBF (± S.D.) (ml · 100 g^{-1} · min^{-1})
Air	5.2	4.8 (2.12)
O_2 100 percent	5.0	3.6 (2.00)
O_2 98.75 / CO_2 1.25 percent	5.2	3.4 (1.95)

[a] Number of subjects: six.
$F_{A_{CO_2}}$ was reduced during oxygen breathing in all experiments.
$ATBF_{air/O_2}$ $P < 0.005$
$ATBF_{air/O_2—CO_2}$ $P < 0.005$
$ATBFO_2/O_2+CO_2$ $P > 0.1$

TABLE V

ATBF AND ALVEOLAR CO_2 CONTENT DURING NORMAL BREATHING AND CONTROLLED HYPERVENTILATION[a]

Breathing Condition	$F_{A_{CO_2}}$ (percent)	ATBF (± S.D.) (ml · 100 g^{-1} · min^{-1})
$Normal_1$	5.6	6.7 (4.31)
Hyperventilation	4.9	6.1 (4.12)
$Normal_2$	5.6	6.5 (4.74)

[a] Number of subjects: six.
$ATBF_{normal_1/hypervent.}$ $P > 0.2$
$ATBF_{normal_2/hypervent.}$ $P > 0.1$

Finally, as a practical consequence of the findings reported here, it is emphasized that there are advantages to maintaining a constant oxygen tension in the breathing medium during experiments on decompression sickness.

ACKNOWLEDGMENTS

This paper was supported by a grant from Statens lægevidenskabelige forskningsråd (No 512-1076), by frk. P. A. Brandt's legat til Fremme af Fysiologisk-patologisk Forskning og Fysiologisk-gymnastisk Videnskab; Carl og Ellen Hertz's legat til Dansk Læge- og Naturvidenskab; F. L. Smidth & Co's jubilæumsfond, Købmand: Odense Johann og Hanne Weimann, F. Seedorff's legat and by Rask-Ørsted Fondet.

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THE EFFECT OF HYPERBARIC OXYGENATION ON THE METABOLISM OF THE LUNG

A. P. Sanders, R. S. Gelein and W. D. Currie

Many studies have been performed on the pathological changes in lung in pulmonary oxygen toxicity. Among these have been ultrastructure studies which have shown changes of cristae, density of the matrix and overall volume of the mitochondria in the great alveolar cells in oxygen toxicity of the lung (4). In addition to the mitochondrial changes, these cells showed an increase in free ribosomes and dilation of the cisternae of the endoplasmic reticulum. Other changes include interstitial and intracellular edema (4, 7, 10, 13, 14, 19), thickening of the oxygen-blood barrier (4, 10, 19) and swelling of mitochondria in alveolar epithelium. The thickening of the oxygen-blood barrier in oxygen toxicity in rat lung has been shown to be due to interstitial edema (3).

Fewer studies have been performed on biochemical changes in the lung in pulmonary oxygen toxicity. Decreased succinic dehydrogenase activity in lung has been reported in rats exhibiting pulmonary oxygen toxicity (9). Decreased sulfhydryl groups and increased disulfide groups have been shown in pulmonary oxygen toxicity in rat lung (8). Marked impairment of tracheal mucus flow in young cats exposed to 100% oxygen could be reversed by administering epinephrine, norepinephrine and adenosine triphosphate (ATP) (11). This was interpreted as an interference by oxygen with carbohydrate metabolism resulting in decreased ATP with consequent marked impairment of tracheal mucus flow. Morgan has suggested that the underlying mechanism in pulmonary oxygen toxicity could be enzyme inhibition, free radical formation, and peroxidation (12).

The ultrastructure studies and the few biochemical studies indicate that gross changes in cell metabolism of lung must occur in pulmonary oxygen toxicity. This paper reports studies of respiration and oxidative phosphorylation, succinic dehydrogenase activity, ATP concentration, ATPase activity, percent free and total cathepsin activity, percent free and total ribonuclease activity, and lipid peroxide levels in the lung from control animals and from rats exposed to 2.0 atmospheres absolute pressure (ata) oxygen for 12, 15 or 18 hours, or to 1.5 ata O_2 for 18, 24, 27 or 30 hours.

Methods

Male Sprague-Dawley rats (150-200 gm) were used in all studies. Animals subjected to the

2.0 ata O_2 exposures were fasted throughout the exposure period. Those animals exposed to 1.5 ata O_2 had standard laboratory rat chow and water ad lib. The exposure chambers used in the study were maintained at 22 ± 1°C throughout the exposure period. Oxygen flow was maintained at 1 liter per minute per animal in the chamber. Soda lime was placed in containers between each of the rat compartments (13) and at both ends of the animal cages. The cage material and separators between compartments were constructed from 3/8" (9.5 mm) square metal cloth so that there was free flow of gas throughout the chamber in all directions. Only three animals were placed in each animal compartment. Immediately after the end of the exposure period, the animals were decapitated and lungs removed and prepared for assay according to the particular biochemical parameter being studied.

Respiration and oxidative phosphorylation were determined on homogenates (5:1) of the lung by the method of Chance and Williams (5), using oxygen electrode systems in temperature-regulated reaction vessels, to determine basal and ADP-stimulated respiration rates and respiratory control ratios for α-ketoglutarate (an NAD-linked substrate to the electron transport chain) and succinate (an FAD-linked substrate). These assays on the lung homogenates were determined at normal air oxygen tensions after the rat had been exposed to the hyperbaric oxygenation levels detailed above. Succinic dehydrogenase (SDH) activity was determined on lung homogenates by the cytochrome *c* reductase method of Cooperstein et al. (6). ATP concentration of lung tissue was determined on homogenates of lung which had been frozen in liquid propane, pulverized, weighed in tared homogenizer in ice, quenched with 0°C 10% TCA, neutralized to pH 7.0 with 0°C NaOH, diluted to volume with H_2O (0-4°C), and a 2.0 cc aliquot used to assay for ATP content via the firefly extract (Sigma Chemical Company) luminescence technique development by Strehler and Totter (16). ATPase activity was determined on homogenates of the lung by the ATP difference before and after incubation in a reaction medium containing a calibrated amount of ATP.

Total activity and percent free cathepsin activity were determined on lung homogenates by the method of Tappel (17). Total and percent free ribonuclease activity were determined by the method of Slater (15). Lipid peroxide levels of lung homogenates were determined by the 2-5 thiobarbituric acid reaction (18).

Results

The results of the respiration and oxidative phosphorylation studies are shown in Fig. 1. The mean ± standard deviation for the basal respiration rate (basal Q_{O_2}) and the ADP Q_{O_2} are shown for succinate and α-ketoglutarate as a function of the time of exposure of the rat to 2.0 ata O_2. Unless indicated, the probability of being the same as controls is less than 1% ($P < 0.01$). There is a significant decrease in the ADP Q_{O_2} and basal Q_{O_2} at the 12-, 15- and 18-hour exposure periods for both succinate and α-ketoglutarate. At the 18-hour exposure, α-ketoglutarate respiration of the lung could not be stimulated above the basal respiration rate. In contrast, succinate respiration, though markedly decreased, still maintained the ability to be stimulated by ADP to a rate significantly higher than the basal rate. This indicates that the NAD-linked pathway in respiration and oxidative phosphorylation in the lung of rat is more sensitive to hyperbaric oxygenation than the succinate FAD-linked pathway.

The data shown in Fig. 1 were obtained from nonperfused rat lungs. When lungs of control animals were perfused there was no difficulty, and clear white lung tissue was

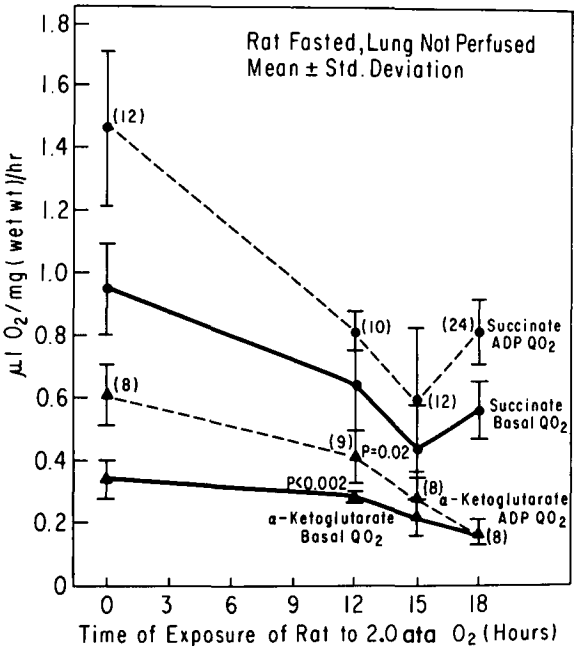

FIG. 1. Rat lung respiration.

obtained for analyses. However, after exposure to 2.0 ata O_2 or 1.5 ata O_2 for the time intervals indicated above, there was a marked difference in the ability to perfuse the lung from such animals. In general, it was impossible to get clean perfusions. Thus, if respiration rate were expressed as a function of mg protein/hour, the data would show a false low due to the inability to perfuse out the protein content of blood in the manner that could be done with controls. However, mitochondrial protein per gram of tissue varies less than 5%, when compared with controls, after 18 hours' exposure to 2.0 ata O_2.

The results of the lung respiration studies from rats exposed to 1.5 ata O_2 are shown in Fig. 2. All values shown are the mean ± standard deviation for each exposure. The probability of being the same as controls was less than 1% ($P < 0.01$) for the 18-, 24-, 27- and 30-hour exposure periods. The marked decreases in basal Q_{O_2} and ADP Q_{O_2} at all exposure periods may be attributed to decreased activity of related enzymes or electron transport chain function.

Bean (1, 2) has suggested that pulmonary oxygen toxicity may be secondary to endocrine stress since gross pathology studies of the lung from adrenalectomized animals subjected to high oxygen exposures reveal less damage than in the lung of normal rats subjected to the same exposure. Consequently, the effects of exposures to 1.5 ata O_2 for 30 hours and 2.0 ata O_2 for 15 hours on lung respiration and oxidative phosphorylation in normal rats and in adrenalectomized rats have been compared. These data are shown in Fig. 3 where both basal Q_{O_2} and ADP Q_{O_2} are expressed as percent of control (nonexposed animals of each type) values for the normal rats and the adrenalectomized rats. It is readily seen that the adrenalectomized rat lung capacity for respiration and oxidative phosphorylation had minimal, if any, protection when compared with normal rats.

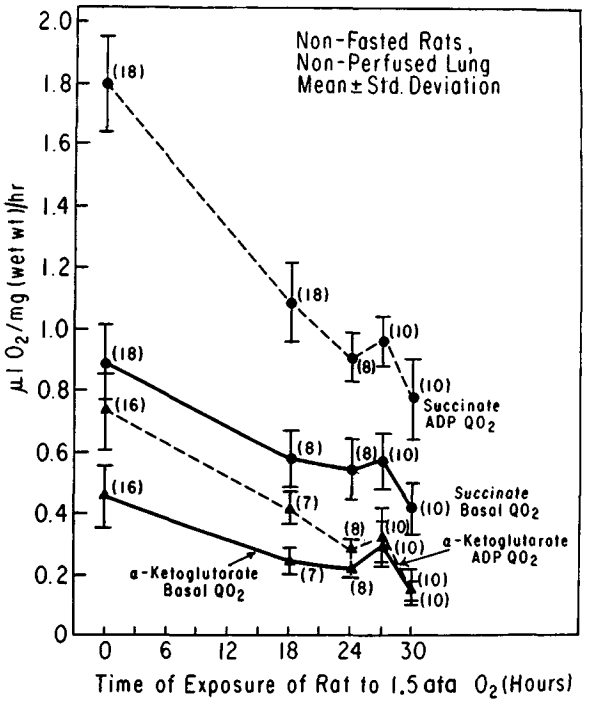

FIG. 2. Rat lung respiration.

Succinic dehydrogenase activity is shown as a function of exposure time for 1.5 ata O_2 and 2.0 ata O_2 exposures of normal rats and for two exposure times at each pressure in adrenalectomized rats in Fig. 4. All SDH activity levels for the different exposure times and pressures has a probability of less than 1% ($P < 0.01$) of being the same as controls. There was a significant decrease in SDH activity as early as 6 hours of 2.0 ata O_2 exposures, and as early as 18 hours for the 1.5 ata O_2 exposures. SDH activity was only 41.5% and 43.5% of controls after 30 hours' 1.5 ata O_2 and 18 hours' 2.0 ata O_2 exposures, respectively. The adrenalectomized animals showed no significant protection of SDH activity at the 1.5 ata O_2 exposures or at the 2.0 ata O_2 exposures.

Figure 5 shows lung ATP concentration as a function of time of exposure to 1.5 ata O_2 and 2.0 ata O_2 for normal and adrenalectomized rats. As would be expected from the respiration and oxidative phosphorylation data and the SDH activity data, there was a marked decrease in lung ATP concentration at 2.0 ata O_2 exposures of 15 and 18 hours (64.1 and 41.4% of controls, respectively), and at 1.5 ata O_2 exposures of 24, 27 and 30 hours (78.0, 63.0 and 51.2% of controls, respectively). Again it is seen that adrenalectomy did not significantly protect the animals from decrease in lung ATP concentration at the 1.5 or 2.0 ata O_2 exposures. ATP concentration levels of the lung for nonexposed control animals were 1.28 ± 0.18 μM/gm wet wt. (n = 49) and 1.31 ± 0.14 μM/gm wet wt. (n = 10) for normal and adrenalectomized rats, respectively.

The decreased ATP levels, observed at the 1.5 and 2.0 ata exposures, made it mandatory that we determine ATPase activity levels at exposure times where ATP concentration was significantly decreased to ascertain if such decreases were due to increased ATPase activity

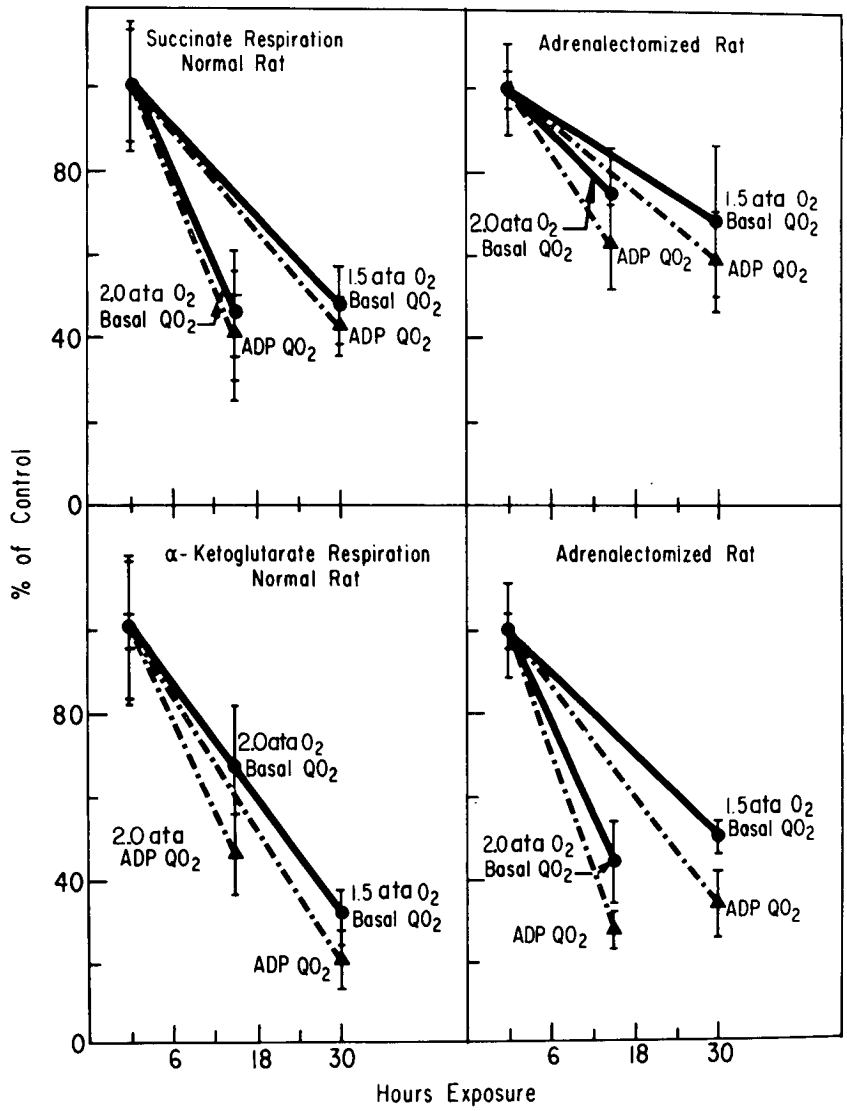

FIG. 3. Lung respiration—normal vs. adrenalectomized.

in addition to the decreased respiration and oxidative phosphorylation. Figure 6 shows the Mg^{++}-stimulated and the nonstimulated ATPase activity of lung of control rats and for 24 hours' 1.5 ata O_2 or 18 hours' 2.0 ata exposure rats. In both instances, the hyperbaric oxygen exposures resulted in a decrease of both nonstimulated and Mg^{++}-stimulated ATPase activity of the lung.

Total and percent free cathepsin activity, and total and percent free ribonuclease activity of the lung are shown as a function of time of exposure to 1.5 ata O_2 or 2.0 ata O_2 in Fig. 7. The percent free activity when expressed as percent of total activity shows no significant

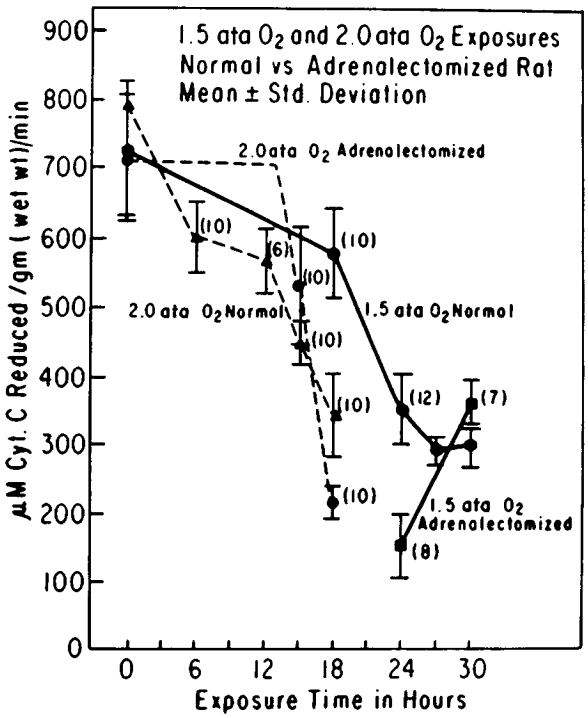

FIG. 4. Rat lung succinic dehydrogenase activity.

change. However, in light of the fact that at the longer exposures to either pressure the total activity of both cathepsin and ribonuclease is markedly decreased, it is apparent that both the total activity and the free activity of cathepsin and ribonuclease of the lung are markedly decreased by prolonged exposures (15-18 hours) to 2.0 ata O_2 and (27-30 hours) to 1.5 ata O_2.

Lung lipid peroxide levels were determined in lungs from control rats, from rats exposed to 1.5 ata O_2 for 24 or 30 hours, and from rats exposed to 2.0 ata O_2 for 15 and 18 hours. (At these exposures, significant decrease was seen in respiration and oxidative phosphorylation, SDH activity, ATP concentration, ATPase activity, free and total cathepsin and ribonuclease activities.) The lipid peroxide levels of lungs from rats subjected to these 1.5 and 2.0 ata O_2 exposure periods did not differ to a statistically significant degree from the lipid peroxide levels of lung from control rats.

Discussion

Pathological studies done simultaneously with the biochemical assays showed that decreased SDH activity, respiration and oxidative phosphorylation, and ATP concentration preceded the earliest observed pathological changes (perivascular and alveolar edema). Such changes could be explained by decreased ATP concentration leading to inability of the endothelial cells of the vasculature to maintain permeability integrity and by the inability of the cells to maintain the sodium pump at normal levels, resulting in the transport of sodium and water across the cell membrane.

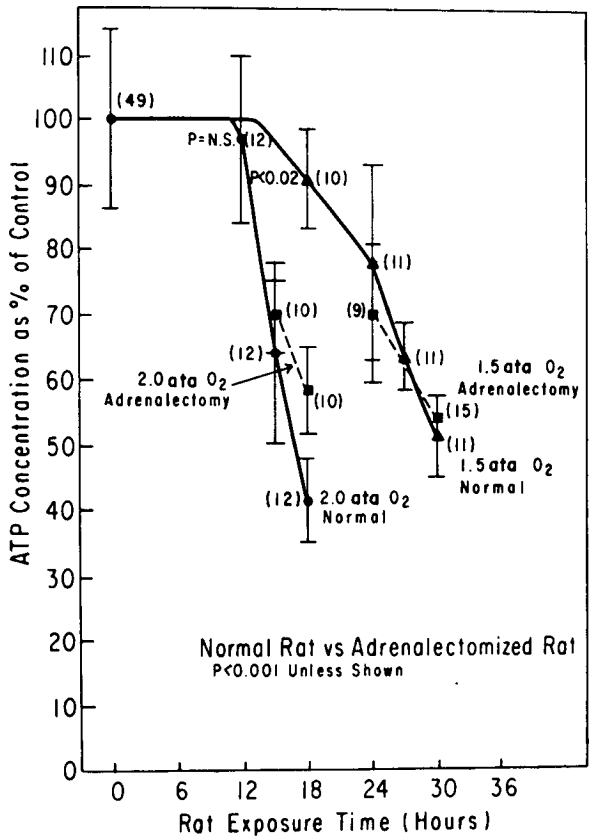

FIG. 5. Rat lung ATP concentration—1.5, 2.0 ata O_2.

The earlier observations by Bean (1,2) that adrenalectomy protects the lung against gross pathological changes seem to be in disagreement with the biochemical assays performed in these studies. We also observed that the gross appearance of the lung from the hyperbaric oxygen-exposed rats was better in the adrenalectomized animals than in the normal rats, as evidenced by a less congested appearance than in normal rats. However, the biochemical assays indicated serious biochemical damage had occurred in the lungs of the adrenalectomized animals even though the outward appearance of the adrenalectomized rat lung was better than rat lung poisoned without adrenalectomy. Consequently, we do not believe that the effects of pulmonary oxygen toxicity are secondary to epinephrine and/or norepinephrine secretion by the adrenal glands under the stress of the hyperbaric oxygenation. Rather, it appears to be a direct toxic action of hyperbaric oxygen on the cells of the lung.

The assay of lung ATP should be briefly discussed. Initially problems had been anticipated in getting the lung out of the thoracic cavity and into the liquid propane fast enough to prevent a major loss of ATP; it was recognized that as soon as the thoracic cavity was opened to atmospheric pressure the lungs would collapse. In other tissues such as brain, liver and kidney, it is essential that the tissue be placed into liquid propane in less than a second to prevent a major decrease in the ATP level. The need to do time sequence ATP

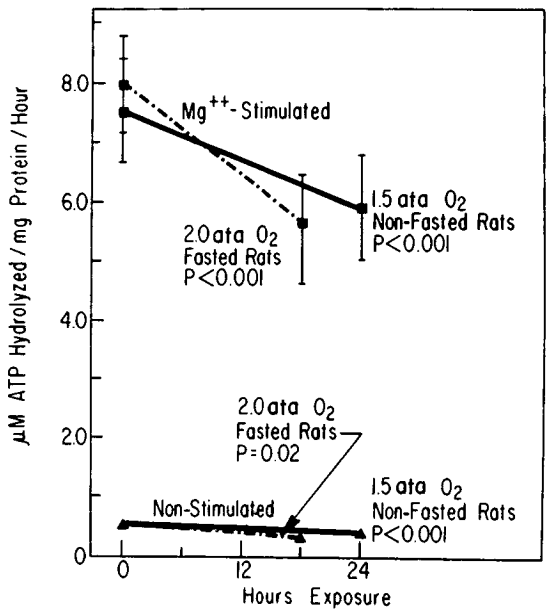

FIG. 6. Rat lung ATPase activity vs. 1.5 and 2.0 ata O_2 exposures.

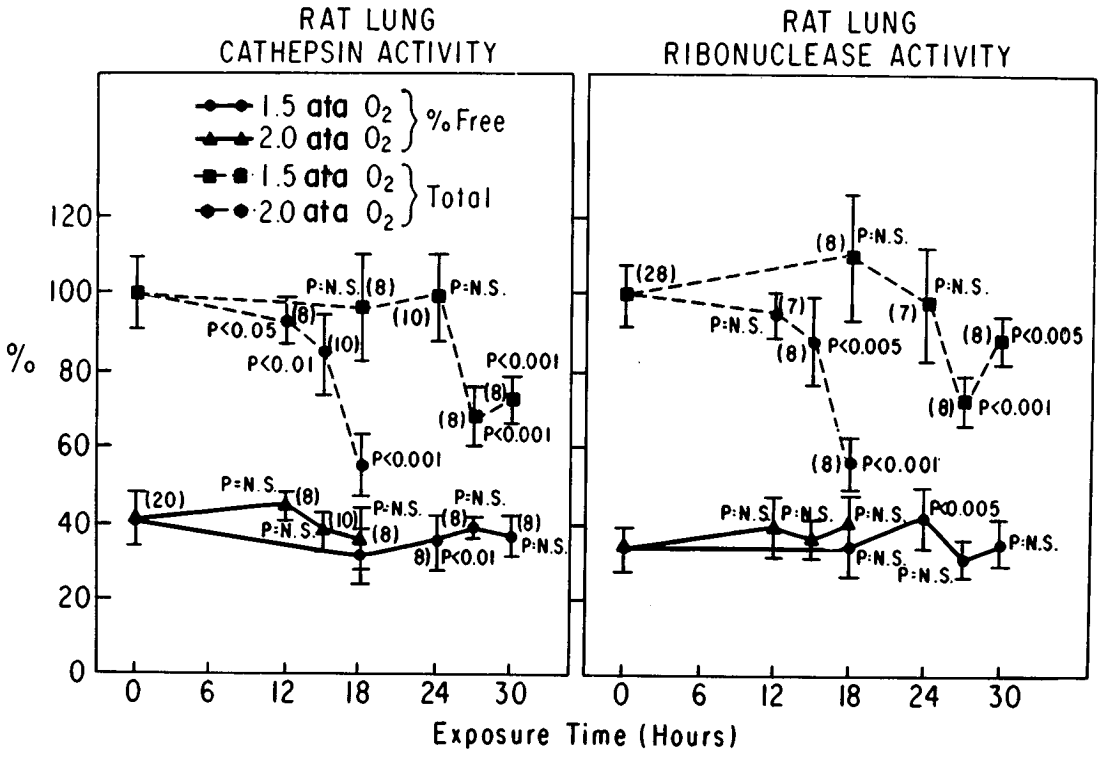

FIG. 7. Left, rat lung cathepsin activity. Right, rat lung ribonuclease activity.

concentration assays which would allow extrapolation back to time zero for quantitation of the true ATP concentration of the lung was anticipated. Surprisingly, however, the ATP concentration of lung tissue frozen at 10, 15, 30, 45 and 60 seconds after the thoracic cavity was opened gave identical results. It was assumed that this was due to the lung trapping sufficient oxygen to sustain it for this relatively long (when compared to other tissues) time period and to low ATPase activity of the lung.

The findings—decreased respiration and oxidative phosphorylation of both NAD- and FAD-linked substrates (with the NAD-linked substrate being more sensitive), and decreased ATP concentration with no increase in ATPase activity in the lung of rats exposed to 1.5 or 2.0 ata O_2 exposure periods—support the hypothesis that the primary site of damage in pulmonary oxygen toxicity is the interference with ATP production, via decreased respiration and oxidative phosphorylation with subsequent decrease in ATP concentration such that the cells are unable to maintain homeostasis and normal cell functions.

Summary

Succinic dehydrogenase activity, respiration and oxidative phosphorylation utilizing succinate and α-ketoglutarate, ATP concentration, ATPase activity, free and total cathepsin activity, free and total ribonuclease activity and lipid peroxide levels were determined in the lung from control rats and from rats exposed to 12, 15, or 18 hours of 2.0 ata O_2, or to 18, 24 and 30 hours of 1.5 ata O_2. Significant decreases (when compared to controls) were observed in (SDH) activity, respiration and oxidative phosphorylation, (ATP) concentration, ATPase activity, and free and total cathepsin and ribonuclease activity in the lung from animals exposed to 1.5 ata O_2 or 2.0 ata O_2.

Adrenalectomy did not give protection as indicated by measurements of respiration and oxidative phosphorylation, SDH activity and ATP concentration of the lung from animals exposed to 1.5 ata O_2 or 2.0 ata O_2. The biochemical changes in the lung in oxygen toxicity do not appear to be secondary to epinephrine or norepinephrine secreted by the adrenal glands, due to the stress of hyperbaric oxygenation, as indicated by the comparison of adrenalectomized rats with normal rats.

Lipid peroxide levels did not vary significantly from controls at these levels. Lipid peroxide levels, cathepsin activity and ribonuclease activity appear to play no role in the changes observed in lung in pulmonary oxygen toxicity at 1.5 ata O_2 or 2.0 ata O_2. The synthesis of ATP in lung by NAD-linked substrate (α-ketoglutarate) is more sensitive to inhibition by a high oxygen environment than ATP synthesis via FAD-linked substrates (succinate). However, both pathways of ATP synthesis are decreased by hyperbaric oxygenation. Thus, the primary site of damage in pulmonary oxygen toxicity is interference with ATP production as evidenced by decreased ATP levels under conditions which decreased metabolic synthesis of ATP and did not increase the ATPase activity.

Acknowledgments

This research was supported in part by Office of Naval Research contract N00014-67-A-0251-002 with funds provided by the Naval Medical Research and Development Command, and National Heart and Lung Institute contract 71-2154.

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THE EFFECTS OF OXYGEN ON PULMONARY PHOSPHOLIPID SYNTHESIS

H. Gilder and C. K. McSherry

High oxygen tensions cause a reduction of pulmonary surfactant as determined by measuring the surface tension of minced lung extracts (16) and by an in vitro assay which measures the incorporation of radioactive palmitic acid into lecithin (7). Pulmonary surfactant is a lipoprotein having a high proportion of lipid which is predominantly the lecithin, dipalmitoyl phosphatidylcholine. In an effort to explore the relationship of surfactant inhibition to the pulmonary lesions induced by high oxygen tensions, studies of the synthetic pathways of lecithin in the lung have been performed.

The major pathway for the synthesis of lecithin is the incorporation of phosphoryl choline into diglyceride via the intermediate cytidine diphosphate choline (14). A quantitatively less important pathway (except in the liver) involves an initial synthesis of phosphatidylethanolamine which is then methylated via *S*-adenosylmethionine (2). An alternative manner in which lecithin is synthesized, and the one concerned here, involves two enzymes, i.e., a phospholipase which hydrolyses lecithin to lysolecithin and an acyltransferase which then re-esterifies the lysolecithin. Preferential localization of saturated fatty acids occurs in the α position and the unsaturated fatty acids in the β position of the lecithin molecule (3). This distribution has been postulated to depend on a monoacyl-diacyl lecithin cycle whereby the fatty acids in the lecithin molecule are replaced with specific fatty acids when needed (21). By this reaction lecithins with particular properties can be synthesized for specific tissues. Thus in lung, nonspecific lecithin may be transformed to the dipalmitoyl lecithin which, combined with a glycoprotein, is particularly efficient in reducing the surface tension of the film lining the alveolar membrane. Although this scheme does not represent de novo synthesis of lecithin, it could be a significant step in the lung and explain the relatively high concentration of saturated fatty acids in lung lecithins (15).

In 1960, Lands described an enzyme in liver microsomes which acylates lysolecithin (11). Since then similar enzymes have been described in brain, red cells, parotid glands and a number of other tissues (19, 20, 22, 25-27).

Phospholipases are present in many tissues. Individual enzymes have been isolated which specifically hydrolyze one or the other or both of the fatty acids in the lecithin molecule (the phospholipases A and B), or release diglyceride or choline (the phospholipases C and D,

respectively) (1, 13). A combined effect of phospholipase A and B with an acyltransferase could produce a selective synthesis of surfactant lecithin.

Our previous study measured radioactive palmitic acid incorporation into the lecithin of lung slices as an indication of surfactant lecithin synthesis by any of the pathways known. The present study attempts to assess the importance of transacylation in surfactant synthesis and to determine whether the enzyme is altered in the lungs of rabbits that have been exposed to increased oxygen tensions. Two sets of enzyme systems have been studied in crude lung homogenates: the acyltransferases which transfer fatty acid in the form of its coenzyme A derivative to lysophosphatidylcholine (lysolecithin) to form diacylphosphatidylcholine (lecithin); and the phospholipases which remove one or both fatty acids from lecithin. This investigation deals with the requirements of these two enzymes for optimal activity and their relative level in the lungs of normal rabbits and of rabbits exposed to high oxygen tensions (OHP). The study reveals that the acyltransferase is depressed by OHP and appears to be a sensitive indication of defects in surfactant, whereas the phospholipase levels in lung are not affected by OHP.

Methods

Rabbits were killed by intravenous injection of sodium pentobarbital, the chest opened, major vessels clamped off, and the lungs removed and inflated via the trachea. Part of the lung was utilized for assay of surfactant lecithin synthesis as previously described (7). For the enzyme studies portions of lung were weighed and ground for 6 minutes in a Potter grinder with Teflon plunger with 9 parts of Krebs-Ringer phosphate buffer, pH 7.4 (4) containing 21 m*M* $MgCl_2$ (KRP buffer). The homogenate was spun at 4°C at 1,000 rpm for 2 minutes, and the supernatant material, about 85% of the total volume, was decanted from the cellular residue and used for the following studies.

For measurement of acyltransferase the lipid substrates, nonradioactive palmitic acid dissolved in ethanol, and lysolecithin* dissolved in chloroform were pipetted into 20-ml screw cap test tubes with two glass beads, and the solvent evaporated off by warming at 35°C under a stream of nitrogen. Except for special experiments described later, the palmitic acid and lysolecithin were in concentrations of 1.6 m*M* and 0.2 m*M*, respectively. The aqueous agents were then added, consisting of adenosine-5-triphosphate (ATP), potassium fluoride, and palmitic acid-1-^{14}C† complexed to albumin (0.5 x 10^6 cpm and 0.033 μmol of palmitic acid) (7). The homogenate representing 0.07 or 0.10 gm of lung in KRP buffer was added to make a final volume of 1 ml. Coenzyme A was added last and the open tubes were incubated with slow shaking at 37°C. (See Tables and Figures for the reagents in specific experiments.) Control tubes at zero time and incubated tubes at 30 minutes were inactivated by the addition of 1.4 ml methanol followed by 2.8 ml of chloroform in preparation for fat extraction.

Phospholipase activity was determined by measuring the decrease of radioactive lecithin in incubates containing uniformly ^{14}C-labeled lecithin.† A crude vegetable lecithin‡ was added

*The lysolecithin (Sigma, L-6626) is derived from egg lecithin and contains primarily palmitic and stearic acids (1).

† Palmitic acid-1-^{14}C (NEC-075) and lecithin-^{14}C (U) (NEC-588) were purchased from New England Nuclear Corp. The ^{14}C-lecithin was prepared from *Chlorella* grown in a $^{14}CO_2$ atmosphere.

‡Vegetable lecithin from Schwarz-Mann (2207). TLC analysis revealed that 50% of its phospholipid was lecithin. Calculations of phospholipase activity took this into consideration. Experiments with this lecithin after purification with TLC did not affect the results.

as a carrier. It was assumed that the preponderance of fatty acid in the two lecithins was the same, both being of vegetable origin; that endogenous lecithin added in the homogenate was constant; and that for the purposes of a crude assay it was permissible to consider them to be in the same pool. The behaviour of the enzyme systems with varying amounts of added carrier lecithin tended to confirm this assumption.

For the phospholipase analysis the lipids were dried under nitrogen as above. To the dried lipids were added ATP, 50 m*M*, glutathione, 0.1 m*M*, homogenate representing 0.07 gm of lung and KRP buffer to make a final volume of 1 ml. The tubes were incubated for 15 minutes.

Lipids were extracted from the incubation mixtures by a modified Folch procedure (5). The final extract was brought to 0.5 ml with $CHCl_3$ and 0.06 ml applied to precoated silica gel F254 sheets on plastic.* The thin-layer chromatography (TLC), the visualization of phospholipid spots, and the analysis by liquid scintillation spectrophotometry have been described previously (6).

For the calculation of transacylation activity, the radioactivity of newly synthesized lecithin was taken as the difference between the cpm of lecithin in the control tube and that in the incubated tube. This figure in terms of cpm per gram per hour (cpm/gm/hr), was divided by the specific activity (SA) of the fatty acid in the incubation mixture to give the μmol/gm/hr of lecithin synthesis.

Phospholipase was calculated from the loss of radioactive lecithin in 15 minutes and the SA of the incubation mixture, the final result being in μmol of lecithin hydrolyzed/gm/hr without regard to whether one or two fatty acids were removed. It was also assumed that other phospholipases, such as the one which attacks the choline moiety, were negligible in the system (1).

Increased O_2 tensions were achieved by means of a Bethlehem Co. hyperbaric chamber (16). In the present series all the rabbits were exposed to 100% O_2 at 3 ata for 3 hours followed by 45 minutes of decompression.

Results

Table I describes a typical acyltransferase experiment showing the importance of the various ingredients of the medium for optimal activity of the enzyme in normal rabbit lung homogenate. The data represent the fatty acid uptake into lecithin in 30 minutes at 37°C and are calculated in terms of μmol fatty acid incorporated per gram of lung per hour. Experiments on the requirement of carrier fatty acid and lysolecithin are shown below. The enzyme system absolutely requires ATP and coenzyme A. Its performance is enhanced by the addition of magnesium and fluoride. Bovine serum albumin and Tween (polyoxyethylene [20] sorbitan monolaurate) were assayed in the system with the intention that these could be added to solubilize the lipids. However, both substances inhibited the system. Glutathione also depressed the activity. With the complete medium, and the quantities of palmitic acid and lysolecithin shown in the table, normal lung homogenates incorporated from 4 to 11 μmol of fatty acid per gram of lung per hour.

The rate of acyltransferase activity is constant for 30 minutes but decreases slightly in the subsequent 30 minutes (Fig. 1).

*Brinkman Instruments, Inc.

TABLE I

TRANSACYLATION SYSTEM: REQUIREMENT OF FACTORS FOR OPTIMAL LECITHIN FORMATION[a]

Incubation Mixture	Acyltransferase[b] μmol/gm/hr
Complete medium	7.7
Omit lysolecithin	3.3
Omit palmitic acid	0.4
Omit ATP	0
Omit Mg	4.1
Omit F	6.0
Double ATP, omit F	7.9
Omit CoA	0

[a] A typical experiment on normal lung homogenate. The complete reaction medium contained lysolecithin, 0.2 m*M*; palmitic acid, 1.6 m*M*; ATP, 10 m*M*; Mg, 21 m*M*; F, 40 m*M*; CoA, 0.04 m*M*; homogenate representing 0.10 gm of lung and KRP buffer to a total volume of 1 ml.

[b] In terms of μmol palmitic acid incorporated into lecithin.

The level of the two substrates, palmitic acid and lysolecithin, affects the activity of the enzyme over a wide range. As palmitic acid is increased from 0 to 3.2 m*M*, the activity increases linearly to about 1.6 m*M* (Fig. 2). Above this level the curve becomes nonlinear probably because of the insolubility of the fatty acid. The level of 1.6 m*M* of palmitic acid where the incubates were clear has been utilized in all the studies.

With the addition of increasing amounts of lysolecithin up to 0.5 m*M*, acyltransferase increases to about four times the level of the sample without added lysolecithin (Fig. 3). Higher levels of lysolecithin appear to inhibit or decrease the enzyme activity.

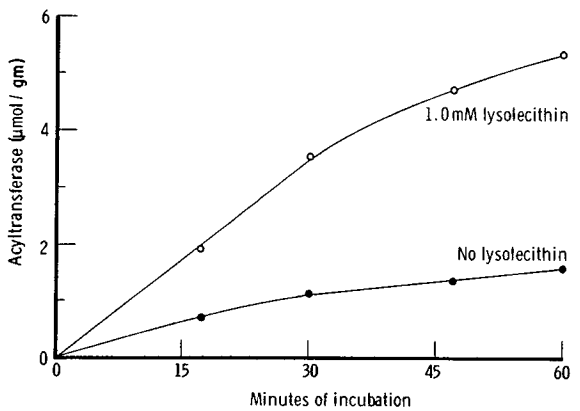

FIG. 1. Acyltransferase activity of lung homogenates with time of incubation. The reaction mixture contained crude homogenate from 0.1 gm of lung in KRP buffer; lysolecithin as shown; palmitic acid, 1.6 m*M*; ATP, 10 m*M*; magnesium, 21 m*M*; fluoride, 10 m*M*; coenzyme A, 0.02 m*M*; and 0.5 x 10^6 cpm palmitic-1-^{14}C in a final volume of 1 ml. Tubes were incubated at 37°C for the times shown.

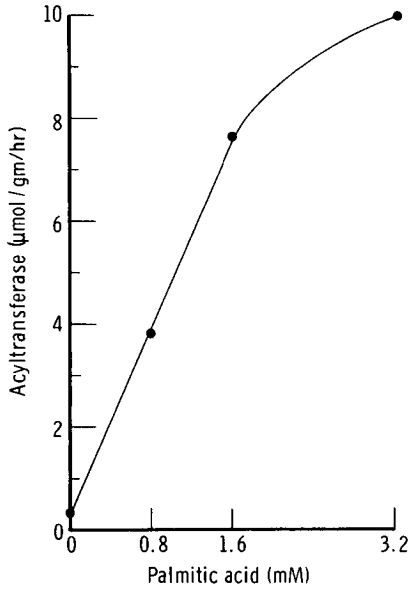

FIG. 2 Effect of variations of palmitic acid concentration on acyltransferase activity. The incubation mixture is the same as that in Fig. 1 except that the nonradioactive palmitic acid is shown in the abscissa and the lysolecithin added is 0.2 m*M*. All tubes were incubated for 30 minutes.

The lysolecithin content of lung has been estimated at 0.002 mmol per gram (9); therefore, the concentration of endogenous lysolecithin in the incubates in the experiment represented in Fig. 3 was about 0.2 m*M*. The acyltransferase activity of the incubates can, therefore, be accounted for by the endogenous lysolecithin in the first sample, and by the sum of endogenous lysolecithin and added lysolecithin in the other samples. The dashed line

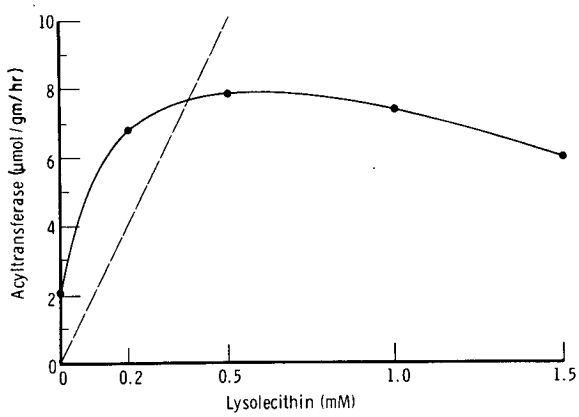

FIG. 3. Influence of the concentration of lysolecithin on acyltransferase activity. The incubation mixture is the same as that in Fig. 1 except that lysolecithin was added in the amounts shown and the crude homogenate represents 0.07 gm of lung. The dashed line represents the calculated acyltransferase if all added lysolecithin had been acylated (see text).

in the figure indicates the lecithin that would have been synthesized if all the added lysolecithin had been utilized. In the presence of 0.2 m*M* added lysolecithin, the enzyme had to utilize endogenous lysolecithin. At higher levels of lysolecithin, lecithin synthesis did not keep up with the lysolecithin present.

Table II summarizes the acyltransferase activity of lung homogenates. When no lysolecithin was added the acyltransferase ranged from 1.2 to 3.3 μmol/gm/hr in lungs of normal rabbits and of rabbits exposed to OHP. When lysolecithin was added to the incubates there was a significant difference between the two groups. The mean acyltransferase activity in the

TABLE II

ACYLTRANSFERASE ACTIVITY OF LUNG HOMOGENATES FROM RABBITS IN AIR AND EXPOSED TO 100% OXYGEN AT 3 ATA FOR 3 HOURS (OHP)[a]

		Acyltransferase μmol/gm/hr Added Lysolecithin	
		None	0.2 m*M*
Normal			
		3.26	7.66
		—	7.34
		1.82	4.77
		1.72	8.06
		3.30	7.46
		2.16	6.80
		1.24	7.04
		1.32	8.34
		1.30	8.80
		2.30	11.24
	Mean	2.05	7.75
	S. D.	± 0.75	± 1.56
OHP			
		2.08	3.61
		1.16	6.68
		1.45	4.06
		1.59	5.11
		1.56	5.58
		1.32	3.97
		1.70	6.28
	Mean	1.55	5.04
	S. D.	± 0.27	± 1.11
	P	> 0.1	< 0.001

[a] The composition of the medium is the same as that of Table I except that the homogenate added represented 0.07 gm of lung. The volume was brought to 1 ml with KRP buffer.

normal rabbits was 7.75 ± 1.56 μmol/gm/hr and in the OHP group 5.04 ± 1.11 μmol/gm/hr.

The phospholipase system measures any phospholipase which destroys lecithin since the disappearance of radioactive lecithin from the TLC-isolated lecithin is being measured. In the incubation mixture that was used, it was found that ATP and glutathione enhanced the activity by about 20%. The presence of added lecithin over the amount in the lung itself also increased the activity. No effort was made to determine the maximum level, which is over 5 m*M*, but instead the arbitrary level of 1.5 m*M* was present in all incubation mixtures of which about one-half was calculated to be endogenous, that is the lecithin present in the homogenate itself. Figure 4 summarizes the phospholipase activity in a series of normal rabbits and rabbits exposed to OHP. It is apparent that there is a wide range of activities in both groups and that OHP does not affect the system as determined in this experimental model.

Surfactant lecithin synthesis was determined by the incorporation of radioactive palmitic acid into lecithin of lung slices (7) on the rabbits in the present series. The exposure to 100% O_2 at 3 ata for 3 hours had previously been shown to produce toxic but not lethal pulmonary damage by this method. Figure 5 depicts the relationship between lecithin synthesis and acyltransferase activity of the same lung. The groups of rabbits exposed to OHP had normal incorporation of fatty acid into the lecithin of lung slices whereas the acyltransferase activity was significantly reduced. It is evident that the latter system is more sensitive to oxygen toxicity than is the measurement of overall lecithin synthesis in lung slices. It leads to the speculation that the acyltransferase pathway in vivo may be particularly sensitive to oxygen toxicity.

Discussion

This work attempts to determine whether the acyltransferase pathway for lecithin synthesis plays any special part in the manufacture of the lecithin of the surfactant system. The

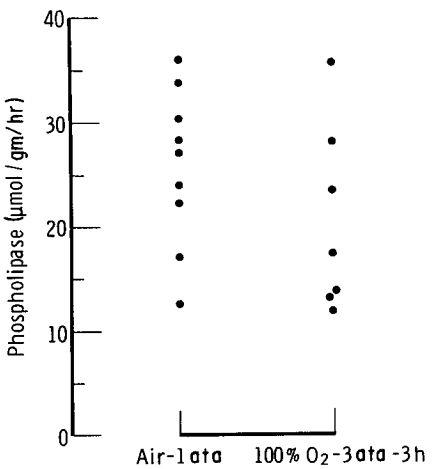

FIG. 4. Phospholipase activity of crude lung homogenates of normal rabbits and rabbits exposed to 100% O_2 at 3 ata for 3 hours. (See text for composition of medium.)

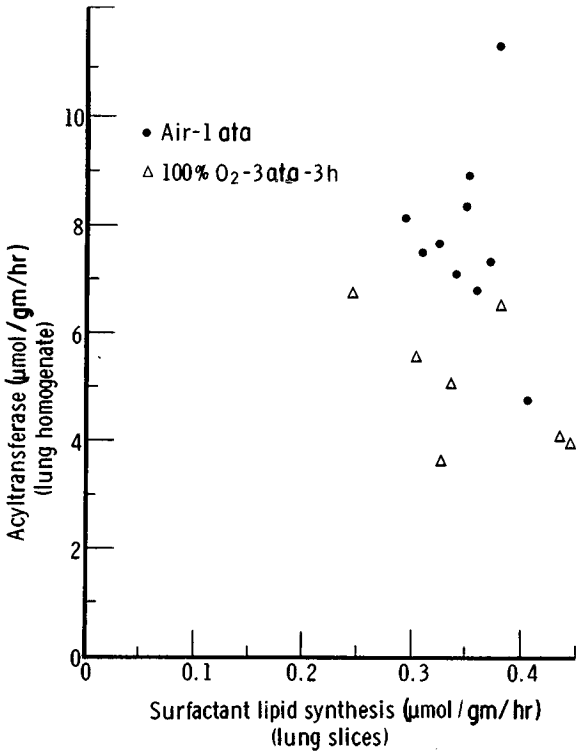

FIG. 5. Correlation between surfactant lipid synthesis as measured by the slice technique (7) and transacylation of lung homogenates in the presence of 0.2 m*M* added lysolecithin. OHP rabbits were exposed to 100% O_2 at 3 ata for 3 hours followed by a decompression time of 45 minutes.

relatively short half-life of lung lecithin, determined by Tierney et al. (23) to be only 14 hours, suggests that this may be the case. Using the 14-hour figure and 20 μmol/gm as the lecithin content of whole lung, it is possible to calculate that the rate of lecithin synthesis is 0.8 μmol/gm/hr. In the acyltransferase system described above, synthesis of lecithin from lysolecithin varied from 1 to 3 μmol/gm/hr when no lysolecithin was added to the medium, so that the potential for acyltransferase is high and the renewal of the fatty acid moiety only of the lecithin molecule may explain the short half-life. The mechanism is important in providing specific appropriate fatty acids in lecithins for specific functions, and this could be particularly true of surfactant lecithin which is known to contain palmitic acid in higher concentration than in other body lecithins (10).

The one obvious fallacy in equating whole lung lecithin synthesis with surfactant lecithin synthesis is that the latter represents only a part of total cell phospholipid synthesis, the remainder going to maintain membranes and to perform other functions in the cells. Nevertheless, lung lecithin is a specialized one. Forty-six percent of the total lung lecithin is dipalmitoyl lecithin while in other organs, such as the kidney and brain, the level is about 30% (9, 15). The composition of lipids extracted from alveolar washings significantly differs from that extracted from whole lung (8, 17, 18, 22-24). Unfortunately, in a study of cellular

enzymes we cannot take advantage of this fact in order to study the specific lecithin synthesized for use in surfactant.

Several workers have identified and studied the acyltransferases in mitochondria or crude homogenates of liver (11), erythrocytes (20), aorta (11), salivary glands (19) and brain mitochondria (26) with and without added lysolecithin, using palmitic acid and/or oleic acid as the acyl donor. The levels of acyltransferase were much lower than the ones shown in the present work because of the absence of carrier fatty acid. Another study by Webster (27) on homogenates of a number of different tissues in the presence of 1.4 m*M* each of lysolecithin and oleic acid showed activities from 3.0 μmol/gm/hr in spleen to 16 μmol/gm/hr in liver, and a figure of 3.5 μmol/gm/hr in lung which is in the same range as in the present study. However, the level of lysolecithin that he used would be inhibitory in these experiments and the absence of a detailed description of Webster's experiments precludes a comparison with the present work.

Lung homogenate phospholipase along with the acyltransferase enzyme was chosen for study because lysolecithin levels are low in tissues and because the acyltransferase activity in the in vitro system was strongly dependent on the amount of lysolecithin added to the medium. It was conjectured that the supply of lysolecithin might be the limiting factor and this could be affected by total phospholipase activity. A correlation of the two enzymes in the same homogenates might be interesting in view of the reduced transacylation in rabbits exposed to OHP. To date, it has not been possible to detect such a correlation between the two enzyme systems in lung homogenates. This confirms Webster (27), who found no apparent correlation between the activity of his acylating system in a study of a variety of tissues and the activity of phospholipase in acetone precipitates in the tissues as described by Gallai-Hatchard and Thompson (6).

The present preliminary study showing that the acyltransferases of lung homogenates are affected when the animals are exposed to OHP suggests that more detailed studies are in order to determine whether this is responsible for the defects observed with exposure to OHP. Lands et al. (12), Van den Bosch and Van Deenan (24) and others have made more detailed observation of selective discrimination of acyltransferases for fatty acids of various chain lengths and degrees of saturation. This kind of study along with investigation of the localization of these enzymes in lung cell organelles should be considered.

Summary

High oxygen pressures are associated with a reduction of the synthesis of pulmonary surfactant lecithin as determined by the rate of incorporation of palmitic acid into lecithin in lung slices. Acyltransferase, the enzyme which transfers fatty acid to lysolecithin to form lecithin, has been studied in crude lung homogenates of rabbits at room air and exposed to 100% oxygen at 3 ata for 3 hours. The method measures the incorporation of palmitic acid-1-^{14}C into lecithin when lysolecithin is added to the medium. For optimal activity the enzyme requires fatty acid (1.6 m*M*), ATP, CoA, Mg, and F. When only endogenous lysolecithin was present, fatty acid incorporation was 2.05 ± 0.75 μmol/gm/hr (mean ± S.D.). The addition of 0.2 m*M* lysolecithin brought the activity to 7.75 ± 1.56 μmol/gm/hr. Exposure to OHP significantly reduced the acyltransferase activity to 1.55 ± 0.27 μmol/gm/hr and 5.04 ± 1.11 μmol/gm/hr when no lysolecithin was added and when exogenous lysolecithin was added, respectively.

A crude phospholipase assay of the same homogenates revealed no significant difference between the normal rabbits and the rabbits exposed to increased oxygen tensions.

These studies reveal that the acyltransferase enzyme system is more sensitive to O_2 toxicity than is a system for measuring overall lecithin synthesis by the incorporation of radioactive palmitic acid into the lecithin of lung slices, and therefore suggest the possibility that the inhibition of the enzyme may be the cause of decreased surfactant lecithin in O_2 toxicity.

The suggestion that the acyltransferases may have an essential role in synthesizing highly saturated lecithins required in the surfactant system is discussed.

ACKNOWLEDGMENTS

This study was supported in part by the Office of Naval Research, Contract N00014-70-A-0412-0001.

The authors gratefully acknowledge the expert technical assistance of Mrs. Mira Valcic and Mrs. Patricia May.

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EFFECTS OF A NORMOBARIC HYPEROXIA ON PULMONARY SURFACTANT IN THE RAT

B. Broussolle, H. Burnet, R. Ricci, C. Boutier, S. Plouvier, R. Hyacinthe, A. Baret and A. Barranx

Pulmonary surfactant is a biological substance which lines the pulmonary alveoli and changes the surface tension at the liquid-air interface. It is produced by particular alveolar epithelial cells, i.e., type II pneumocytes. It is composed of: 1) phospholipids, in particular dipalmitoyl-lecithin, which lowers surface tension; 2) nonphosphated lipids, the role of which is to protect the phospholipids against oxidation; and 3) proteins, forming a skeleton which gives cohesion to the other constituents.

The surface tension at the alveolar surface is responsible for about two-thirds of the retraction force of the lung, and the inherent elasticity of the lung parenchyma for one-third. The physiological importance of the pulmonary surfactant is, therefore, evident.

Animals exposed to 100% oxygen at normobaric or hyperbaric pressures for appropriate periods show lung lesions characterized by an edematous alveolitis and areas of atelectasis. This is the syndrome of pulmonary oxygen poisoning. Such lesions are compatible with a decrease in production or an impairment in activity of pulmonary surfactant, either secondary to or contemporaneous with lesions of the pulmonary capillaries. The present study is concerned with an investigation of changes in pulmonary surfactant in rats exposed to normobaric pure oxygen.

Experimental Procedure

After approximately 60 hours' constant exposure to normobaric hyperoxia, pulmonary lesions led to the death of all animals exposed. However, it is possible to lengthen the survival time considerably if, after 48 hours' exposure, the animals are put back into air for 5 hours. They seem then to have adapted since they will now survive for more than 20 days if subsequently exposed to a pure oxygen atmosphere.

This technique allows the study of changes in the quality and quantity of surfactant and of pulmonary lesions under such hyperoxic conditions. It is hoped that a better understanding of these processes will allow eventual production of a pharmacological means of preventing pulmonary oxygen toxicity.

Pulmonary surfactant from the experimental animals was studied physically by measuring the surface tension of minced lung extracts, and by chemical analysis of the liquid obtained from lung lavage to estimate phospholipid concentration. Individual phospholipid components were then identified by thin-layer chromatography on silica gel. The results obtained are compared with a histopathological study of the pulmonary tissue itself.

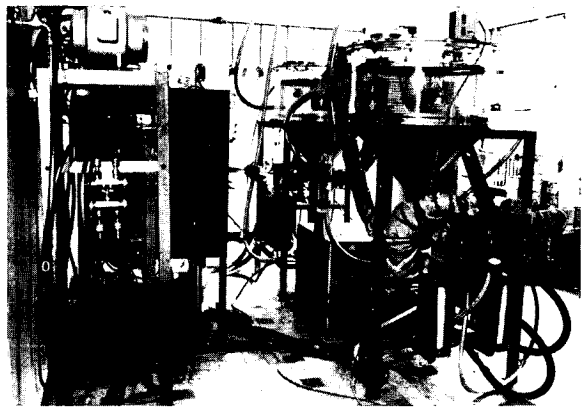

FIG. 1 Oxygen chamber at atmospheric pressure.

TECHNIQUE OF ANIMAL EXPOSURE TO PURE OXYGEN

The same exposure technique was employed throughout, regardless of subsequent investigations. Male albino rats weighing between 250 and 300 gm were used. They were exposed to pure oxygen in a sealed chamber designed for animal experiments and large enough to accommodate 20 to 30 rats (Fig. 1). The chamber is equipped with a closed ventilation circuit supplying oxygen at an over-pressure of a few cm of water; a cooling system maintaining an average internal temperature of 25°C; an average partial pressure of water vapor of 25 mm Hg; and a soda-lime filter to scrub out carbon dioxide. Water and food were allowed at will, the food being in a granular form. Experiments were performed on approximately 400 rats in all.

Figure 2 outlines the procedure used. The first set of rats was exposed to pure oxygen for 48 hours only. The second set was exposed for 48 hours, adapted by 5 hours' exposure to air, and then re-exposed to pure oxygen for the remainder of the time. The animals of the first set were killed immediately following the 48-hour exposure. A set of control animals exposed to air at atmospheric pressure for 48 hours was killed at the end of the exposure. The animals in the second set, which were adapted, were killed at various times during the

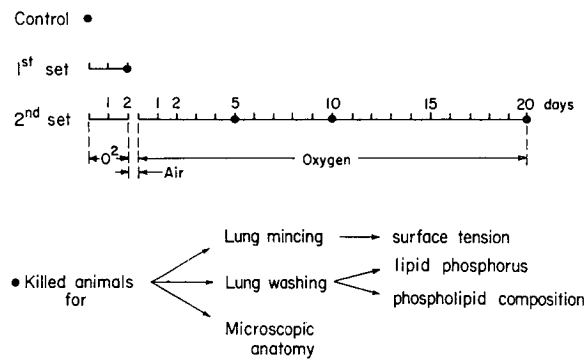

FIG. 2. Cahn electrobalance connected to an *X*, *Y* plotter.

re-exposure to oxygen, i.e., after 5, 10 and 20 days' exposure. The actual method employed to kill the animals in each group depended upon the nature of the subsequent investigation to be performed. Such details will be described for each method of study.

Study of the Surface Tension of Minced Pulmonary Tissue Extracts

After anesthesia the rats were exsanguinated via the carotid artery. The lungs were withdrawn, examined visually and then minced. The surface tension of the minced extracts, diluted with physiological serum (1), was studied according to Clement's technique by means of a Cahn's electrobalance.

On an *X, Y* recorder the surface tension of the extract in dynes per centimeter was plotted against the percentage of the surface area of the film (from 25% to 100% of the surface) (Fig. 3).

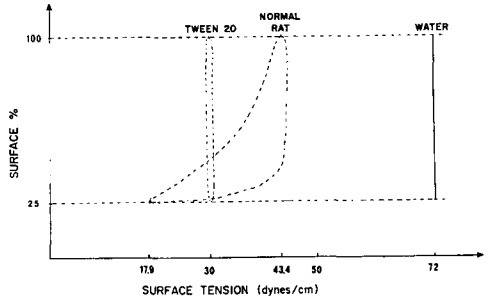

FIG. 3. Diagram of superficial tension versus surface for: water, Tween 20, and smashed lung of control rat.

It is known (17) that pulmonary surfactant causes variations in surface tension of the film as a function of its surface area (hysteresis). More important, however, the greater the quantity of surfactant present the more is the *average* surface tension of the hysteresis loop decreased. Pulmonary surfactant of good quality produces a great difference between the maximum surface tension (γmax) for the largest surface, and the minimum surface tension (γmin) for the smallest surface. Hence, the surface area of the hysteresis loop is large. Figure 3 shows the surface tensions of water (about 72 dynes/cm) and of water after the addition of a common surfactant product (Tween 20) (about 30 dynes/cm) with little variation in γ with surface area.

Description of the curves obtained. Clements, in discussing the relationship between the stability of the alveolar structure on the one hand, and the quantity of pulmonary surfactant on the other, has established an index of such stability as shown in Eq. 1:

$$\bar{S} = \frac{2(\gamma max - \gamma min)}{\gamma max + \gamma min} \tag{1}$$

where γmax = maximum surface tension
γmin = minimum surface tension

The mean surface tension may be determined by Eq. 2:

$$\gamma\text{mean} = \frac{\gamma\text{max} + \gamma\text{min}}{2} \tag{2}$$

γmean is an index of quantity. The difference between maximum and minimum surface tensions (Eq. 3) represents the characteristic property of the pulmonary surfactant hysteresis. Δ_γ is an index of quality of the surfactant:

$$\Delta_\gamma = \gamma\text{max} - \gamma\text{min} \tag{3}$$

The stability index can now be written:

$$\overline{S} = \frac{\Delta_\gamma}{\gamma\text{mean}} \tag{4}$$

In evaluating the hysteresis loops of surface tension plotted against percent area, we have studied: 1) maximum and minimum surface tensions (γmax and γmin); 2) mean surface tension (γmean); 3) the difference of surface tension, i.e., the difference between maximum and minimum surface tensions (Δ_γ); 4) the Clements' index of stability, $\overline{S}$, which takes into account 2) and 3); and 5) the surface area of the hysteresis loop itself. Also, each lung was weighed in order to estimate the importance of any pulmonary edema present.

Figure 4 shows a surface tension hysteresis loop from a control rat and similar loops from adapted rats which were re-exposed for 10 and 20 days, respectively. The loop recorded after 10 days of re-exposure to oxygen showed the greatest qualitative impairment in the properties of surfactant (Δ_γ) and the effect of an increase in quantity of surfactant (γmean decreased).

Results. Results are shown in Table I for both nonadapted and adapted rats.

Nonadapted rats. When compared with control rats the nonadapted rats, exposed continuously to pure oxygen for 48 hours can be subdivided into two distinct populations: A and B.

For population A, which constituted 67% of the whole group, Δ_γ, i.e., the difference in surface tension, stability index $\overline{S}$ and the total surface area of the hysteresis loop were all

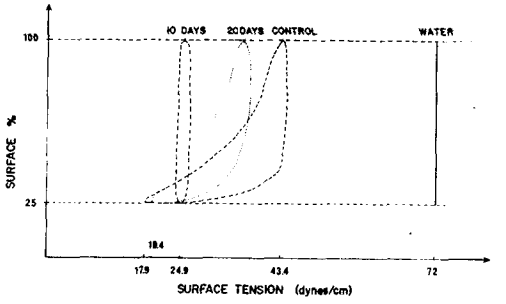

FIG. 4. Average curves of superficial tension versus surface for: smashed lung of control rat; smashed lung of trained rat, exposed 5 days at O_2; smashed lung of trained rat, exposed 10 days at O_2; and smashed lung of trained rat, exposed 20 days at O_2.

TABLE I

EXPLOITATION OF SURFACE TENSION CURVES

	Weight of Lungs (gm)	γmax (dynes/cm)	γmin (dynes/cm)	Δ_γ (dynes/cm)	γmean (dynes/cm)	Stability Index	Area of Hysteresis Loops (cm^2)
Nonadapted							
Control	1.16	43.4	17.9	25.5	30.6	0.83	79.4
n = 10	± 0.03	± 1.4	± 0.9	± 1.8	± 0.8	± 0.06	± 7.8
48^{H}A 67%	± 1.98[b]	44.8[ns]	13.7[a]	31.1[a]	29.3[ns]	1.05[a]	82.0[ns]
n = 10	± 0.03	± 1.2	± 0.6	± 1.0	± 0.8	± 0.02	± 3.6
48^{H}B 33%	2.10[b]	28.6[b]	23.0[a]	5.5[b]	25.8[a]	0.21[b]	40.7[a]
n = 5	± 0.12	± 1.5	± 0.8	± 1.3	± 1.0	± 0.04	± 3.3
Adapted							
5 days	2.29[b]	35.8[a]	17.2[ns]	18.7[a]	26.5[ns]	0.70[ns]	94.4[ns]
n = 7	± 0.21	± 3.2	± 1.2	± 2.2	± 2.1	± 0.03	± 10
10 days	2.97[b]	25.5[b]	24.9[b]	0.5[b]	25.1[b]	0.024[b]	30.0[b]
n = 10	± 0.31	± 0.4	± 0.4	± 0.1	± 0.4	± 0.004	± 2.8
20 days	1.90[b]	36.5[a]	19.0[ns]	17.4[a]	27.8[ns]	0.64[ns]	60.7[ns]
n = 10	± 0.11	± 1.7	± 1.9	± 2.6	± 1.2	± 0.11	± 7.6

[a] $P < 0.05$.
[b] $P < 0.001$.

increased, but the mean surface tension (γ_{mean}) did not vary significantly. These observations would indicate that the quality of the surfactant studied has indeed improved, but its quantity has remained unchanged.

For population B, that is 33% of the whole group, Δ_γ, $\overline{S}$ and the surface area of the loop were considerably decreased, indicating marked impairment in the quality of the surfactant examined. The mean surface tension (γ_{mean}) decreased, showing that the quantity of surfactant produced had increased as well.

Lung weight was significantly greater in the nonadapted but exposed groups in comparison with lung weight in controls, indicating that pulmonary edema is to some extent present after 48 hours' continuous exposure to pure oxygen. There is no significant difference in lung weight between populations A and B in the nonadapted group.

In two-thirds of the rats exposed to pure oxygen for a 48-hour period, the pulmonary surfactant actually appears to have improved in quality but its quality is impaired in the remaining third. The quantity of surfactant present seems to have increased in population B.

Adapted rats. After 5 days' re-exposure, only one population can be identified, the results of which are situated between those of populations A and B in the 48-hour nonadapted group.

After 10 days' re-exposure, the Δ_γ is practically nil, there being little difference between the γ_{max} and the γ_{min}. The stability index is very low indicating considerable qualitative impairment in the properties of the surfactant. γ_{mean} has decreased to about the same level

as was seen in population B of the nonadapted 48-hour exposure group. The results indicate that although the quality of the surfactant produced is increased, the increase in weight of the lungs in this group suggests that pulmonary edema may be more extensive.

After 20 days' re-exposure the hysteresis loops obtained appear more normal in shape, with Δ_γ and stability index increased above the 10-day re-exposure levels. γmean also tends to come back to control levels. Such results indicate both an improvement qualitatively in surfactant over the 10-day levels and a decrease in quantity of surfactant produced. From the lung weights, pulmonary edema appears to be of the same order as was seen in populations A and B in the nonadapted 48-hour exposed group.

Chemical Analysis of Liquid Obtained by Lung Lavage

Groups of rats were exposed as above. Following the relevant exposure to oxygen, the rats were weighed, anesthetized with Nembutal by intraperitoneal injection, and their tracheas cannulated. Each animal was exsanguinated via the carotid artery. Endobronchial washing via the cannula with 3 ml of 9% sodium chloride solution was repeated 4 times allowing the collection of almost all pulmonary surfactant. This technique was developed by Brown in 1964 (2).

Lipid phosphorus concentration. Pulmonary surfactant represents the only possible source of lipid phosphorus from the washing liquid, provided contamination by blood is avoided. After centrifugation of the cells in suspension and precipitation of the proteins and nonlipid substances, the residue consists of lipid phosphorus the concentration of which was measured according to the Fiske and Subbarow technique (7).

Results. Table II shows the lipid phosphorus concentration expressed in μg per rat lung. Each value corresponds to a series of 15 rats of similar weight between 250 and 300 gm. In comparison with the values from the control rats (34 μg), the 48-hour exposed rats show a slight increase in lipid phosphorus concentration of little significance (48 μg per lung). It is not possible to demonstrate two populations in the nonadapted exposed rats. In the adapted re-exposed groups of rats, lipid phosphorus concentration was increased at 5 days' re-exposure (144 μg per lung), increasing to 156 μg per lung after 10 days' exposure. However, by 20 days the lipid phosphorus concentration returned to almost normal control values.

Identification of phospholipid components in surfactant. Lung lavage liquid was collected as described and centrifuged. The proteins were precipitated and recentrifuged. After dis-

TABLE II

Dosage of Lipid Phosphorus

	Nonadapted		Adapted		
	Control	48 Hours	5 Days	10 Days	20 Days
Number of animals	(12)	(10)	(10)	(11)	(9)
Lipid phosphorus[a] (μg per lung)	34 ± 2.5	48[b] ± 3.3	144[c] ± 18	156[c] ± 16	51[b] ± 4

[a] Values are means ± standard deviation.
[b] $P < 0.05$.
[c] $P < 0.001$.

carding the supernatant, the "button" consisting of protein and phospholipid was dissolved in a 1:4 volume for volume solvent mixture of chloroform and methanol. After centrifuging, 300 μl of supernatant was deposited on silica gel plates 250 μ thick. Separation of phospholipids from proteins on the plates was made in a solvent of petroleum ether, ethyl-ether, acetic acid in 80:20:1 volume for volume concentrations. This procedure allows the extraction of purified phospholipid which stays at the point of origin under these conditions while the proteins migrate away with the solvent front. After drying the plates, the areas containing phospholipid were scraped off and the phospholipid was stirred for 15 minutes at 4°C in a tube containing 0.5 ml of the chloroform-methanol mixture. After slight centrifugation in order to settle out the gel, 300 μl of supernatant containing the phospholipid were deposited on gel plates 250 μ thick. The separation of individual phospholipid components was made by the use of a solvent of chloroform, methanol and water in a 65:25:4 volume for volume-ratio solution, respectively. After drying of the plate, the phospholipid spots appear blue on a white background when revealed by treatment with sulphomolybdic reagent. From the point of origin of the solvent front on the plate, one can identify, respectively, lysolecithin, sphyngomyelin, lecithin, phosphatidyl dimethylethanolanine and phosphatidylethanolamine.

Quantitative measurements of the concentrations were obtained by passing the chromatography plates through a Vernon reflection densitometer. By integration of the peaks obtained, the percentages of the different constituents were estimated. The results showed no significant variation in composition of the phospholipids obtained. The only significant variation was in the change in total quantity of phospholipid as estimated by the lipid phosphorus concentration measurements; this showed an increase by a factor of 5 in the rats adapted to pure oxygen and re-exposed for 10 days.

Discussion

An increase in the quantity of pulmonary surfactant material can be shown in the rats adapted and re-exposed to oxygen since there was not only a decrease in the average surface tension of the minced pulmonary tissue extracts, but also an increase in the lipid phosphorus content in the lung wash-out fluid by the 5th day of re-exposure. This surfactant material progressively lost its principal quality, as demonstrated by the decrease in the difference between maximum surface tension and minimum surface tension.

Numerous studies have been reported on changes in pulmonary surfactant by oxygen poisoning, often with contradictory results (5, 8, 9). However, there is agreement on the qualitative impairment of the surfactant but not on the changes in quantity.

Several histopathological arguments support an increase in quantity of the surfactant. The earliest histological lesions caused by oxygen poisoning appear at the capillary endothelial level and not at the type II pneumocyte level, which secretes the surfactant (12). Pariente et al. (15) observed, in electron microscopy of the lungs of rats exposed for 72 hours at 720 mm Hg Po_2, that the type II pneumocytes were undamaged. Moreover, they stated that secretion of surfactant was maintained normally throughout the experiment, and they concluded that, if surfactant synthesis had been altered in the animals, it was more in the direction of over-production than in the decrease or disappearance of the material. Harrison and Rosan (11) described in young rats exposed for several weeks to pure oxygen, the

appearance of numerous myelin figures in mucosal cells which resembled type II pneumocytes. Such myelin figures could be the origin of surfactant (16). Harrison and Weibel (10) have also described, in rats exposed to 100% oxygen for 1 to 2 weeks, numerous "tubular myelin figures", also called "membranous components" in the alveolar exudate. They found similar material in macrophages as well. They noted that the quantity and complexity of these inclusions increased with the time of oxygen exposure. These intra-alveolar membranous components consisted of phospholipid and as such should be aggregates of surfactant. They are of similar appearance to the inclusions in type II pneumocytes, mixed with cellular debris. We ourselves observed in electron microscopy of the lungs of rats adapted and re-exposed to oxygen for 5 days, a rise in the number of cells having the characteristics of type II pneumocytes but abounding in numerous myelin figures. Such type II pneumocytes were bounded by numerous microvilli which protruded into the alveolar lumen (Fig. 5).

A good argument exists on biochemical grounds in support of the increase in quantity of surfactant. Lipid synthesis is activated under the influence of oxygen in vivo in the liver and on adipose tissue slices in vitro (6). Niinikoski et al. (14) noted, in agreement with the present study, an increase in lipid phosphorus in the fluid obtained from pulmonary lavage after rats had been exposed to pure oxygen (Fig. 5).

In our study, this increase in lipid phosphorus corresponds well to the lowering in average surface tension of the minced lung extracts and, therefore, to an increase in the quantity of surfactant. Figure 6 depicts mean surface tension, the ordinate of which lies to the right of the figure, decreasing with increasing re-exposure time to 10 days and, subsequently,

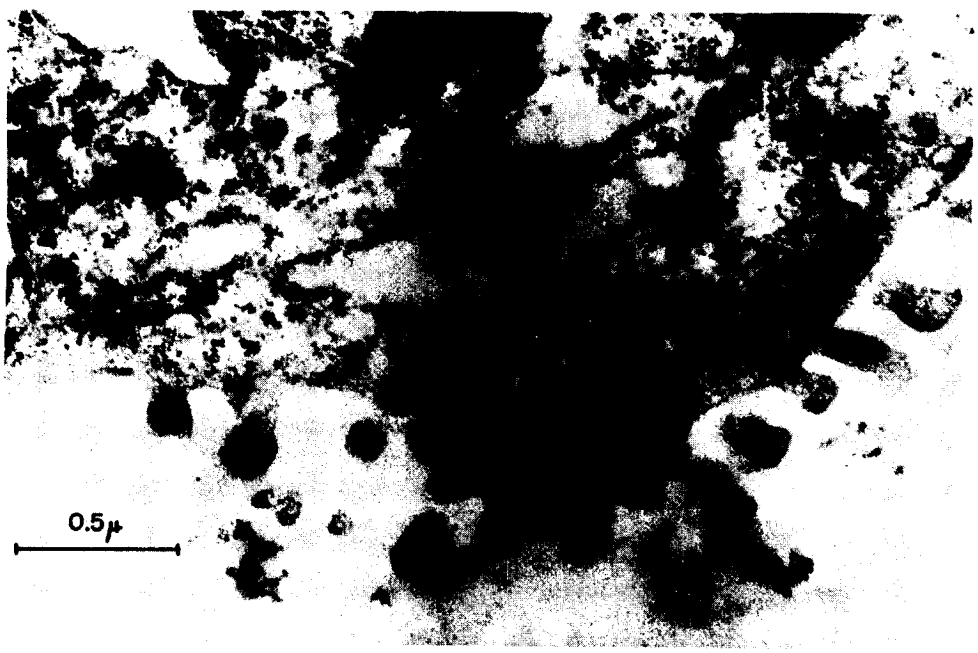

FIG.5. Lung of rat adapted and re-exposed to oxygen for 5 days (electron microscopy). Rise in the number of type II pneumocytes, abounding in numerous myelin figures, and bounded by numerous microvilli.

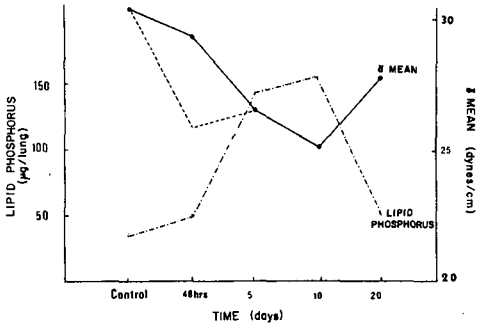

FIG. 6. *Evolution of average superficial tension (average γ, in dynes/cm) and of the concentration of lipidic phosphorus.*

increasing at day 20. The dotted line represents the course for population B among those animals exposed to pure oxygen for 48 hours only. The time course for the change in lipid phosphorus concentration is depicted in open circles joined by a dotted and dashed line. Corresponding to the decrease in mean surface tension there is an overall rise in lipid phosphorus content, indicating a rise in surfactant production most marked between the 5th and the 10th day of re-exposure, but falling again by day 20.

If the quantity of the surfactant produced is increased, why is its quality impaired? One can advance the hypothesis of a modification in surfactant metabolism under the influence of hyperoxia leading to a surfactant of different composition. Niinikoski et al. (14) noted, from silica gel chromatography, a product isolated from lung washings after oxygen exposure; they called this "product X" and it could have been phosphatidyl dimethylethanolamine.

The metabolism of the surfactant is now relatively well known (13, 20). Two pathways exist for the biosynthesis of the lecithin (or phosphatidyl choline) of the pulmonary surfactant: the first is the triple methylation of the phosphatidylethanolamine (with the phosphatidyl methylethanolamine [PME] and the phosphatidyl dimethylethanolamine [PDME] as intermediates) by the *N*-methyltransferase; the second is the cytidine diphosphocholine (CDP-choline) pathway. The first pathway makes a relatively minor contribution to total lecithin synthesis (10% to 20% according to the authors); it is susceptible to high pressure which partially depresses the methylations (especially the last one, with an increase of the intermediate components' percentages). The second pathway is practically insensitive to oxygen partial pressure variations (13).

However, our chromatography on silica gel results indicate the same percentage of PDME for control and oxygen-poisoned rats. The loss of the quality of the surfactant would also be due to a different composition in fatty acids of the lecithin; the lecithin synthesized would remain inactive.

Another hypothesis, which perhaps seems more valid, could be a modification in the assembly of the different constituents of the surfactant, proteins as well as lipids. The work of Pattle et al. (16) shows that the osmophilic bodies which are the source of the surfactant found in type II pneumocytes leave the pneumocytes and spread in a monomolecular layer throughout the internal surface of the alveolar lining. During oxygen poisoning, lamellar structures which look like osmophilic bodies are found in the cells and also in the alveolar

spaces (10). However, they have a structure much more complicated than that found normally, the lamellar forms having a thickness of 85 Å instead of 40 Å. They apparently stay in block form and, therefore, are not distributed in a monomolecular layer of surfactant over the alveolar surface.

Conclusions

By adaptation of the rats to oxygen exposure their survival can be prolonged from normobaric pure oxygen poisoning. We have shown in such conditions an increase in surfactant production, but it is a surfactant which has lost those specific properties normally associated with its function. The explanation of this impairment cannot yet be established by strong evidence. On day 20 of re-exposure to oxygen both quantity and quality of surfactant tended to return to normal.

By day 20, pulmonary edema has decreased but the lesions characteristic of alveolitis have become fibrotic while the capillaries show necrotic lesions with large thrombi. It would appear, therefore, that the close association between the histological lesions observed and the functional changes measured in surfactant no longer holds by the 20th day.

ACKNOWLEDGMENTS

We wish to thank Dr. H. V. Hempleman, and Dr. J. N. Miller of the Royal Naval Physiological Laboratory for their assistance in the preparation of the manuscript in English.

This research has been supported in part by contract with the "Direction des Recherches et Moyens d'Essais" (D.R.M.E. n° 71488).

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PREVENTION BY LITHIUM OF ACUTE HYPERBARIC OXYGEN TOXICITY AND ASSOCIATED CHANGES IN BRAIN GAMMA-AMINOBUTYRIC ACID LEVELS*

M. W. Radomski, J. Rowe and W. J. Watson

The administration of certain simple cations to animals modifies their susceptibility to acute oxygen toxicity (27). Magnesium delays the onset of convulsions and manganese delays the development of lung edema, while a mixture of these two cations protects against both symptoms of oxygen poisoning. The anticonvulsant action of magnesium, however, appears to be due to peripheral rather than central nervous system (CNS) inhibition (27), since magnesium does not cross the blood-brain barrier (17). If such is the case, an oxygen lesion in the CNS of the magnesium-treated animal might still be occurring despite the inhibition of convulsions, a situation analogous to that of anesthetics and oxygen convulsions (3). This would contraindicate its use in acute oxygen toxicity.

One cation which has not been tested to date on oxygen toxicity is lithium. This cation is of particular interest since it has been used effectively in humans to control the manic phase of manic-depressive psychosis (14, 30), and it does cross the blood-brain barrier (8, 29). Although the mechanism of lithium action is unclear, its anti-manic activity has been attributed to its effects on ionic gradients, cerebral monoamines and amino acids in the brain (1, 9, 10, 14, 15, 30).

Acute oxygen toxicity, like mania, is characterized by hyperexcitability and by derangements in brain monoamine (2, 12, 13) and amino acid metabolism (33, 34). Although the mechanism of acute oxygen toxicity is not settled, derangements in brain γ-aminobutyric acid (GABA), a CNS neuroinhibitor, have been strongly implicated in the etiology of oxygen convulsions (34, 35). A consistent relationship between changes in brain monoamines and the development of oxygen toxicity has not been reported (12). This may be a result of species and oxygen pressure differences between studies.

The efficacy of lithium pretreatment of animals exposed to oxygen at high pressure (OHP) on, 1) the development of convulsions and lung damage, 2) brain GABA levels, and 3) brain norepinephrine (NE) and serotonin (5-HT) levels and turnover, is reported here.

Methods

Animals

Nonfasted, male Wistar rats (200-220 gm), fed laboratory chow and water ad libitum, were used in all experiments.

* DCIEM Research Paper No. 878.

Exposure to OHP

Animals were pressurized in individual lucite chambers (3 L capacity) at 2.5 ata/min to a total pressure of 5.0, 5.5 or 6.0 ata of oxygen. They were maintained at this pressure for up to 60 minutes depending upon the experiment and then decompressed rapidly within a 2-minute period. All chambers contained a layer of soda lime to absorb carbon dioxide and were vented at 2.5 L/min while at pressure.

Convulsions and lung edema were measured by standard techniques (27). The 50% convulsion time (CT_{50}) was estimated by the method of Miller and Tainter (25) and the convulsion reduction factor (CRF) calculated from the ratio of the CT_{50} for treated animals to the CT_{50} for corresponding control groups. The CRF is the factor by which the inherent resistance of the animal to OHP has been increased and is analogous to the dose reduction factor used in characterizing agents which modify radiation effects.

Drug Treatment

All drugs were administered intraperitoneally prior to OHP exposure. Control animals received a similar volume of the drug carrier. Where not indicated, the carrier was water. The drugs used were: lithium chloride, 4.7 and 9.4 mM/kg (4% and 8% solutions); magnesium sulphate, 1.0 mM/kg; manganese sulphate, 0.1 mM/kg; zinc sulphate, 0.5 mM/kg; pargyline, 80 mg/kg; α-methyl tyrosine (α-MT), 100 mg/kg in 1 *N* NaOH with the pH adjusted to 9.2 with 1 *N* HCl.

Lithium in serum and brain extracts was assessed by Schou's technique (29).

Brain GABA Levels

In animals exposed to OHP, only nonconvulsed animals were used for measurement of brain GABA. After decompression, the animals were immediately decapitated and the heads allowed to drop directly into liquid nitrogen. Techniques for the preparation and treatment of brain extracts and assay of GABA have been described previously (35).

Brain NE turnover was studied according to the method of Brodie et al. (4). Animals received a single dose of α-MT (100 mg/kg, i.p.) and were killed at 0, 1/4 and 3/4 hour thereafter. Lithium (9.4 mM/kg) was injected 3/4 hour prior to α-MT and animals were exposed to OHP 1/4 hour after α-MT for 1/2 hour.

For 5-HT turnover studies (32), pargyline (80 mg/kg, i.p.) was used and animals were killed at 0, 1/4 and 3/4 hour. Lithium and OHP were administered as above.

The rats were killed by decapitation and the brains were assayed for NE and 5-HT.

Results

Distribution of Lithium in Brain and Serum

Lithium peaked in the serum and the brain 1/4 and 24 hours after injection, respectively (Fig. 1). Doubling the dose of lithium increased peak levels in the serum and brain by at least twofold.

OHP Convulsions and Lung Edema

Maximal protection against the development of convulsions was observed with the 9.4

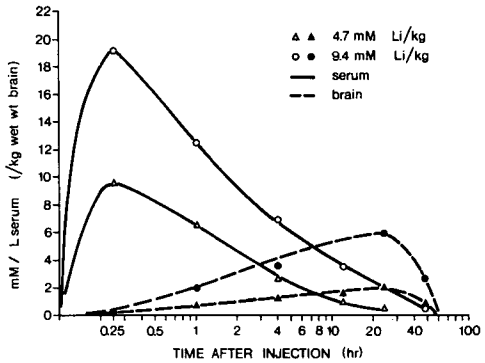

FIG. 1. Distribution of administered lithium with time between serum and brain. Two doses of lithium were administered: 4.7 and 9.4 mM/kg.

mM/kg dose of lithium given 1/4 or 1 hour prior to exposure (Fig. 2); some protection was also evident with the lower dose at 1 hour prior to OHP. Neither dose was protective when administered 24 hours prior to OHP. The CT_{50}s and CRFs for lithium and the other cation treatments are shown in Table I. Lithium was the most effective against convulsions. The protection afforded by magnesium was significant but not as great as the high dose of lithium. Manganese and zinc had no effect.

Comparison of Fig. 1 with Fig. 2 and Table I shows that peak protection against convulsions by lithium was evident at peak levels of lithium in the serum and not the brain.

The high dose of lithium was also effective in inhibiting the development of lung edema induced by OHP (Fig. 3).

BRAIN GABA AND CONVULSIONS

Lithium, magnesium, manganese and zinc did not alter brain GABA levels in normal animals not exposed to OHP (Table II).

Exposure to OHP (5.5 ata) for 20 minutes produced a 19% decrease in GABA in control rats (Table II). Lithium, when administered 1/4 and 1 hour prior to exposure but not 24 hours, inhibited significantly the OHP-induced decrease in GABA. In fact, brain GABA remained unchanged in exposed animals given 9.4 mM/kg of lithium 1 hour prior to exposure.

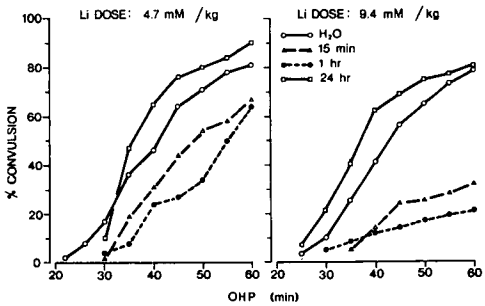

FIG. 2. The effect of two doses of lithium on the convulsions induced by OHP (5.5 ata). Lithium was administered at 15 minutes, 1 hour or 24 hours prior to exposure.

TABLE I

EFFECT OF VARIOUS CATIONS ON THE SUSCEPTIBILITY OF RATS TO OXYGEN-INDUCED CONVULSIONS

Group	Dose (mM/kg)	Pretreatment Time (hr)	N	CT_{50}[a] (min)	Convulsion Reduction Factor	Level of Significance[b]
Water	0	1/4	201	42 ± 1.5[c]		
Li	4.7	1/4	48	49 ± 3.9	1.17	n.s.
		1	68	55 ± 3.2	1.31	< 0.001
		24	20	37 ± 2.5	0.88	n.s.
	9.4	1/4	63	108 ± 9.1	2.57	< 0.001
		1	42	156 ± 10.5	3.71	< 0.001
		24	24	39 ± 4.0	0.93	n.s.
Mg	1.0	1/4	93	62 ± 4.2	1.48	< 0.001
Mn	0.1	1/4	63	51 ± 5.6	1.21	n.s.
Zn	0.5	1/4	21	51 ± 4.8	1.21	n.s.

[a] CT_{50} represents convulsion time for 50% of the animals.
[b] Levels of significance are for the difference from water group.
[c] Values are means ± S.E.

TABLE II

EFFECT OF CATIONS ON BRAIN GABA IN NORMAL AND OHP-EXPOSED RATS

Group	Dose (mM/kg)	Pretreatment Time (hr)	Brain GABA		Level of Significance for % Change
			Nonexposed[a] (μM/gm)	OHP[b] (% Change)	
Water	0	1/4	1.60 ± 0.03[c]	−19	< 0.001
Li	4.7	1/4		−13	< 0.001
		1		− 9	< 0.05
		24	1.61 ± 0.02	−16	< 0.001
	9.4	1/4		− 3	n.s.
		1	1.56 ± 0.04	0	n.s.
		24	1.55 ± 0.03	−16	< 0.001
Mg	1.0	1/4	1.52 ± 0.03	−13	< 0.001
Mn	0.1	1/4	1.64 ± 0.03	−14	< 0.001
Zn	0.5	1/4	1.53 ± 0.05	−16	< 0.001

[a] In nonexposed groups, animals were killed 20 minutes after the pretreatment time.
[b] Following pretreatment time, animals were exposed to 5.5 ata of oxygen for 20 minutes and then sacrificed.
[c] Values are means ± S.E. Levels of significance represent statistical difference from nonexposed groups for % change in brain GABA.

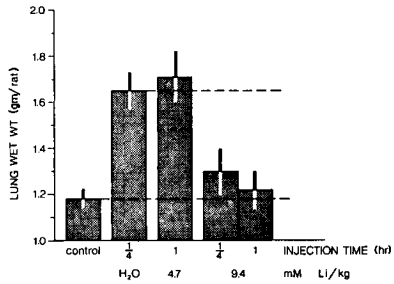

FIG. 3. Effect of lithium on lung edema induced by exposure to OHP (5.5 ata for 60 minutes). Injection time represents time prior to exposure. Control weight is the normal lung weight for a nonexposed rat weighing 200 gm. Values are mean ± S.E.

Magnesium, zinc and manganese did not modify significantly the OHP-induced decrease in brain GABA.

Figure 4 illustrates the increase in the CRF and the corresponding decrease in brain GABA for each pretreatment. It is obvious that, for lithium, the increase in the CRF was inversely related to the magnitude of the decrease in brain GABA. The highest CRF (280% increase) occurred at the lithium dose (9.4 mM/kg at 1 hour) which prevented completely a

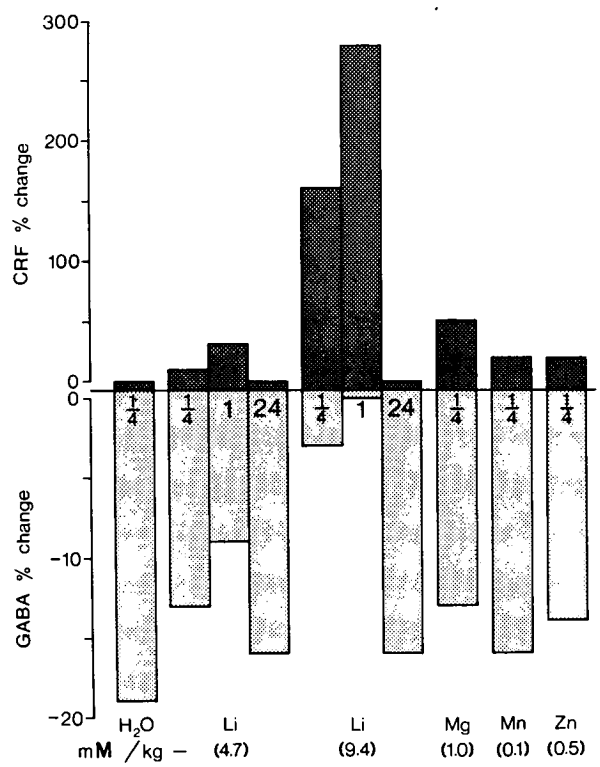

FIG. 4. Comparison of the convulsion reduction factor to the corresponding decreases in brain GABA for various cation treatments. The numbers in the bars are the times (hours) of cation injection prior to OHP.

decrease in GABA. At 24 hours after lithium when there was no increase in CRF, GABA dropped as in the water group. Magnesium, on the other hand, elevated the CRF without modifying the drop in GABA.

Turnover of Brain NE and 5-HT

The brain NE level was not altered at 5.0 ata but decreased significantly at 5.5 and 6.0 ata (Fig. 5). Lithium pretreatment (9.4 mM/kg at 1 hour) did not modify the changes in NE induced by OHP. No dose-effect relationship was evident between oxygen pressure and brain NE. An elevation in 5-HT occurred at all three pressures but was not significant. Lithium pretreatment plus OHP, however, elevated significantly 5-HT levels at all three pressures.

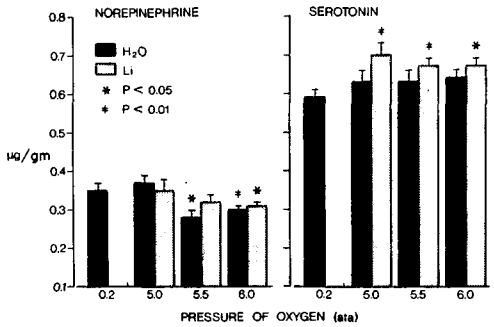

FIG. 5. Brain norepinephrine and serotonin levels in water- and lithium-treated groups exposed to OHP (5.0, 5.5 and 6.0 ata). The lithium dose was 9.4 mM/kg given 1 hour prior to OHP. Values shown represent the mean ± S.E. and the *P* values the significant difference from the sea level (0.2 ata oxygen) group.

The turnover of brain NE, measured following α-MT (5, 23), was assessed in rats exposed to OHP (5.0 and 6.0 ata) and pretreated with lithium (9.4 mM/kg, 1 hour pre-OHP) (Table III). In control rats at ambient pressures of oxygen, the turnover rate (rate of synthesis) was 0.076 μg/gm/hr which falls in the range of 0.021-0.095 reported by others (6). Oxygen at 5.0 and 6.0 ata increased the rate of degradation, *k* (200% and 164%), decreased the turnover time (— 67% and — 63%), and accelerated the turnover rate (205% and 169%) of NE. Lithium did not modify the turnover of NE at ambient or high pressures of oxygen. No dose-effect relationship was evident between oxygen pressure and NE turnover.

The rate of 5-HT synthesis, measured after monoamine oxidase (MAO) blockade by pargyline (80 mg/kg), was accelerated by OHP at 5 and 6 ata (88% and 76%), by lithium alone (48%), and by lithium + 6 ata of oxygen (142%) (Table IV). The effects of lithium and OHP appeared to be additive in accelerating 5-HT synthesis. Increasing the pressure from 5 to 6 ata did not produce a further increase in 5-HT synthesis.

Discussion

This study has demonstrated that lithium, which is an effective anti-mania agent in humans, significantly inhibits the development of convulsions and lung edema induced by breathing oxygen at high pressures. Of the cations tested in this and an earlier study (27), lithium proved to be the most effective in counteracting both effects of acute oxygen toxicity.

TABLE III

TURNOVER RATES AND TURNOVER TIMES OF BRAIN NOREPINEPHRINE IN RATS EXPOSED TO OHP AND GIVEN LITHIUM[a]

Group	Rate Constant of Amine Loss (k)[b] (hr^{-1})	Turnover Time ($1/k$)[c] (hr)	Turnover Rate[d] ($\mu g/gm/hr$)
Control	0.22	4.6	0.076
OHP—5 ata[e]	0.66 (200)[f]	1.5 (—67)	0.232 (205)
OHP—6 ata	0.58 (164)	1.7 (—63)	0.204 (169)
Control + lithium[g]	0.22 (0)	4.6 (0)	0.076 (0)
OHP—6 ata + lithium	0.74 (236)	1.4 (—70)	0.260 (242)

[a] Values were calculated by blocking norepinephrine synthesis with α-MT (100 mg/kg).

[b] Values for k were measured over the period of OHP exposure. The value k represents the fraction of the total NE that is formed and lost per unit time (fractional turnover rate).

[c] The turnover time, $1/k$, is the time interval required for the biosynthesis of an amount of NE equal to that stored in the tissue.

[d] Rate of synthesis of NE.

[e] OHP exposure was initiated 15 minutes after α-MT and continued for 30 minutes. Only nonconvulsed rats were assayed.

[f] Values in parentheses represent percent change from controls.

[g] Lithium (9.4 mM/kg) was given 60 minutes prior to OHP.

TABLE IV

EFFECT OF OHP AND LITHIUM ON THE RATE OF 5-HT SYNTHESIS MEASURED AFTER PARGYLINE

Group	5-HT, $\mu g/gm/hr$	
	Water[a]	Lithium[a]
Ambient Air	0.33	0.49 (48%)[b]
5 ata O_2	0.62 (88%)[b]	
6 ata O_2	0.58 (76%)[b]	0.80 (142%)[b,c]

[a] Values in parentheses are percent change from the control (ambient air-water).

[b] $P < 0.01$ vs. control group.

[c] $P < 0.01$ vs. lithium-ambient air group.

Surprisingly, the effect of lithium was maximal at high serum concentrations of lithium and not at peak brain levels.

Although the lithium concentration which proved to be effective in this study is 6-10 times the serum concentration that is chronically maintained to alleviate mania, a study of its anticonvulsant action may help to elucidate the etiology of acute oxygen toxicity. Abnormalities of biogenic monoamines and amino acids in the brain have been postulated to be causative factors both in mania (9, 14, 30) and in acute oxygen toxicity (2, 12, 34), and lithium has been found to alter biogenic monoamines (9, 14, 30) and amino acids (1, 10) in the animal brain.

It was found that the anticonvulsant action of lithium coincided with its ability to prevent the well-known decrease in brain GABA which precedes OHP convulsions. This finding could be interpreted as support for the hypothesis of Wood et al. (35) that a cause-effect relationship exists between a derangement in GABA metabolism and the development of oxygen convulsions. Wood and co-workers have amassed impressive evidence in support of such a relationship and the present findings with lithium support Wood's hypothesis.

Oxygen may act to decrease brain GABA by 1) an inhibition of intracellular glutamic acid decarboxylase (GAD), the enzyme synthesizing GABA, and/or 2) an increase in the uptake of GABA into the neuron for subsequent degradation. It is unlikely that lithium acts by protection of intracellular GAD against oxygen since maximal protection against convulsions occurs when lithium is primarily extracellular (1/4 to 1 hour after injection) (8) rather than intracellular (24 hours after injection).

Since the neuronal uptake of GABA is dependent upon the sodium concentration (18, 22, 28), and OHP increases the concentration of sodium in brain slices in vitro (19, 20), OHP may conceivably accelerate the uptake of GABA by such a process and thereby accelerate its degradation. Lithium may inhibit such an OHP-accelerated uptake of GABA by substitution of lithium for sodium or by decreasing brain sodium, processes known to inhibit GABA uptake under normal conditions (18, 22, 28). Such an effect of lithium would tend to maintain the GABA concentration in the brain under OHP as found in this study and thereby prolong its neuroinhibitory action. This hypothesis would explain the observed relationship between time of injection of lithium and protection against convulsions. The finding that lithium does not alter GABA levels in normal nonexposed animals, in accord with others (10, 15), but does counteract OHP effects on GABA, suggests that lithium protects by directly counteracting the effect of OHP on the brain rather than by producing an altered state in the animal prior to exposure.

Attempts to implicate abnormalities in cerebral monoamines in the etiology of acute oxygen toxicity have not been successful to date. Whereas some workers have found no changes in brain NE and 5-HT under OHP (2, 21), others have reported decreases in both monoamines (7, 12, 16). Elevation or depression of NE and 5-HT by various compounds prior to OHP did not modify convulsion susceptibility (2, 13). Furthermore, Faiman and co-workers (12) could not find any relationship between OHP-induced changes in the concentration of NE and 5-HT and convulsion susceptibility.

In this study, OHP decreased NE only at 5.5 and 6.0 ata and not at 5.0 ata in rats. This is in accord with Blenkarn et al. (2) who found no decrease at 5.0 ata and also with Häggendal (16) and Cross and Houlihan (7) who reported decreases at 6 ata in rats. It appears that only above a certain pressure are NE levels modified by OHP. Levels of 5-HT were not significantly altered in this study in accord with some workers (2, 4) but not with

Faiman et al. (12) who found a decrease in mice. The present results support the conclusion of others (2, 12) that no apparent relationship exists between changes in brain NE and 5-HT levels and convulsion susceptibility.

As is well known, turnover rates more accurately reflect the activity of a system than do levels of a compound. Although 100% oxygen at ambient pressure (1 ata) increased the turnover of NE (26) and 5-HT (11) by almost twofold, no turnover studies had been carried out at pressures (> 3 ata) at which acute oxygen toxicity occurs. This study has shown that oxygen at 5 and 6 ata elevates the turnover rate of both NE (200% and 164% increase) and 5-HT (88% and 76% increase). From a comparison of turnover rates at 1 (26), 5 and 6 ata, it is evident that there is no relationship between convulsion time (susceptibility) and change in turnover rate. This suggests that the changes in NE and 5-HT turnover, although altered by OHP, are not involved in the etiology of OHP convulsions.

Although lithium did not alter NE levels and turnover in rats exposed to OHP, it did significantly elevate the level and turnover rate of 5-HT over that produced by OHP alone. The significance of this observation with respect to the mechanism of the anticonvulsant action of lithium toward OHP is not clear. It is known that elevation of 5-HT in the brain decreases convulsion susceptibility to electroshock and pentylenetetrazol convulsions (24), but this relationship does not hold for OHP (2, 13). An increased turnover of 5-HT seen in lithium-pretreated rats is not necessarily associated with an increased release of 5-HT (31) but may reflect merely enhanced intraneuronal metabolism and not be related to convulsions.

Summary

Further evidence is presented in support of derangements of GABA in the etiology of OHP convulsions. The protection afforded by lithium appears related to its effects on GABA metabolism rather than on monoamine metabolism. It does not appear that alterations of monoamine levels and turnover are involved in the etiology of OHP seizures. The effects on oxygen toxicity of chronically administering to rats moderate amounts of lithium, which would simulate human therapeutic levels in the treatment of mania, have not yet been assessed.

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EFFECT OF OXYGEN AT HIGH PRESSURE ON CELLULAR ULTRASTRUCTURE AND SOME GLYCOLYTIC AND CITRIC ACID CYCLE ENZYMES

E. W. Banister, N. M. G. Bhakthan, A. J. Davison and C. Asmundson

In organisms exposed for long periods to oxygen at high pressure (OHP), it has been suggested that toxic effects, including convulsions and death, involve a variety of mechanisms (1, 14): 1) tissue hypoxia resulting from spasm of the diaphragm, lung damage accompanying disruption of the surfactant action of the lungs, and resulting from atelectasis; 2) interference with levels of neurotransmitters or agents such as gamma aminobutyric acid (GABA) which control nervous impulse transmission in the brain; and 3) oxidation of the thiol (SH) groups of rate-determining enzymes, in both the glycolytic and tricarboxylic pathways, or of enzymes in the respiratory chain, the inhibition of which subsequently extinguishes energy-linked electron flow. The accompanying deficit in the production of ATP necessary to maintain the ultrastructural integrity of the cell might have important consequences in central nervous system (CNS) metabolism.

Many of the changes observed, including much of the pulmonary damage, may be secondary to metabolic alterations induced by generalized convulsions after exposure to OHP, as has been suggested by Bean et al. (4).

Despite a great deal of research, the primary etiology of oxygen toxicity remains obscure, and to date studies involving the correlation of biochemical, ultrastructural and overt toxic characteristics in animals subjected to hyperoxic conditions have not been undertaken.

Recent literature (14, 26) has linked the protective action of certain chemicals, singly or in combination, to their effect 1) in providing a reducing environment which protects susceptible thiol groups of the enzyme from being oxidised, thus preventing inactivation, and 2) in stimulating ATP production necessary for essential metabolism via metabolic pathways less inhibited by hyperoxia.

On the other hand, in the case of a protective agent of a different kind, GABA, Wood and Watson and Wood et al. (34-37) have previously suggested that its role in depressing nervous transmission, when applied directly to neurons, is closely allied to its efficiency in protecting the organism against OHP.

Ballentine and Gutsche (2) described the following ultrastructural changes in animals exposed repeatedly to high pressure oxygen until paralysis developed: 1) type A lesions

characterized by focal necrosis of individual neurons within certain nuclear groups and 2) type B lesions, more general effects involving complete or partial necrosis of groups of neurons with damage to myelin, axons, and glia within the grey matter. Both types of lesion appeared bilaterally and symmetrically. Cytologic changes in type A lesions were clumping of nuclear chromatin, pyknosis, cellular swelling and lysis. Necrosis and evidence of repair is noticeably greater in type B lesions. Selective necrosis and destruction is sometimes apparent with remaining cellular ultrastructures relatively unaffected.

Von Schnakenburg and Nolte (28) describe ultrastructural changes in response to 8 ata oxygen exposure similar to those of Ballentine and Gutsche (2) occurring in five regions: the medullary reticular formation, the medio-lateral area of the cervical spinal grey matter, the mammilary body, the cochlear nuclei, and the inferior colliculi. These authors also described type A and B lesions, the ultrastructural changes in the former being characterized by osmophilia and deformities of the nuclei and cytoplasm of cells which included some dendrites. Cisterns of the endoplasmic reticulum and Golgi region are distended and mitochondria are extremely large and often damaged. The cytoplasm is extensively vacuolated. Type B lesions included clumping of the nuclei and plasmolysis.

In one of the above studies (2), serial exposure to 100% O_2 at 6 ata was carried out on several days until a certain degree of paralysis was developed before sacrifice. In another study (28), the animals were sacrificed, after exposure to 8 ata 100% O_2 for periods of 1 hour, on each of 6 days after exposure. In the last study no precise time schedule was defined but it was concluded that the incipient ultrastructural changes may be important in the etiology of the convulsive state due to OHP (28). Similarly, no simultaneous biochemical and ultrastructural correlates of overt toxic effects have been obtained in experiments to date which might give insight into the sequence of events accompanying oxygen toxicity, paralysis and convulsion.

The present experiments have been designed to investigate the significance of the biochemical and ultrastructural changes accompanying the development of oxygen toxicity and convulsion in rats exposed to 6 ata 100% O_2 for extended periods.

Methods

Animals

Male Sprague-Dawley rats (250-300 gm) were maintained on a diet of standard rat lab chow and water ad libitum. Prior to the experiment, they were fasted for 14 hours. Thirty-five rats were randomly assigned to four groups: 1) a normal group (N), sacrificed at sea level after no pressurization; 2) a hyperbaric air group (HA), sacrificed after exposure to 6 ata air for 60 min; 3) a small-convulsion hyperoxic group (SC, 6 ata, 100% O_2), decompressed after one small convulsion and immediately sacrificed; and 4) a large-convulsion hyperoxic group (LC, 6 ata, 100% O_2), sacrificed after several generalized convulsions which often continued throughout decompression. A small convulsion was characterized by minor clonic contractions whereas a large convulsion was characterized by violent, complete and repeated tetany.

Chamber Operation

A small animal chamber, of about 100-liter capacity was used in the hyperoxic and

hyperbaric experiments. The two ends of the chamber were made of thick plexiglass for easy viewing of the inside.

A single animal was placed in the chamber. Before pressurization, either 100% O_2 (hyperoxic group) or air (hyperbaric group) was flushed through the chamber at a flow rate of 5 L/min for 5 minutes. Following this, the chamber was pressurized to 72.5 psig (6 ata) over a period of 6-8 minutes. The chamber was sufficiently large and exposure time sufficiently short that CO_2 accumulation could be neglected. Gas flow throughout the experiment was maintained at 5 L/min, 1 atmosphere equivalent. Decompression took place over an 11-minute period with successive pauses at 40 psig (5 minutes), 30 psig (1 minute), 15 psig (1 minute) and 3 minutes from 15 psig to surface.

Blood Enzymes and Glucose

All animals, including the normal sea level group, were lightly anesthetized with ether and the abdominal cavity immediately opened. The animals were killed by exsanguination by withdrawing the maximum blood obtainable from the bifurcation of the abdominal aorta using a needle and syringe. Two ml of this blood was heparinized and immediately centrifuged (9000 G, 15 minutes). Plasma was removed and stored at 4°C for later LDH analysis by the method of Hochella and Weinhouse (15). The use of plasma alleviated the variability in serum LDH analysed by the usual methods (22). The remaining blood was allowed to clot and, after centrifugation at 900 G for 15 minutes, the serum was separated and stored at 4°C for later analysis of creatine kinase (CPK), aspartate transaminase (SGOT), glucose, and alkaline phosphatase. CPK activity was measured by the method of Rosalki (24), SGOT by the method of Morgenstern et al. (21), glucose by a modification of the methods of Brown (7) and Bittner and McCleary (6), alkaline phosphatase by the method of Morgenstern et al. (20).

Tissue Biochemistry (5)

Immediately after the rapid removal of small pieces of tissue for electron microscopy, the brain and skeletal muscle were placed in wash beakers cooled in ice. Wet weights of the tissues were taken after three separate washings and blottings. Muscle tissue was cut into small pieces and homogenized at full speed on a Virtis model 23 homogenizer for 2 minutes at 30-second intervals. Brain was homogenized in a Tenbrook homogenizer. The homogenization vessels were always cooled in ice. The homogenates were made up in phosphate buffer (0.01 molar pH 7.4), a volume of 9 ml/gm of tissue being used. Homogenates were centrifuged at 2800 rpm for 10 minutes at 0°C to remove connective tissue and gross cell debris. The supernatant was poured through glass wool into 1-oz plastic reagent bottles and frozen in liquid nitrogen for storage in dry ice for up to 2 weeks before analysis. On analysis, the supernatants were thawed in a water bath at 30°C. Lactate dehydrogenase activity was measured by the method of Kornberg (18), NADH dehydrogenase [cytochrome *c* reductase (31)] by the method of King and Howard (16), and cytochrome oxidase by the method of Smith and Camerino (29). The LDH and NADH dehydrogenase were followed by measuring absorbance at 340 nm or 630 nm on a Unicam SP600 UV spectrophotometer with a temperature-controlled cell compartment. Reactions were recorded on a Heath EU 205-11 potentiometric strip chart recorder. In the cytochrome oxidase reaction, oxygen consumption was followed with a YSI model 53 polarographic oxygen monitor with a Clark

type oxygen electrode. All reagents except the supernatants were incubated in a water bath at 30°C. The supernatants were stored in ice.

Electron Microscopy

Very small pieces of tissue were removed rapidly from the liver, adrenal cortex, gastrocnemius muscle and brain for examination. Liver tissue was taken from the anterior lobule of the right lobe, myocardial tissue from the left ventricle midway between the base and apex, and brain tissue from the right temporal lobe of the cerebral cortex and the hypophysis. The tissue was immediately fixed in 3% gluteraldehyde in cacodylate buffer and postfixed in 2% buffered osmium tetroxide at pH 7.4 for 2 hours. After dehydration through graded ethanol, the tissue was embedded in araldite. Sections were cut with a diamond knife held in a Reichert OmU2 ultramicrotome and mounted on uncoated copper grids. They were then stained with uranyl acetate and lead citrate and examined in a RCA EMU-3H electron microscope at an accelerating voltage of 100 kV.

Results

General

No signs of toxicity were apparent in the rats (five animals) subjected to 60 minutes of exposure to 6 ata air. The mean time of exposure to hyperoxia (6 ata 100% oxygen) of small-convulsed rats (11 animals) was 43 ± 7 (SEM) minutes, and for the large-convulsed group (10 animals) was 61 ± 7 (SEM) minutes. These mean times and standard errors reflect the marked variability of the toxic response to hyperoxia in rats, previously reported by other investigators (2, 28).

Overt behaviour preceding convulsion in both groups of hyperoxic rats was uniform in nature and in sequence and included: 1) quiet immobility interspersed with hyperactivity; 2) kangaroo-like posturing; 3) ataxia in the hind limbs; 4) circling movements; 5) loss of balance and righting ability; 6) frequent urination; and 7) immediately preceding convulsion, head shaking and body twitching. Occasional gasping was thought to indicate pulmonary damage, but this was not confirmed by macroscopic examination of the lungs. Arterial blood samples, which were always bright red in color, excluded the possibility of marked hypoxemia. Two rats, which suffered massive convulsions throughout decompression and died shortly after anesthesia, were discarded from the analysis.

Blood Enzymes and Glucose Levels

Table I shows the comparison of several constituents of normal blood both in the serum and plasma. Blood glucose levels were significantly elevated after hyperoxia (small-convulsed 386 mg/100 ml and large-convulsed 198 mg/100 ml vs. normals of 137 mg/100 ml), as has been noted previously. There was no elevation above normal in hyperbaric air (158.0 mg/100 ml) although the Po_2 (1.26 atm) was substantially greater than normal (0.21 atm). Elevated levels of alkaline phosphatase above normal (188.2 IU/L) indicate some degree of liver damage in all hyperbaric conditions, air (268.0 IU/L) as well as oxygen (small-convulsed 336.4 IU/L and large-convulsed 290.0 IU/L). There are, however, no quantitative differences between any of the hyperbaric conditions with regard to this

TABLE I

COMPARISON OF BLOOD LEVELS OF GLUCOSE, ALKALINE PHOSPHATASE, LACTATE DEHYDROGENASE, ASPARTATE TRANSAMINASE AND CREATINE KINASE IN NORMAL (N) HYPERBARIC AIR (HA, 6 ATA AIR), SMALL-CONVULSED (SC, HYPEROXIC 6 ATA, 100% OXYGEN) AND LARGE-CONVULSED (LC, HYPEROXIC, 6 ATA 100% OXYGEN) RATS

	Condition	N	$\overline{X}$	SD	Comparison	σ Value	Level of Significance
	N	5	136.7	16.3	N vs HA	1.38	None
Glucose	HA	5	158.0	30.9	N vs SC	4.07	0.01
mg/100 ml	SC	10	286.4	80.0	N vs LC	1.79	0.1
	LC	5	198.3	74.9	HA vs SC	3.42	0.01
					HA vs LC	1.12	None
					SC vs LC	2.22	0.05
	N	5	188.2	25.5	N vs HA	3.56	0.01
Alkaline	HA	5	268.0	43.1	N vs SC	2.79	0.05
Phosphatase	SC	11	336.4	115.6	N vs LC	3.08	0.05
IU/liter	LC	6	290.0	69.6	HA vs SC	1.26	None
					HA vs LC	0.61	None
					SC vs LC	0.89	None
	N	5	130.0	18.7	N vs HA	4.31	0.01
Lactate	HA	2	355.0	134.3	N vs SC	1.97	0.1
Dehydrogenase	SC	10	296.0	184.6	N vs LC	3.31	0.05
IU/liter	LC	5	296.8	104.2	HA vs SC	0.42	None
					HA vs LC	0.74	None
					SC vs LC	0.10	None
	N	5	338.0	45.5	N vs HA	4.89	0.01
Aspartate	HA	5	163.0	65.9	N vs SC	0.08	None
Transaminase	SC	11	336.8	116.7	N vs LC	0.88	None
IU/liter	LC	6	295.0	100.7	HA vs SC	3.02	0.01
					HA vs LC	2.51	0.05
					SC vs LC	0.68	None
	N	5	74.0	51.1	V vs HA	0.55	None
Creatine	HA	5	55.5	50.5	N vs SC	0.69	None
Kinase	SC	11	98.0	67.9	N vs LC	0.77	None
IU/liter	LC	6	94.3	29.1	HA vs SC	1.24	None
					HA vs LC	1.60	None
					SC vs LC	0.13	None

enzyme. Plasma lactate dehydrogenase is significantly elevated above normal (130.0 IU/L) in both hyperbaric air (355.0 IU/L) and oxygen (small-convulsed, 296.0 IU/L; large-convulsed 296.8 IU/L). Although the elevation in the group of rats in air was greater than in oxygen, (355.0 IU/L compared to 296.0 and 296.8 IU/L for the small- and large-convulsed groups, respectively), these differences were not statistically significant. The source of the total lactate dehydrogenase was not established by isoenzyme determination. Serum aspartate transaminase (SGOT) was significantly depressed in hyperbaric air (163.0

IU/L) below normal levels (338.0 IU/L); in conditions of hyperoxia (336.8 IU/L small-convulsed and 295.0 IU/L large-convulsed, respectively) these differences were not observed. Serum creatine kinase was not significantly altered from normal values (74.0 IU/L) in either hyperbaric air (55.5) or hyperbaric oxygen (98.0 IU/L, small-convulsed or 94.3 IU/L, large-convulsed).

Tissue Enzymes

Table II shows the activity of lactate dehydrogenase, cytochrome oxidase and NADH dehydrogenase (cytochrome *c* reductase) of brain and muscle in normal rats and after their exposure to hyperbaric conditions of air and oxygen. Brain lactate dehydrogenase activity is significantly decreased below normal levels (230 units; unit = $\mu M\ min^{-1}$ gm wet $weight^{-1}$) in both small (185.5 units) and large (185.7 units) convulsed rats. No significant decrease in activity was observed in rats subjected to hyperbaric air (232.0 units) nor was there significant difference between the degree of depression in the groups of hyperoxic animals. Muscle lactic dehydrogenase was unchanged from normal values in all hyperbaric groups. Although brain NADH dehydrogenase tended to be decreased in both small- and large-convulsed rats (834 and 931 units, respectively) below normal (1086 units) and hyperbaric air (1142 units), in no case was the difference significant. Similarly with muscle NADH dehydrogenase, there was no definitely established change from normal activity (194.0 units) in any experimental group, hyperbaric air (209 units), small-convulsed rats (196 units) and large-convulsed rats (211 units), respectively. In another study, liver lactate dehydrogenase, cytochrome oxidase and NADH dehydrogenase levels were not decreased (within experimental error) by similar exposure to hyperoxia, nor were liver, muscle or brain levels of glyceraldehyde-3-phosphate dehydrogenase or isocitrate dehydrogenase.

Ultrastructure

Gastrocnemius muscle

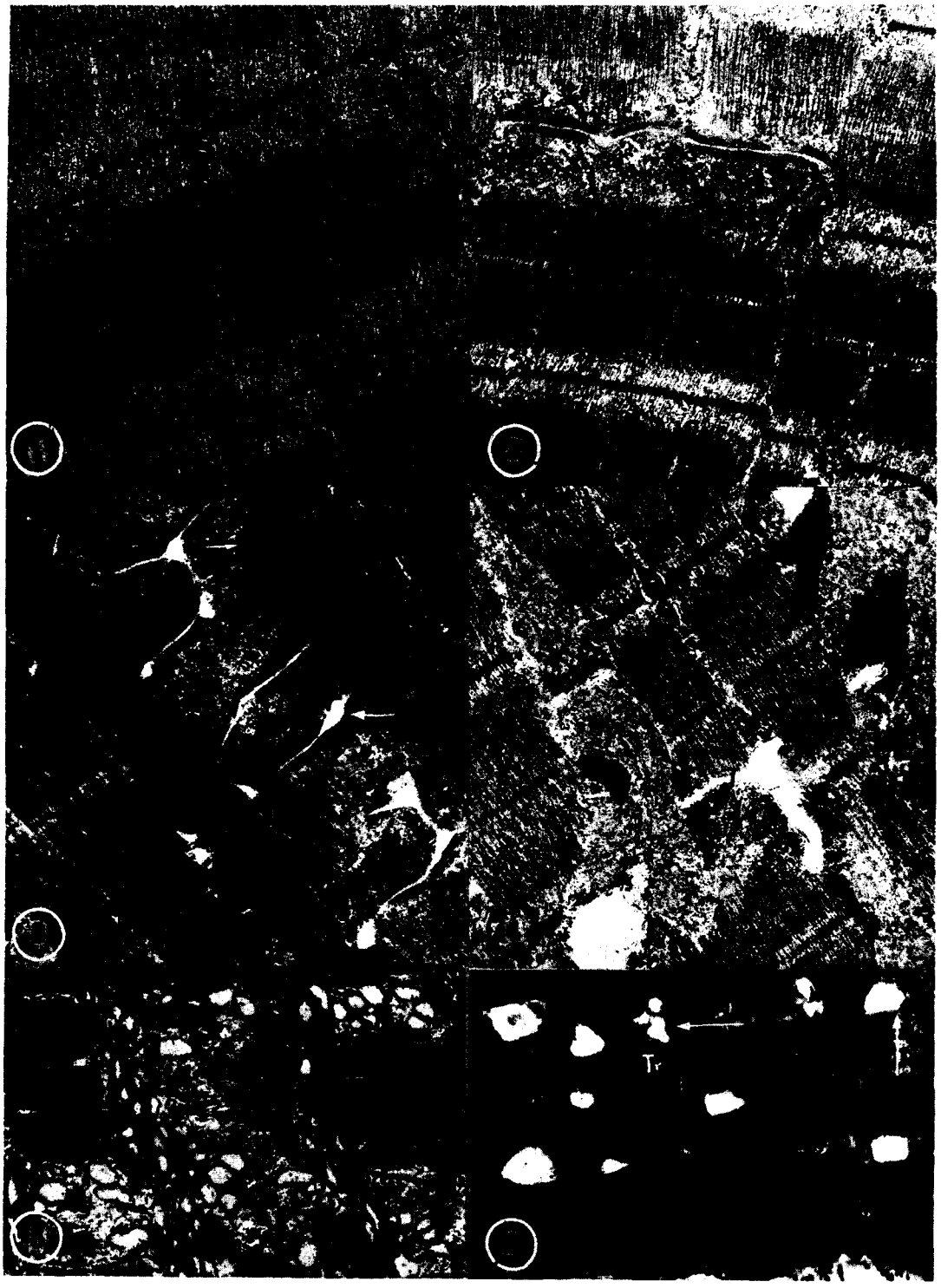
Normal. Although most mammalian skeletal muscles are mixed with type I, type II and intermediate types of fibres in the body of the rat, gastrocnemius muscle type I and intermediate type fibres predominate. The typical ultrastructure of type I fibre shows small mitochondria, few or no lipid inclusions and a preponderance of glycogen distributed between the myofibrils, mitochondria and the sarcoplasmic reticulum. Figure 1 shows a portion of a fibre with the typical arrangement of the intracellular structures described above. Figure 2 is similar but enlarged to show glycogen granules as well as the nature and size of the transverse system tubules which usually run through the myofibril at the I band near the A and I band junction. Where a triad is formed, the T-system tubule is slightly more dilated than when it traverses the fibrils.

OHP-convulsed rats. Muscle tissue obtained from rats exposed to OHP but only small-convulsed shows contracted myofibrils, conspicuously dilated T-system tubules (Fig. 3) and considerably more glycogen granules (Fig. 4) than controls. The mitochondria are less dense and the definition of the membranes is fuzzy. Rats which were allowed to convulse longer, have supercontracted myofibrils in several areas in the peripheral regions

TABLE II

COMPARISON OF BRAIN AND SKELETAL MUSCLE TISSUE LACTATE DEHYDROGENASE (μM MIN^{-1} GM WET WEIGHT), CYTOCHROME OXIDASE (μM MIN^{-1} 100 GM WET WEIGHT^{-1}) AND NADH DEHYDROGENASE (CYTOCHROME *c* REDUCTASE) (μM MIN^{-1} 100 GM WET WEIGHT) ACTIVITY IN NORMAL (N), HYPERBARIC (HA 6 ATA AIR), SMALL-CONVULSED (SC, HYPEROXIC 6 ATA 100% OXYGEN) AND LARGE-CONVULSED (LC, HYPEROXIC, 6 ATA 100% OXYGEN) RATS

	Condition	N	$\bar{X}$	SD	Comparison	σ Value	Level of Significance
Brain	N	6	230.5	19.9	N vs HA	0.09	None
Lactate	HA	5	232.0	31.5	N vs SC	2.61	0.05
Dehydrogenase	SC	10	185.5	38.9	N vs LC	2.56	0.05
	LC	9	185.7	39.3	HA vs SC	2.31	0.05
					HA vs LC	2.25	0.05
					SC vs LC	0.01	None
Muscle	N	5	1251.3	130.4	N vs HA	0.28	None
Lactate	HA	5	986.7	162.6	N vs SC	0.30	None
Dehydrogenase	SC	10	1274.7	149.4	N vs LC	0.21	None
	LC	9	1350.5	370.8	N vs SC	0.57	None
					HA vs LC	0.26	None
					SC vs LC	0.60	None
Brain	N	5	2.08	0.27	N vs HA	3.42	0.01
Cytochrome	HA	5	1.63	0.17	N vs SC	2.88	0.05
Oxidase	SC	10	1.72	0.23	N vs LC	2.49	0.05
	LC	9	1.69	0.31	HA vs SC	0.81	None
					HA vs LC	0.40	None
					SC vs LC	0.24	None
Muscle	N	5	0.364	0.05	N vs HA	2.30	0.05
Cytochrome	HA	5	0.273	0.07	N vs SC	3.78	0.01
Oxidase	SC	11	0.255	0.05	N vs LC	1.65	None
	LC	9	0.290	0.09	HA vs SC	0.55	None
					HA vs LC	0.36	None
					SC vs LC	1.05	None
Brain	N	6	1085.7	188.5	N vs HA	0.31	None
NADH	HA	4	1142.8	393.5	N vs SC	0.157	None
Dehydrogenase	SC	10	834.0	359.8	N vs LC	2.02	0.10
	LC	10	930.0	121.2	HA vs SC	1.42	None
					HA vs LC	1.61	None
					SC vs LC	0.81	None
Muscle	N	5	194.0	19.8	N vs HA	0.76	None
NADH	HA	5	209.0	39.6	N vs SC	0.10	None
Dehydrogenase	SC	9	195.6	31.0	N vs LC	0.75	None
	LC	10	211.2	48.4	HA vs SC	0.71	None
					HA vs LC	0.09	None
					SC vs LC	0.83	None


Tr

where the T-system tubules appear normal while the sarcoplasmic reticulum is more dilated (Fig. 5). Where the fibrils are not supercontracted, the T-system tubules are vacuolated (Fig. 6). These electron microscopic changes observed in the experimental rats were focal, the deeper parts of the fibre being less affected than the peripheral region.

Hyperbaric air. Rats exposed to hyperbaric air (6 ata) alone show no distinguishable electron microscopic changes in skeletal or cardiac muscle. However, there is some edema in the brain around the capillaries (Fig. 7) but no detectable disruption of motor neurons or astrocytes. In the hepatocytes, there is some small depletion of glycogen and swelling of mitochondria but no damage to the endoplasmic reticulum (Fig. 8).

Myocardium

Normal. Cardiac muscle fibres are attached to each other through the intercalated disc which becomes denser while passing through the Z line of the myofibrils. Normally, the disc is made up of the cell membranes of the adjacent fibres with little space in between them (Fig. 9).

OHP-convulsed rats. Myocardium from long-convulsed rats showed disruption of the intercalated disc, and swollen and vacuolated mitochondria (Fig. 10). As in the skeletal muscle, the transverse system tubules as well as the sarcoplasmic vesicles were dilated and often damaged (Fig. 11).

Liver

Normal. The liver acts as a central store and clearing house of the body, the hepatocytes normally containing large stores of glycogen (seen as β granules), some lipid globules, large numbers of mitochondria and several dense lysosomes and microbodies. The nucleus is usually spherical, and both the granular and agranular reticulum are present in the same cell (Figs. 12a and b). The hepatocytes are closely packed and, where the bile capillaries are formed, the cell membranes show dense structures resembling desmosomes. Few studies of O_2 effects have been done (27).

FIG. 1. Portion of a muscle fibre from gastrocnemius (type I) showing the normal ultrastructure of the sarcomeres, mitochondria and the sarcoplasmic and transverse system tubules. M, Mitochondria; T, Transverse system tubule; Tr, Triad; Z, Z-line. X11610.

FIG. 2. Portion of normal type I fibre showing the size and structure of the transverse system (T-system) tubule passing through the sarcomeres as well as the store of glycogen granules. T, T-system tubule; G, Glycogen. X16420.

FIG. 3. Gastrocnemius muscle type I fibre from short-convulsed rat. Note the dilation of the T-system (arrow) tubules especially where the triad is formed. The sarcomeres are more contracted showing a small I band. X11610.

FIG. 4. From a different rat similarly treated showing ill-defined mitochondria and large accumulation of glycogen. The T-system is seen dilated in several places. G, Glycogen; M, Mitochondria; T, T-system tubule. X16420.

FIG. 5. Portion of type I fibre from large-convulsed rat showing supercontracted sarcomeres and vesiculated sarcoplasmic reticulum. SR, Sarcoplasmic reticulum. X11610.

FIG. 6. From the same muscle where fibrils are not supercontracted showing largely dilated triads and less glycogen storage. Tr, Triad. X7780.

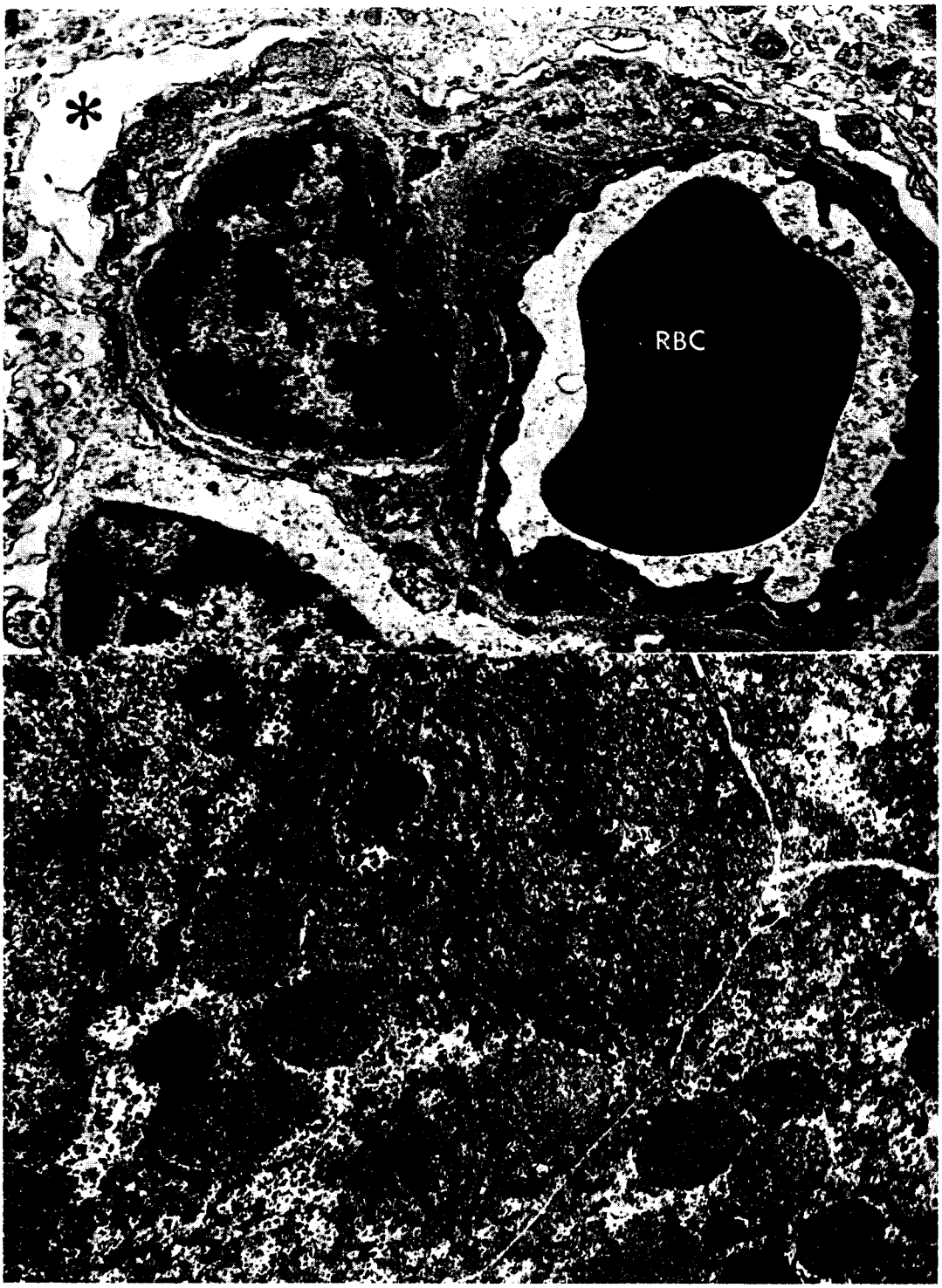


FIG. 7. Part of cerebral cortex from rats kept in 6 ata of air showing some space (asterisk) between capillary and the axonal processes with no apparent damage to the cells. GC, Glial cell; RBC, Red blood cell. x23320.

FIG. 8. Part of liver cells from rats under pressure and air. Some depletion of glycogen and swelling of mitochondria but no apparent effect on endoplasmic reticulum. x23320.

FIG. 9. Portion of myocardial fibres showing the normal arrangement of myofibrils, mitochondria and intercalated disc. ID, Intercalated disc; M, Mitochondria. X11610.

FIG. 10. Similar region from a long-convulsed rat showing swollen and often damaged mitochondria and the intercalated disc showing discontinuity. V, Vacuolated mitochondria. X7780.

FIG. 11. Part of the myocardial fibre enlarged to show the changes in the transverse tubule and sarcoplasmic reticulum. Pinocytotic vesicles can be seen forming from the T-tubule (arrow) and several sarcoplasmic vesicles show damaged membranes. X46640.

OHP-convulsed rats. In the hepatocytes from the small-convulsed rats, much of the glycogen is depleted and there is more space between cells (Fig. 13). Dense lysosomelike bodies and mitochondria appear normal while the granular endoplasmic reticulum appears to be slightly deranged with more ribosomes free than attached to the membranes. In the large-convulsed rats, in addition to complete depletion of glycogen, there is conspicuous dilation of the reticular systems (Figs. 14 and 15). The red blood cells congregate in rouleaux (Fig. 15). The dilation of the reticular systems and the swelling and matrix disintegration of the mitochondria are clearly seen in the more magnified portion of the micrograph shown in Fig. 16.

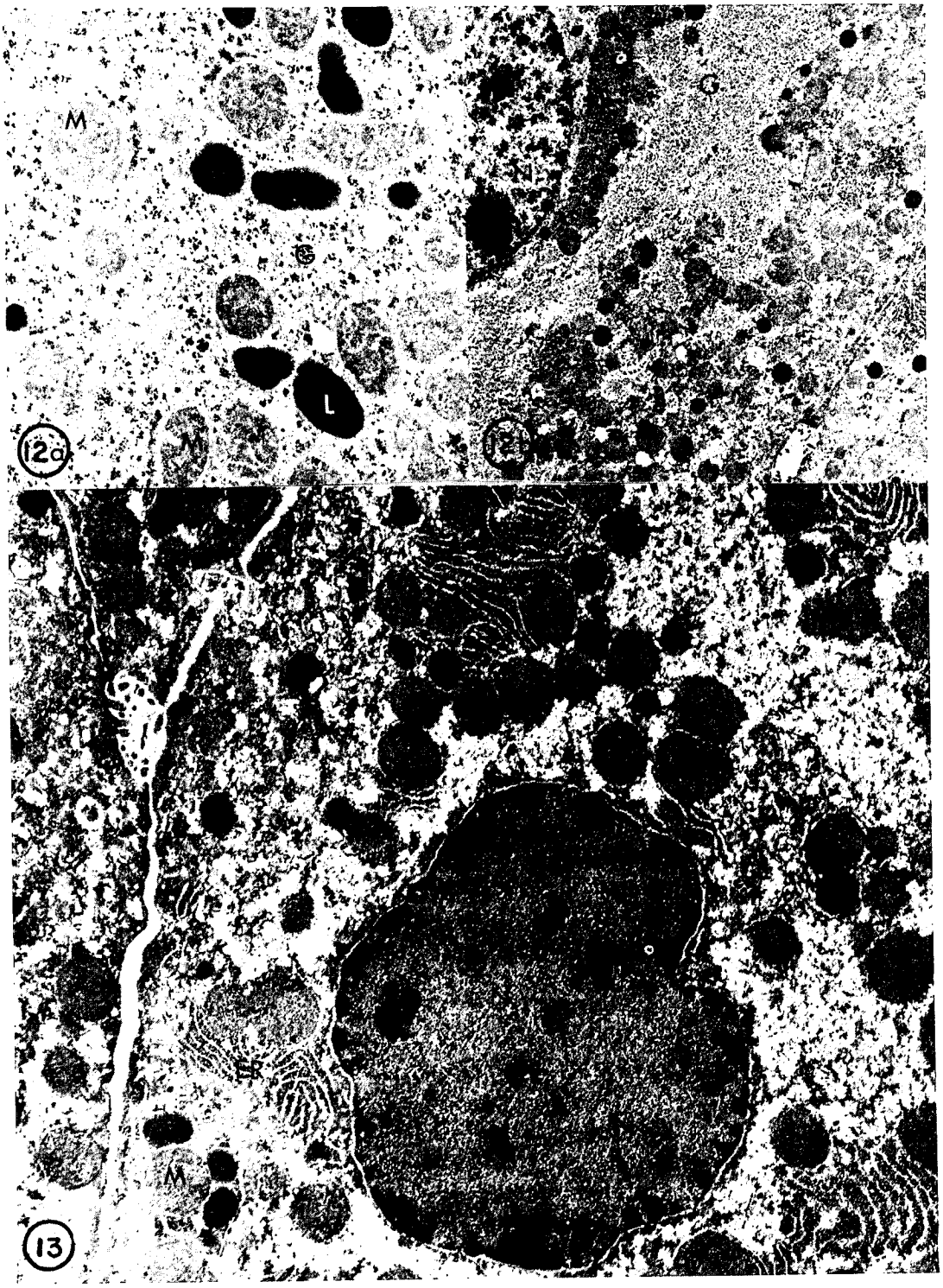
Cerebral cortex

Normal. The cerebral cortex consists largely of neurons of various kinds with a diverse function. In the present investigation, concentration of interest was on structural differences (the astrocytes, motor neurons and the blood capillaries) of the cortex. Figure 17 shows a part of the cortical region where an astrocyte and two microglial cells may be seen. The astrocytes are usually denser than other neurologia containing lysosomelike bodies while the microglial cells possess very little cytoplasm. The motor neuron (Fig. 18) is less dense and contains a large nucleus with a conspicuous nucleolus. The cytoplasm contains granular endoplasmic reticulum, ribosomes in polysomal configuration, one or two well-developed Golgi bodies and slender mitochondria (Fig. 19). The neurofilaments which are found in the cytoplasm become more numerous toward the axon hillock, and the ribosomes and mitochondria become sparse throughout the axon.

OHP-convulsed rats. Small convulsions disrupt the polysomal arrangement of the ribosomes and the Golgi bodies vesicles, and the mitochondria become swollen (Fig. 20). The cytoplasm of the glial cell close to the blood capillaries is often disintegrated though no destruction of the nucleus is apparent. Inside the capillary, the red blood cells are very closely packed to form dense rouleaux (Fig. 21). There are also large spaces and damaged neuronal processes near the blood capillaries (Fig. 22). The astrocytes are much more dense than normal with severe structural damage to other intracellular organelles like the mitochondria, Golgi bodies and the endoplasmic reticulum (Figs. 23 and 24). In the astrocytes, as well as in some axons, most mitochondria are disrupted, cell nuclei are pyknotic, and the Golgi bodies become highly dilated (Fig. 25). The motor neurons are also severely affected in the large-convulsed rats with mitochondrial disruption, severe vesiculation of the endoplasmic reticulum (Fig. 26), dilated Golgi bodies and free ribosomes (Fig. 27). The above-mentioned cellular damage is greater in the vicinity of blood capillaries which consequently appear to be surrounded by large spaces and damaged astrocyte processes.

FIG. 12 a & b. From normal rat liver showing hepatocytes with spherical nucleus, large store of glycogen, several mitochondria and some lysosomelike bodies. A bile capillary can be seen in Fig. 12b. ER, Endoplasmic reticulum; G, Glycogen; L, Lysosomelike bodies; M, Mitochondria; N, Nucleus. X16420 and 4140, respectively.

FIG. 13. From short-convulsed rat showing depletion of glycogen granules, more dense nucleus and slight derangement in the endoplasmic reticulum. There is also increased intercellular space between the hepatocytes. ER, Endoplasmic reticulum; M, Mitochondria and N, Nucleus. X23320.


M
G
L
12a
12b
ER
M
13

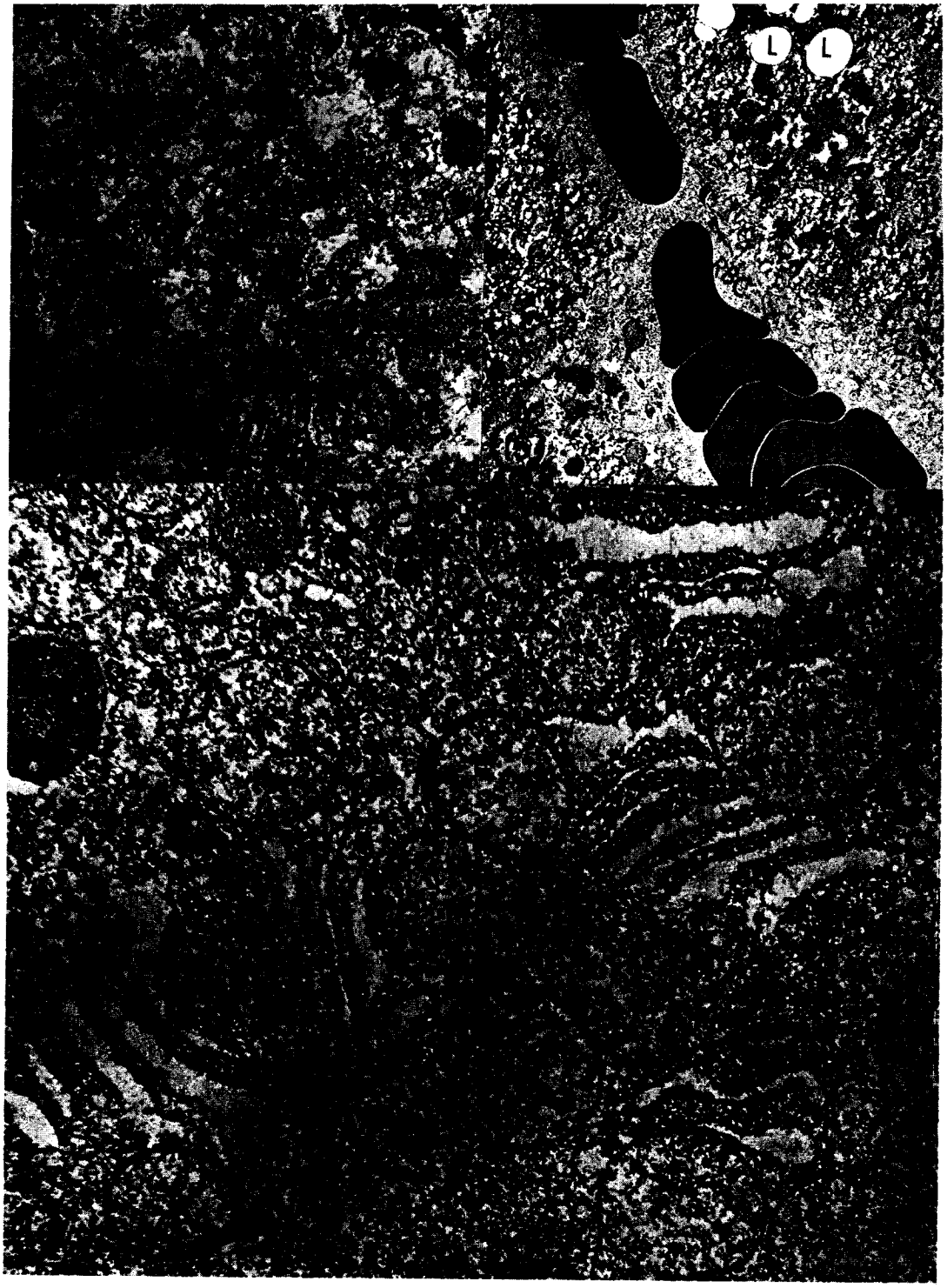


FIG. 14. Part of hepatocyte from large-convulsed rat showing dilated endoplasmic reticulum, swollen mitochondria. VER, Vesiculated endoplasmic reticulum; M, Mitochondria. x5044.

FIG. 15. A blood capillary showing very closely packed RBC similar to rouleaux formation and the cells on either side showing depletion of glycogen and no apparent lysis of the lipid globules. L, Lipid; RBC, Red blood cells. x4140.

FIG. 16. A portion of liver cell from long-convulsed rat to show the vesiculation of the granular endoplasmic reticulum, equally dilated soft reticulum and swollen mitochondria with disrupted matrix and cristae. M, Mitochondria. x32840.

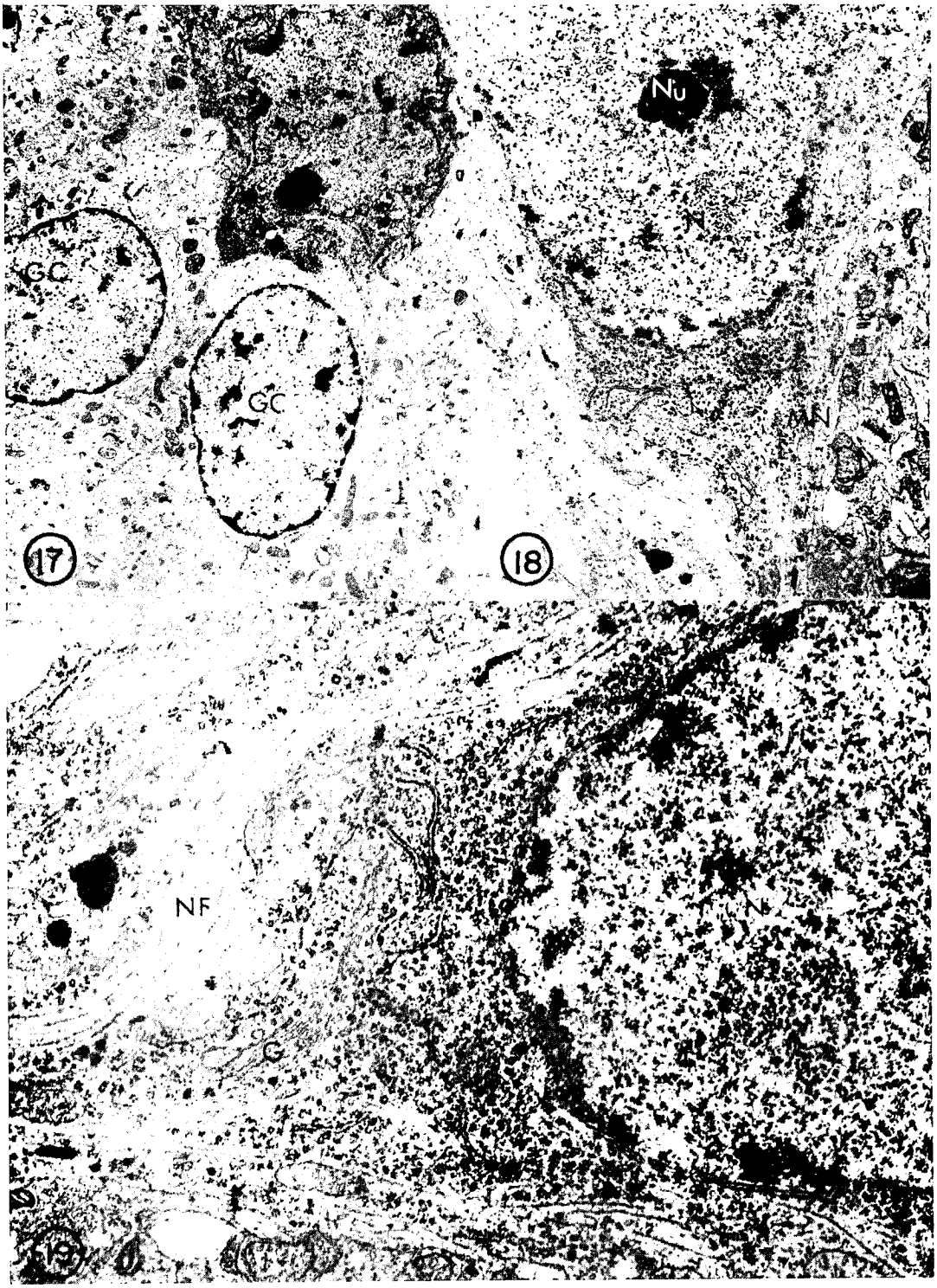


FIG. 17. Cortical region from control rat. Showing an astrocyte and two glial cells leaving little intercellular space between them. AC, Astrocyte; GC, Glial cell. x4140.

FIG. 18. Part of a motor neuron showing well-developed nucleus, nucleolus, granular endoplasmic reticulum, Golgi bodies and mitochondria. Several synapses can be seen adjacent to the neuron. MN. Motor neuron; N, Nucleus; Nu, Nucleolus. x7780.

FIG. 19. Part of Fig. 18 enlarged to show the polysomal arrangement of the ribosomes, Golgi region and neuronal filaments. G, Golgi bodies; N, Nucleus, NF, Neural filaments. x32840.

FIG. 20. Part of a motor neuron from short-convulsed rat showing the dilated Golgi body, damaged mitochondria and the free ribosomes. x46640.

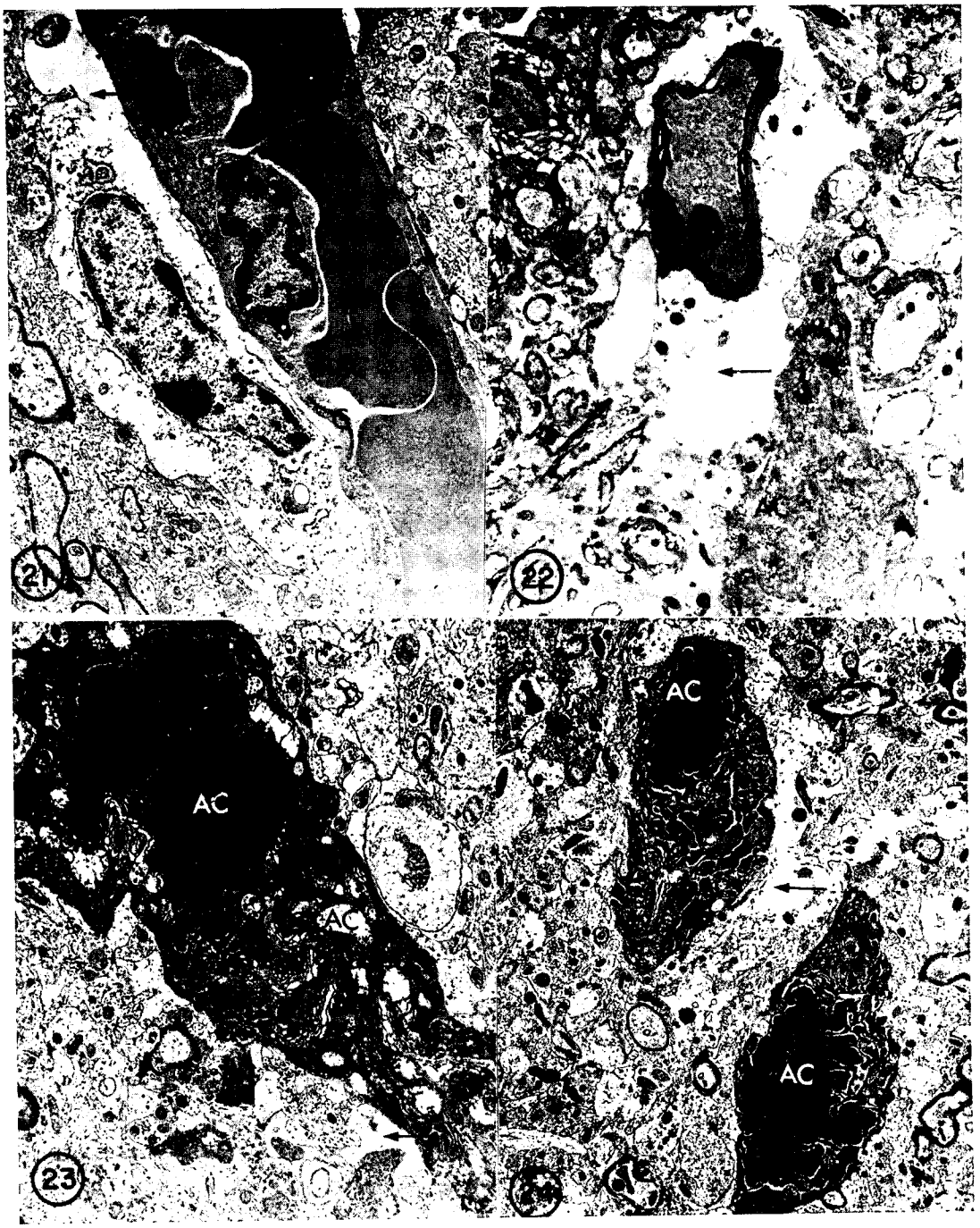


FIG. 21. Cerebral cortical region from long-convulsed rat showing a capillary packed with RBC and completely cytolyzed cytoplasm of a dendrite with no apparent damage to the nucleus. Some axonal processes are also damaged (arrow). C, Capillary; RBC, Red blood cell. X7780.

FIG. 22. From a different rat similarly treated showing large space (arrow) around the capillary and some disorganization of the myelin structures of axons. X7780.

FIG. 23. An astrocyte from a severely convulsed rat for long time showing pyknotic nucleus, swollen and damaged mitochondria and dilated Golgi bodies. X7780.

FIG. 24. Shows two astrocytes in more condensed and dense condition than normal. X4140.

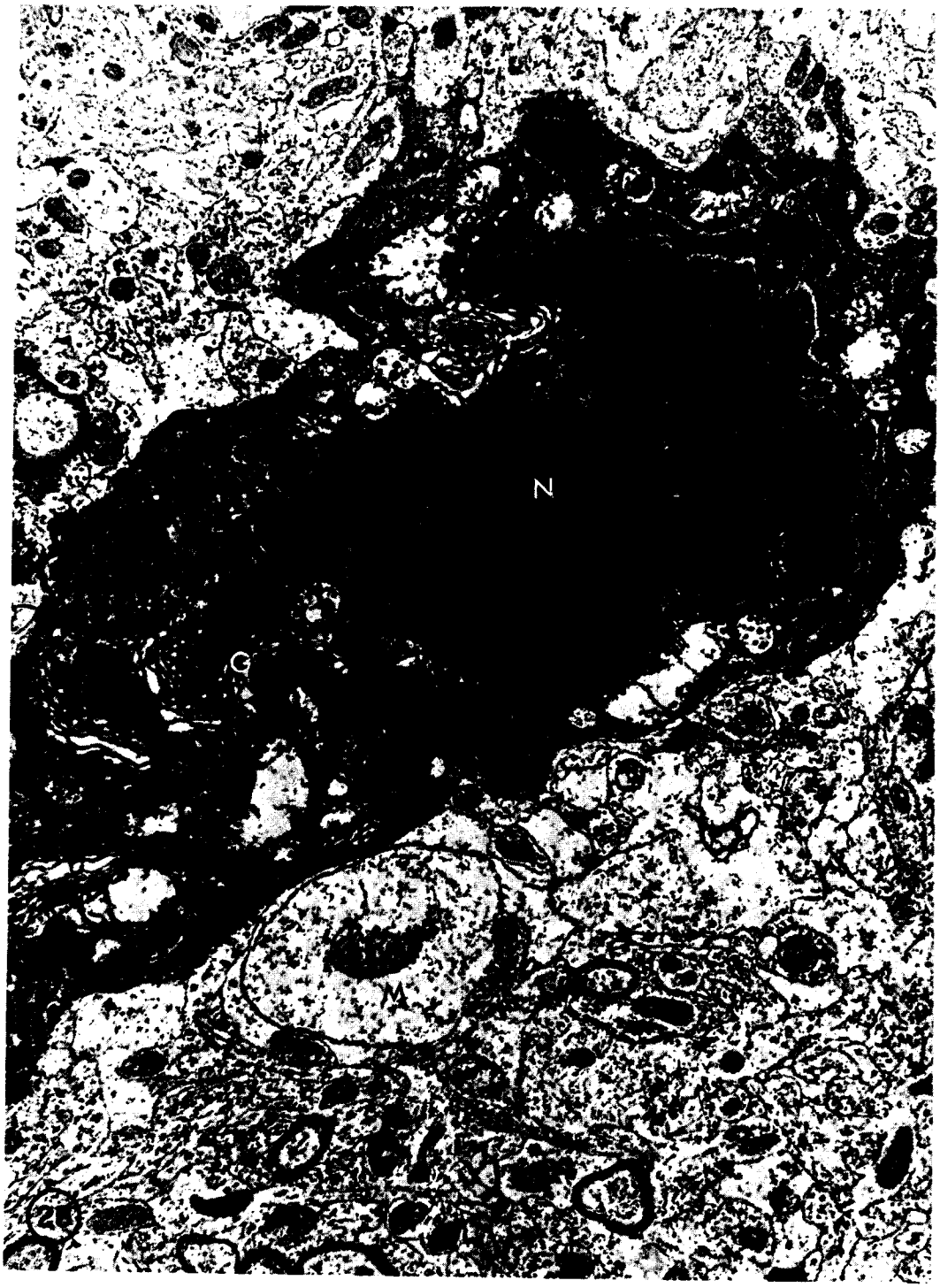


FIG. 25. Part of Fig. 23. Enlarged to resolve the disruption in the astrocyte. Note the damaged mitochondria, the dispersion of the granulated endoplasmic reticulum and the dilated Golgi bodies. N, Nucleus. x32840.

FIG. 26. A part of a motor neuron from large-convulsed rat showing severe vesiculation of the endoplasmic reticulum, mitochondrial damage and separated synapse. VER, Vesiculated endoplasmic reticulum; M, Mitochondria. S, Synapse. x46640.

FIG. 27. A different motor neuron showing similar damage as seen in Fig. 26 along with dilated Golgi body. x46640.

Hypophysis

Normal. Cells in control rats are packed with secretory granules (Fig. 28).

OHP-convulsed rats. Both acidophils as well as the basophils of the adenohypophysis are affected in convulsed animals. Those from the large-convulsed rats have fewer secretory granules, the reticulum has become vesiculated, and the mitochondria show swelling and disruption of the inner membranes and matrix (Figs. 29 and 30).

Adrenal cortex

Normal. Unlike the peptide hormones of the pituitary, the steroid hormones of the adrenal cortex are not stored as granules, and it is believed that the mitochondria in the cortex play an important role in the synthesis and perhaps storage of the corticoid hormones. Hence, the mitochondrial structure is different in adrenocortical cells (Fig. 31). The cortical cells from large-convulsed rats have swollen mitochondria without any definition of their outer membrane. There is separation of the outer nuclear envelope as well as a general depletion of materials from the cells (Fig. 32).

Discussion

The overall picture of changing blood composition, tissue biochemical change and ultrastructural alteration is one of severe disruptions in rats subjected to oxygen at high pressure, in contrast to the mild consequences of nonconvulsive exposure to hyperbaric air.

Hyperbaric Air (6 ata) Effect on Blood and Tissue Enzymes and Ultrastructure

No unnatural behaviour was observed in rats breathing air at 6 ata. Plasma lactate dehydrogenase was not significantly elevated above normal. The tissue enzyme activity of both brain and muscle cytochrome oxidase was significantly depressed but the accompanying ultrastructural changes in heart, skeletal muscle and brain tissue were small. Blood glucose levels remained normal, and only serum aspartate transaminase (SGOT) unaccountably decreased significantly. It would seem, therefore, that compared to normobaric control animals, the effects of hyperbarism with slightly elevated oxygen pressure (P_{O_2} 1.2 ata in 6 ata air) without the attendant complicating features of narcosis and decompression sickness are mild at the pressures attained in this investigation.

Blood, Tissue and Ultrastructural Changes in Small- and Large-Convulsed Rats

Blood glucose levels in convulsed rats were significantly elevated above normal and hyperbaric air rats. It has been noted that long oxygen exposure at atmospheric pressure has a similar effect on carbohydrate metabolism generally. Simultaneously, lactate and pyruvate concentrations decreased. Similarly, Weglicki et al. (32) noted decreased lactate levels in dogs exercising at 3 ata oxygen. These observations have been taken to be indicative of decreased glycolysis during oxygen exposure. It is noteworthy that the ultrastructural evidence that glycogen accumulates in skeletal muscle supports this hypothesis. The depletion of liver glycogen observed is in accord with previous investigations (27) and suggests

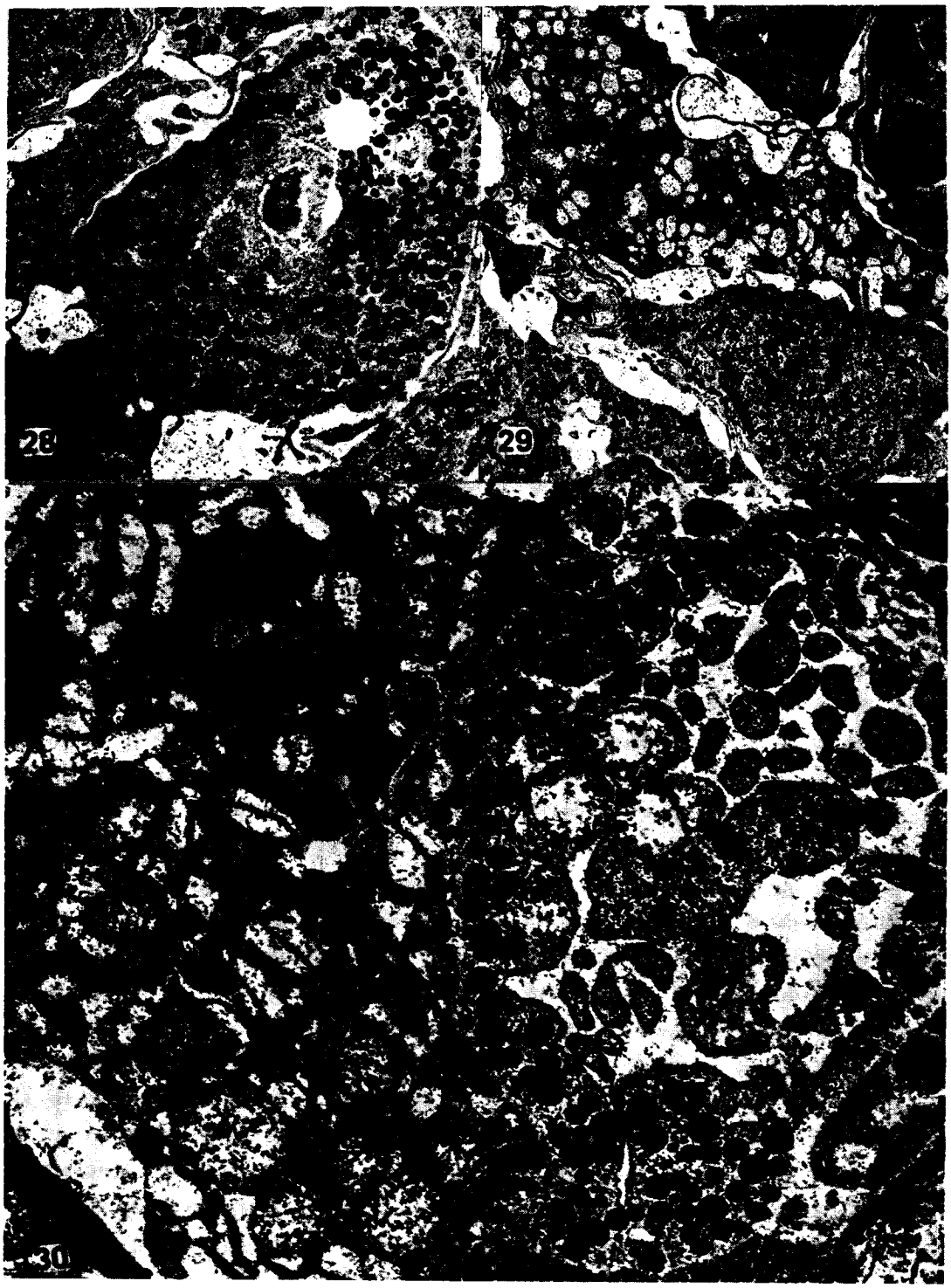


FIG. 28. Portion of the anterior pituitary showing the different cell types filled with secretory granules. x7760.

FIG. 29. From large-convulsed rat showing depletion of secretory granules as well as swollen mitochondria in acidophil. x4140.

FIG. 30. A magnified portion of an acidophil cell showing vesiculation of the reticulum as well as the swollen and damaged mitochondria. SG, Secretory granules; M, Mitochondria. x32840.

FIG. 31. Adrenal cortical cell showing the peculiar structure of mitochondria and large lipid globules. N, Nucleus; M, Mitochondria; L, Lipid. x23320.

FIG. 32. Portion of cortex from large-convulsed rat showing the loss of density in the mitochondria as well as the disruption of mitochondria. The nucleus appears to have shrunk from the outer membrane (arrow). M, Mitochondria; N, Nucleus. x46640.

that liver glycogenolysis, perhaps stimulated by circulating hormones released during or prior to the convulsion, contributes to the hyperglycaemia.

Since the depletion of glycogen, swollen disrupted mitochondria and dilation of the reticular formation in the liver suggest impairment of its function, alternate detoxifying metabolic pathways and new sources of substrates may be utilized by the organism at this time. These, as a consequence, may precipitate convulsive action.

A major substrate and regulator of neuronal activity in the central nervous system noted by Wood and Watson (34) and others is GABA, the precursor of which in the brain is glutamate. Brain glutamic acid is normally produced from other amino acids or from carbohydrates via pyruvate and oxaloacetate or malate which then serve as a continuing source of α-ketoglutarate in the transamination reactions with α-amino acids forming glutamate (13, 38). The detoxifying action of glutamate involves its amidation to glutamine catalysed by glutamine synthetase and requiring ATP (13).

In circumstances where ammonia released in various organs is not removed by the liver, it is possible that an intolerable drain is placed upon brain glutamate concentrations and, hence, subsequently on brain and citrate cycle intermediates, which a poisoned glycolytic sequence and oxidative pathway cannot maintain. In consequence, brain glutamate levels would be depleted and normal glutamate metabolism, both as a source of GABA in neuronal regulation (37) and as a bypass of the citrate cycle for α-ketoglutarate to succinate, would cause the FAD-linked electron transfer (26) to be compromised. Sanders et al. (25) have argued that the citrate cycle bypass, forming glutamate from α-ketoglutarate via succinate semialdehyde, is advantageous since ATP produced in the oxidation of succinate occurs via a FAD-linked electron transport system rather than one adversely affected by hyperbaric oxygen as is the NAD linkage (9, 10, 30). In addition, Sanders et al. (25) have proposed but not confirmed that deficient brain ATP production plays a significant role in seizure genesis mediated by hypoxia, or the convulsive drugs Metrazol and hydroxylamine, although this concept has been challenged by Collins (11).

Cardiac function has been shown to be impaired during exposure to hyperbaric oxygen by Kioschos et al. (17) and the electronmicrographic details of heart muscle from large-convulsed animals of this investigation provide supporting ultrastructural evidence for such overt mechanical impairment. Dilation of the transverse tubules and sarcoplasmic vesicles would result in poor activation and spread of excitation together with sustained depolarization, whereas swollen, damaged mitochondria contribute to deficient energy production for maintenance of membrane integrity and the sodium pump mechanism.

Recent investigations on hyperoxic convulsions in rats (4) have shown a precipitate loss of cerebral oxygen regional vascular control, resulting in secondary vasodilation and parallel regional blood flow prior to and accompanying the convulsive state. This is accompanied, overall, by a sharply increased flow through the brain and a greatly elevated tissue P_{O_2}. The prime factor in the induction of this sudden loss of vascular control remains uncertain although it is proposed that excessive oxygen (or an unknown metabolite) in the extravascular spaces and tissues causes accumulated toxic effects, resulting in breakdown of vasoconstrictive control of cerebral regional blood flow. Supportive evidence for these ideas is the progressively disrupted neurologia close to the blood capillary in small- and large-convulsed rats. Glial cell cytoplasm is often disintegrated, and there are large spaces and damaged neuronal processes in the vicinity of the capillary, together with swollen and disrupted mitochondria. Alternately, the loss of vascular control may be triggered by local

hypoxia since the red blood cells inside the capillary form rouleaux, perhaps resulting from damage by hyperoxia to the lipid membrane of the erythrocyte resulting in increased "stickiness" (8). Decreased flow in the precapillary, resulting from the original vasoconstrictive control and the stasis associated with obstruction of flow by rouleaux, would be expected to cause local deficiencies in the supply of oxygen, as well as some edema resulting from the loss of plasma protein to the extravascular space through damaged capillary walls. These observations together with the significantly depressed respiratory chain activity of the brain (cytochrome oxidase significantly depressed in both small- and large-convulsed rats and NADH dehydrogenase activity significantly depressed in large-convulsed animals), prompt description of these events leading to the convulsive state as hyperoxic histohypoxia in addition to hypoxia related to the apneic state previously described as hyperoxic anoxia (3).

Integrity of the Hypophysis and the Adrenal Cortex

Probable widespread imbalance of circulating hormones in convulsed rats is reflected in the ultrastructural details of the hypophysis and adrenal cortex from these animals. Although cells of control rats from the anterior pituitary abound with full secretory granules, these granules are sparse in large-convulsed animals and those remaining are largely empty cells, indicating that the target glands for both basophils (ACTH, TSH, gonadotrophins) and eosinophils (growth hormone) are acutely maximally stimulated. In addition, there is organelle damage as indicated by the vesiculated reticulum and the swelling and disruption of the mitochondria. Other investigators have found no changes (12).

The hyperactivity and disruption of the adrenal cortex cells confirms this. The cortical cells from large-convulsed animals have swollen mitochondria and ill-defined or absent delineation of their outer membranes. There is general depletion of material from the cells. Secretions from the adrenal cortex have widespread stimulatory effects on many metabolic processes including formation of increased blood sugar levels and formation of sugar from protein, with accompanying deamination of amino acids and increased glycogen storage which is observable in muscle tissue from small-convulsed rats. Detoxification of ammonia from the blood must load the brain glutamate system as described previously. A potent effect of growth hormone and cortisone is upon lipolysis, via cyclic AMP, of the fat storage depots (23). This may be a factor in the general swelling and disruption of mitochondria since Wojtczak et al. (33) have presented evidence of the regulatory and disruptive effect of long-chain fatty acids on the energy-producing and energy-requiring processes in mitochondria and upon the permeability of mitochondria to adenine nucleotides. Although not directly investigated, it is likely that the hormones of the adrenal medulla are also involved in the general metabolic imbalance ensuing from the hyperoxic conditions and other stress inherent in the procedures.

The widespread derangement of the ultrastructural integrity of several tissues of the animal organism due to OHP, observed in this study, serves to indicate the need, proposed by Lambertsen (19), for further integrated studies of the etiology of the convulsive syndrome throughout the body, modifying the disproportionate emphasis on central nervous system and pulmonary effects.

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PART VII. OXYGEN*

DISCUSSION

J. D. Wood, Chairman

Dr. Lundgren: I would like to comment on the paper on control of ventilation at 4 ata. As I understood it, you created an increasing CO_2 stimulus by rebreathing into a spirometer. When you studied the simultaneous effect of hypoxia you let the oxygen concentration decrease over a period of 7 or 8 minutes; during this period you measured ventilation.

This makes me concerned because CO_2 stimulation is mainly through central receptors, which require some time before they respond because CO_2 has to pass through the cerebrospinal fluid. Therefore you have dealt here with a nonsteady state and that would make the absolute figures that you have measured uncertain in relation to CO_2 stimulation. I would think this would also be true with regard to the hypoxic stimulus, although it has a much faster action since it is on the peripheral chemoreceptors.

Dr. Doell: The 1 atmosphere and increased pressure studies were done the same way, providing comparative control for the CO_2 response curve method. We also calculated lag for circulation time and found there was no significant difference between the results we obtained and results using a calculation for circulation lag to account for the CO_2 difference that you are talking about.

Dr. Lundgren: I am not talking about circulation time between lungs and the receptors, but delay in response due to the CO_2 having to pass through the cerebrospinal fluid.

Dr. Clark: Dr. Sanders, your work showed that the biochemical changes which you observed in oxygen-poisoned lung were not prevented by adrenalectomy, and yet it has been well established that adrenalectomy does in fact retard the development of pulmonary oxygen toxicity. Can you reconcile these findings?

Dr. Sanders: Have not most of these studies simply involved gross observations of pathology rather than biochemical measurements? I believe the investigators looked at the lungs, and the lung looked better, but they did not do biochemical assays.

Dr. Clark: It's true they did not do biochemical assays. The studies primarily compare survival times and gross pathology. But biochemical changes do in fact lead to the development of pulmonary oxygen toxicity; we are not sure of the exact biochemical changes we should be looking at. Somewhere adrenalectomy has to help.

Dr. Sanders: When we saw this, it came to our surprise. However, we then went back and looked at convulsions. We have done much work on convulsions in our previous oxygen toxicity studies. We compared adrenalectomized and normal animals at 5 atmospheres of 100% oxygen. Where the normal rat convulses at an average of about 62 minutes, the adrenalectomized animals convulsed at 178 minutes. The adrenalectomy definitely gives a tremendous protection to something in the CNS.

I think any protective effect of adrenalectomy is where there is CNS involvement and maybe pulmonary effects secondary to the CNS involvement. At least we did not see what we could call a real protection biochemically in the lung.

Dr. Clark: The protective effect of the adrenalectomy has also been observed at 1 atmosphere.

Dr. Sanders: We have not studied oxygen at 1 atmosphere with adrenalectomies.

Dr. Lambertsen: I suggest that we use the term "masking effect" instead of "protecting effect" for adrenalectomy.

Dr. Radomski: I wonder whether by adrenalectomy we rule out the sympathetic nervous system and activation of that and its effect on certain biochemical changes.

**Panelists*: M. W. Radomski, H. Gilder, A. P. Sanders, J. Madsen, E. W. Banister, H. V. Hempleman, D. Doell.

Dr. Sanders: Dr. Wood has posed several questions relating to some of our previous work and I would like to offer answers. On the lung ATP, there is a very reproducible decrease in high oxygen exposures. The paper he refers to is one where we exposed animals to 5 atmospheres of 100% oxygen in a small non-temperature-controlled chamber, and then did ATP assays on those that were alive at the end of the 90 minutes. A drop was found.

We compared these with anesthetized controls. This was done because if you use an awake animal and decapitate that animal into liquid propane and measure ATPs, you will find a significantly lower level of CNS ATP than in an anesthetized animal. Even further, if you hyperventilate an anesthetized animal you will get an even higher ATP.

If we measured ATP in the course of the oxygen study at 30 minutes and 60 minutes, we found elevated ATPs and do not know when the subsequent drop occurred.

The time that you take for the ATP assay in an animal is extremely critical because we know that a drop in ATP can occur within a matter of 20 seconds. For this reason we compared values obtained from unanesthetized animals at 5 atmospheres prior to convulsion with normal animals and, as Dr. Wood said, no difference in ATP is found.

With regard to the relation of GABA and succinate, GABA is part of the glutamate shunt.

No one has yet confirmed the hypothesis that succinate is going to push the shunt direction back two steps. Moreover, Eugene Roberts says that GABA cannot penetrate the blood-brain barrier.

You do get anticonvulsive protection from GABA in oxygen toxicity, as Dr. Wood has reported. Similarly, GABA provides protection from convulsions induced by thiosemicarbazide or Metrazol. We have repeated these experiments with succinate and got protection against Metrazol convulsions with succinate.

But then if the GABA protection is to hold, the stress is increased to force the convulsions to occur more rapidly, if the GABA inhibitor is functioning, then a degree of protection should remain. So we raised the dose of Metrazol to 17.5 mg per kilo and we found the GABA protection disappeared and the succinate protection remained.

We then did a series of experiments to compare glutamate, GABA and succinate in O_2 toxicity and increased the pressure of oxygen to where the convulsion times occurred rapidly; again the succinate was the sole remaining protection. So obviously we are leaning in the direction of the succinate stimulus to ATP production.

Why do we say succinate stimulus? If you give exogenous succinate or if you take tissue slices or tissue homogenates of brain (which is the most important tissue here), as you go up scale—you can increase the ATP production with exogenous succinate by roughly 70%. If the animal gets larger where the metabolic rate goes lower, that increase goes up drastically to where you can get an increase of 350% of ATP production in the brain of human by administering succinate.

Dr. Banister: In response to Dr. Sanders' comment about pushing succinate through two previous states back to GABA, it would seem that would be impossible with falling ATP levels.

Dr. Gottlieb: We too have studied lithium effects in O_2 toxicity in mice and found protection against convulsions as noted by an increase in the preconvulsive latency period. However, we did not find any lung protection and I think that was because we kept all our animals in for a constant period of time, 4 or 5 minutes. Under those conditions there is no protection of the lung indicated from measuring lung weight/body weight ratio.

I would like to ask Dr. Banister: The type of changes you saw with oxygen convulsions—would you see those same changes using any convulsant agent?

Dr. Banister: We have seen those changes incipiently in other kinds of experiments done in hypoxia and, as has been said, Metrazol and hypoxia will cause these kinds of changes too.

In 1940 Bean proposed a term which he called "hyperoxic anoxia." We have proposed in this study to call this hyperoxic histohypoxia, which reflects the inability, although it is in the presence of high oxygen atmosphere, of the cell to use that oxygen in conditions of oxidative phosphorylation or any other kind of process that the cell needs to function.

Dr. Danziger: We also can substantiate Radomski's finding on the lithium. We had seen a paper on bioamine-mediated hyperexcitability induced by morphine in mice in which lithium protected and rubidium tended to aggravate the hyperactivity. We tried lithium.

We also looked at the inhibition by O_2 of alpha-ketoglutamate decarboxylase and pyruvate decarboxylase, in collaboration with Dr. Neptune, and found that lithium, as we had possibly expected, did not reverse the inhibition of these enzymes.

Part VIII. INERT GASES AND HYDROGEN

PHARMACOLOGICAL EFFECTS OF INERT GASES AND HYDROGEN

P. B. Bennett

This review will consider primarily, the research since the last Symposium (56) on the measurement, causes and mechanisms of the narcosis produced by the physiologically inert gas series: helium, neon, nitrogen, argon, krypton and xenon. During this period, helium has been of considerable interest due to the significant increase in experimental deep diving beyond 1000 feet. The results from the many measurements made during these simulated dives have been reported in detail previously at this Symposium. This paper will therefore be restricted mainly to research at depths less than 1000 feet. Furthermore, the gases xenon and krypton, due to their strong narcotic properties, are primarily of academic interest so far as diving is concerned; also, due to their expense, they have not been studied in connection with inert gas narcosis in recent years. Neon ~~will be~~ considered extensively later in the Symposium and ~~will~~ not be dealt with in any detail here (76).

However, hydrogen has received additional study since the work of Brauer et al. (30), which showed that the relative narcotic potency of hydrogen in mice is about a quarter that of nitrogen. This confirmed earlier work by Zaltsman and his co-workers in Leningrad, reported in 1961 (87) and 1968 (88), which describes the very considerable amount of research carried out in Russia over the last decade in connection with hydrogen, inert gas narcosis, the high pressure nervous syndrome (HPNS) and oxygen toxicity.

Hydrogen

Hydrogen has been considered as a diluent in the breathing gases since the early forties (27, 34, 58, 87–89) principally because it is the lightest gas available and, therefore, the gas of choice to permit effective pulmonary ventilation in very deep diving at depths where even helium may be causing respiratory embarrassment. In addition, hydrogen may have a decompression advantage over helium (52). The fact that an oxygen–hydrogen mixture, when the oxygen is in excess of 4%, is spontaneously explosive at 585 °C, has discouraged great interest in this mixture in diving. As a result of recent research on its flammability (41), however, there is reason to believe that mixtures of 3–4% oxygen can be used with reasonable safety (44).

Consideration of the narcotic potency and flammability limits led Brauer and Way (28) to suggest that oxygen–hydrogen would need to be restricted to a depth zone between 300 and

750 feet, depths at which hydrogen would have little or no respiratory advantage over helium. Conversely, Brauer et al. (29), showed also that the convulsions in mice and squirrel monkeys, due to HPNS, occur at higher pressures with hydrogen than with helium and that addition of hydrogen to a helium-oxygen mixture raised the convulsion pressure. Since nitrogen and nitrous oxide had a similar effect, the protective action was believed to be due to the narcotic properties of these gases.

This stimulated further experiments with hydrogen by the French (83) as reported by Chouteau (35). Five rabbits were compressed in an oxygen-hydrogen atmosphere to 908 feet (29 ata) with an oxygen partial pressure of 0.3 ata, but after 7–12 hours all had died. Similarly, Zaltsman and co-workers (88) exposed mice to hydrogen at pressures up to 50–90 ata and found that convulsions occurred at higher pressures than with helium but that after 30 minutes a number of the animals also died.

Yet Brauer et al. (30) have exposed a number of monkeys to 58 ata with 0.5 ata oxygen and the remainder hydrogen for periods of 24 hours without effect, and eight other monkeys survived exposures of 6–20 hours at 60–75 ata, as did mice to 90 ata for considerable periods, without ill effect. It is possible that the cause of the deaths in the Russian and French work was hypothermia for the chambers were not heated to 32–34 °C, as was Brauer's, and hydrogen (like helium) has a high thermal conductivity. Whether, as has also been suggested, hydrogen interferes with cellular metabolism at these pressures needs further research.

Rostain and Naquet (75) twice successfully exposed a Papio-papio baboon to hydrogen at 1000 feet for 24 hours. In spite of convulsions and early modifications of the electroencephalogram, no deaths resulted. Furthermore, at the COMEX laboratories a Papio-papio baboon was exposed to hydrogen three times within a month in dives at 1000 feet and 1650 feet for 2 hours, and for several minutes at 2200 feet at which point convulsions were seen. At COMEX these convulsions occurred with either hydrogen or helium at about 2300 feet, and the protective action of hydrogen against the HPNS was not confirmed.

The only human exposures with hydrogen, since the work of Zetterstrom (89) in the late 1940s, have been carried out by Edel (42–45). In 1967 Edel made two successful simulated dives to 200 feet for 10 and 20 minutes while breathing 97% hydrogen and 3% oxygen. During 1970 and 1971, 12 additional human studies were made to 200 feet, while the subjects breathed this mixture for times varying from 10 to 108 minutes. In half of these experiments pre- and postdive blood and urine samples were collected for analysis. Similarly, Edel and Fife exposed dogs to 300 feet for 24 hours and 1000 feet for 39 hours and made blood and urine studies to learn if longer exposures might cause irreversible tissue damage.

The results showed many similarities with oxygen-helium diving. It was found from these studies that, for comfort, the chamber temperature needed to be kept between 88–89 °F. As with helium, much deeper initial decompression stops were required than with air. There was no significant difference in speech between the diver breathing hydrogen and one breathing helium. The biomedical data, which included full serum biochemistry, hematology and—in the case of a dog exposed to 200 feet oxygen-hydrogen—a detailed postmortem examination including a microscopic study of the major organs, revealed no significant changes.

Whether or not oxygen-hydrogen will eventually be used operationally by divers remains to be seen, but on the basis of the experimental evidence to date, hydrogen appears to have

little practical advantage over the more safe helium at depths to 2000 feet, the point at which the HPNS currently sets the limit for deep diving.

Measurement of Inert Gas Narcosis

Measurement in Man

The difficulties in application of psychological tests to quantification of inert gas narcosis are well-recognized (9, 10, 50, 84). Although these have continued to be used in recent years, the advent of the computer has led to application of more sophisticated neurophysiological techniques involving measurement of the electrical activity of the brain or electroencephalogram (EEG) and sensory evoked cortical potentials.

Thus Bennett, Ackles and Cripps (14) measured the percentage decrement at arithmetic multiplication *(ab × c)* due to nitrogen narcosis at 50-foot increments between 100 feet and 300 feet while the subjects breathed compressed air. In addition, the results were compared with helium-oxygen at 300 feet and later oxygen alone at pressures to 3 ata (66 feet) (13). During these experiments, a computer of average transients was used to record the potential evoked at the cortex by synchronised auditory 60 dB bi-aural clicks presented at intervals of one second for 1 minute or 5 minutes. The level of narcosis, measured by the reduction of the N_1P_2 spike height, was correlated with arithmetical performance.

Compressed air caused decrements in both the auditory evoked response (AER) and arithmetic efficiency which correlated with the depth. Helium-oxygen caused a small decrement in the N_1P_2 response which was considered to be due to the oxygen partial pressure of 2 ata (Fig. 1).

Hyperbaric oxygen from 1 to 3 ata also depressed the AER but did not affect arithmetic performance. This was taken as an early indication of oxygen narcosis prior to sufficient inhibition of enzyme mechanisms, such as gamma aminobutyric acid, to cause convulsions (13). This therefore appeared to afford a new method for quantification of inert gas narcosis free of the subjective motivation and learning problems of psychological tests.

A year later Kinney and McKay (54) used visual evoked cortical responses (VER) to a patterned visual target, presented at either 2 flashes/sec or 16 flashes/sec, in four subjects exposed to 100 feet, 200 feet and 250 feet, breathing compressed air together with detailed

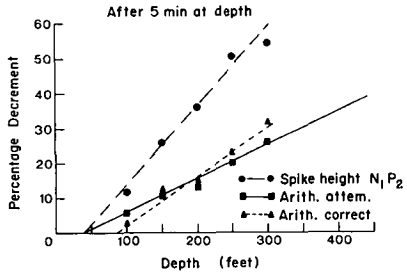

FIG. 1. Mean percentage decrement in the spike height of the N_1P_2 complex of the AER and the number of arithmetic sums (96 × 8 = 768) correct and attempted in men exposed to increased pressures of compressed air at 50-foot intervals between 100 feet and 300 feet (14).

TABLE I

MEAN AMPLITUDES OF VISUAL EVOKED RESPONSE[a]

Gas Breathed	Surface	100 Ft	200 Ft	250 Ft	Surface
Air	2.27	1.96 (0.86[b])	—	—	2.16 (0.98[b])
Air	2.56	—	1.66 (0.65[b])	—	2.32 (0.95[b])
Air	2.58	—	—	1.54 (0.60[b])	2.52 (0.99[b])
$He-O_2$	2.53	—	—	2.12 (0.84[b])	2.51 (0.99[b])

[a] From Kinney and McKay (54).
[b] Relative Amplitude = Amplitude at depth/Amplitude predive.

control studies of the effects of temperature variations as high as 37–39 °C, of the noise of compression by carrying out a dive to 10 feet followed by extensive venting of the chamber, and an empirical assessment of time per se and also helium-oxygen exposures at 250 feet. Of the two methods employed, the 16 flashes/sec proved the more useful. The mean amplitude of the 16 responses to the light decreased with depth for each diver breathing compressed air, while the variability of response increased. The helium-oxygen mixture, as with the AER studies, showed only a marginal decrement in the VER (Table I).

In addition, temperature variation had apparently no effect on the VER. Although there was some loss in mean amplitude due to noise, there was no decrement due to the duration of time. Kinney and McKay noted that, despite the obvious differences between the present visual method and earlier auditory technique (14), remarkably similar results were achieved between the two experimental methods; the absolute sizes of the decrements in AER and VER expressed as percentage decrement in amplitude, fell on the same regression line.

During experiments to compare the physiological properties of nitrogen, helium and crude neon, Schreiner, Hamilton and Langley (76) used both psychological tests and the AER at 200 feet, 300 feet and 400 feet. Among the performance tests used were reaction time, tapping, nut and bolt test, arithmetic and a counting task. Signs and symptoms of the HPNS (22, 23), such as tremor, were also recorded. The AER was produced by a 1 KHz tone frequency presented every 2 seconds.

Their results agreed too with the earlier work of Bennett et al. (14) and Kinney and McKay (54). Thus, the arithmetic multiplication test showed no reduction of efficiency in subjects exposed to the helium-oxygen mixture. Using a mixture of crude neon (75% neon–25% helium) and oxygen also caused no decrement in performance (Fig. 2), which agrees with earlier research on neon by Bennett (9) and Hamilton et al. (48). Again the N_1P_2 spike height of the AER indicated a marginal reduction with the helium-oxygen and the neon-oxygen mixtures but a marked reduction with compressed air (Fig. 3).

Bevan (26) compared the effects of compressed air intoxication on the human AER with the Contingent Negative Variation (CNV) or expectancy wave in the EEG. Although little is known at present concerning the mechanism of the CNV, it has been suggested that there is a coincidence in time between the appearance of the CNV and the blocking of the alpha rhythm of the EEG, and perhaps that both manifestations have the same origin.

Bennett and Glass (20) have reported an abolition of the alpha-blocking response to

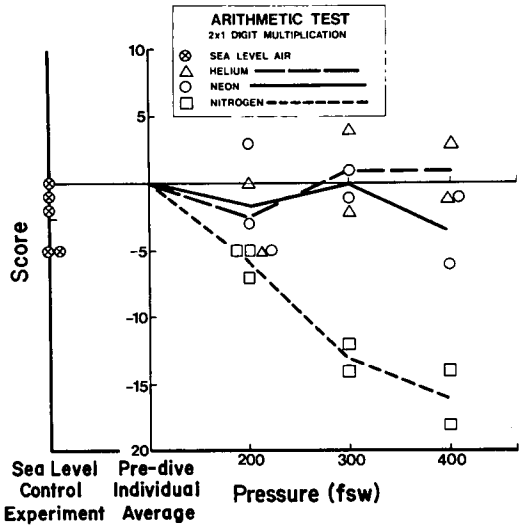

FIG. 2. The effect on an arithmetic test of exposing men to nitrogen-oxygen or a helium-oxygen or neon-oxygen mixture at depths of 200 feet, 300 feet, and 400 feet. Only the nitrogen-oxygen causes a significant decrement in performance (76).

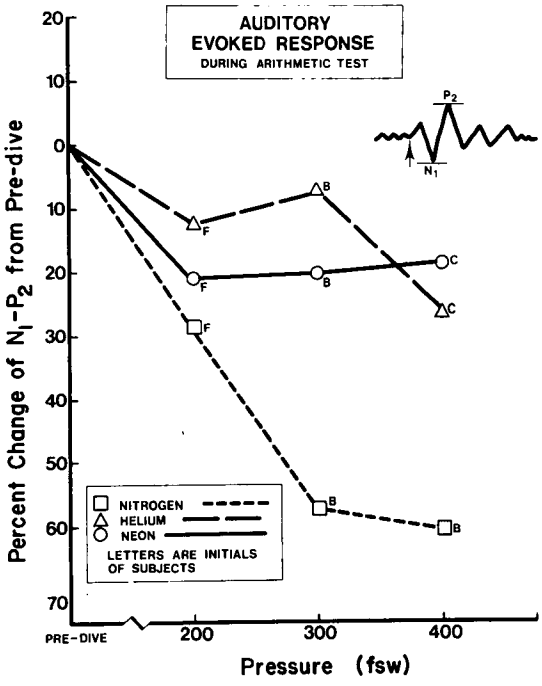

FIG. 3. Percentage change in the spike height of the N_1P_2 complex of the AER in subjects breathing either nitrogen-oxygen or helium-oxygen or neon-oxygen mixtures at depths of 200 feet, 300 feet, and 400 feet (see Fig. 2). Only the nitrogen-oxygen mixture causes a marked decrement of N_1P_2 (76).

mental arithmetic in men exposed to compressed air at depths between 50 to 200 feet, but a number of other workers have not been able to report this finding (81). However, this seems to be due primarily to a question of definition of alpha blocking. Under the generally accepted definition, the classical alpha block may be considered a measure of attention, whereas the Bennett and Glass interpretation is, as Townsend et al. (81) point out, more directly related to maintenance of continuous mental effort, and is a complex function of inert gas narcosis, subject motivation and the task, rather than a physiological indicator of the perception of a stimulus by a subject. Nevertheless, Bevan (26) reasoned that the relationship of the CNV to alpha blocking might make it sensitive to inert gas narcosis and therefore studied the effect of compressed air on the CNV at 300 feet. The conditional stimulus was provided by a 65 dB binaural click and the imperative stimulus followed 0.7 second later as two flashes of light 50 msec apart. On the imperative stimulus the subject was required to respond by depressing a hand-held button. At 300 feet of air the N_1P_2 component of the AER showed a 63.5 ± 4.5% decrement in size compared with 55.5 ± 10.5% of Bennett et al. (14). However, the CNV remained unaffected by the exposure (Fig. 4).

The different reactions of the AER and CNV were considered by Bevan to indicate that there was no decrement at the cortical level as reflected by the CNV, since it is a self-propagating phenomenon initiated by the interaction between the specific and nonspecific paths and the associated thalamic nuclei. CNV is therefore at least partly independent of the probable changes in the ascending reticular activating system, such as a blockage exerted on the ascending signals through this system or a similar effect on the subcortical afferent synapses, which may account for the decrement in the AER or VER (39).

Ackles and Fowler (1) further examined the evoked response reaction to inert gases in two series of experiments. In the first, the AER was measured together with arithmetical performance in subjects breathing either air or 20–80% oxygen–argon mixture at 100 feet and 200 feet. The arithmetic test (47) consisted of 30 problems of the $ab \times c$ type presented at a paced rate, and the evoked potentials were produced by a binaural auditory signal of 500 Hz of 400 msec duration, repeated at intervals of 2 seconds. The mean number of errors at the paced arithmetic task indicated a nonlinear reduction with increasing depth and a greater decrement with argon than with nitrogen (Fig. 5), which is in agreement with their narcotic potencies (9, 10). This nonlinearity poses problems in relating narcotic potency to a physical parameter such as lipid solubility. In addition their results did not support an *S*-shaped pharmacological dose–response curve (40). More important, perhaps, analysis of the N_1P_2 amplitude of 8 of the 10 subjects indicated that, while the magnitude of the AER does decrease with increasing depth or pressure, the degree of change is independent of whether or not nitrogen or argon is breathed (Fig. 6).

In a second series of experiments, the VER was measured in addition to the AER with the subjects again breathing either oxygen–argon or air at 200 feet. In this series, neither the nature of the stimulus (i.e., visual or auditory) nor the nature of the gas breathed affected the reduction of the evoked potentials at 200 feet which remained the same throughout.

A similar study has been made by Bennett and Towse, in unpublished experiments, which also compared the decrement in visual evoked potentials with ability to perform a number of mental and psychomotor tests in six men, breathing either air or oxygen–argon (20–80%) at 150 feet. Although there was no difference in the reduction of the evoked potential, both

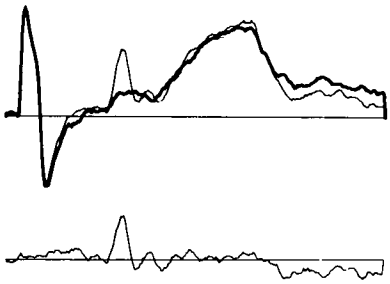

FIG. 4. Averaged AER and CNV for 12 subjects. Thin line shows the mean of controls at atmospheric pressure; the thick line shows the mean of all records at 300 feet compressed air. The lower trace is the computed difference between the two traces above. The AER has disappeared at 300 feet but the CNV is unaffected (26).

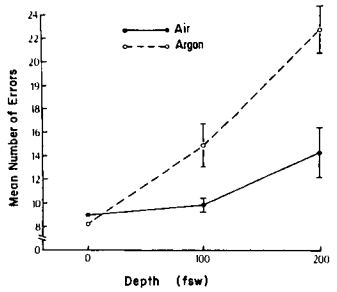

FIG. 5. The mean decrement in efficiency measured by the number of errors at a paced arithmetic task in men breathing either compressed air or 20–80% oxygen-argon at 100 feet (4 ata) and 200 feet (7 ata). The argon-oxygen shows a greater decrement than nitrogen-oxygen and also indicates a nonlinear reduction with increasing depth (1).

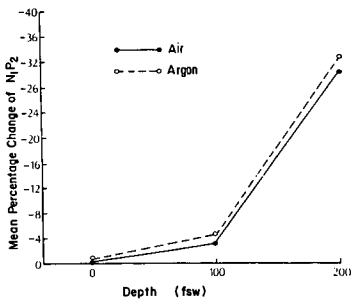

FIG. 6. The mean decrement in AER in subjects breathing either air or 20–80% oxygen-argon at 100 feet and 200 feet. The same reduction of AER with depth occurs independently of the gas breathed (1).

TABLE II

CORTICAL VISUAL EVOKED RESPONSE (VER) AND PERFORMANCE TESTS DURING FIRST 7 MINUTES AT 150 FEET[a]

Test	Percent Change from Surface in 20–80% Oxygen–Argon	Percent Change from Surface in Air
VER	−21.9 ± 6.87	−20.1 ± 8.05
Arithmetic correct	−62.1 ± 6.54	−28.6 ± 8.80
Arithmetic attempted	−47.6 ± 8.88	−22.9 ± 6.76
Visual analogy	−33.3 ± 5.22	−04.2 ± 2.57
Ball bearing	−18.1 ± 8.91	+00.6 ± 6.89

[a]From Bennett and Towse (24).

mental and motor tests emphasised the stronger narcotic properties of the argon mixture (Table II), in agreement with Ackles and Fowler (1).

These results suggest a very complex relationship between inert gases at high pressures and evoked cortical potentials, especially since the work of Ackles indicated no correlation between the evoked response and the arithmetic test. This is inconsistent with the previous work of Bennett et al. (14), who found a high correlation using mean data. However, the results of Ackles and Fowler are based on individual scores. A recalculation of the earlier data, using single point values rather than mean values, agrees with the lack of correlation.

What, then, does the decrement in evoked responses mean? Since oxygen–helium and oxygen–neon have little or no effect, and what decrement there is may well be due to the fact that oxygen at raised pressures also depresses the evoked response, pressure itself would not seem to be the prime factor. It would seem that the apparently nonnarcotic gases helium and neon have no effect on the evoked response while argon, nitrogen and oxygen do. The latter gases adsorp to lipid membranes and are considered to increase electrolyte transport across neurone membranes causing narcosis (15, 17, 21). Neither helium nor neon adsorp to model membranes in this way, which has been taken as the reason for their lack of narcotic properties. Perhaps the reduction in evoked response represents an all-or-none response to narcotic concentrations at its site of origin, either in the ascending reticular activating system or afferent synapses, whereas the differential effect of performance and sensation of gases of different potencies is related to a differential effect on the neurones and synapses of the cortical mantle. More research is required to resolve this problem.

Frequency analysis is another technique of specific interest in electroencephalography today, which has been applied recently to the study of hyperbaric narcosis in efforts to quantify the condition. Some of the stimulus for this approach came from the need for frequency analysis in order to identify the theta (4–8 Hz) changes in deep oxygen–helium dives.

Townsend et al. (81) have examined the effects of hyperbaric, normoxic mixtures of helium, nitrogen and neon on the EEG and simple reaction time at 4.02, 7.05 and 8.57 ata.

Only nitrogen showed evidence of narcosis, the reaction time increasing as a function of pressure. This was accompanied by a small increase in alpha frequency with increased nitrogen pressures.

A similar increase was noted by Bennett and Towse (24) in a study of the EEG, tremors and mental efficiency of men compressed at 100 feet/minute to 100 feet, 200 feet, and 300 feet, breathing either air or oxygen-helium in connection with study of the HPNS. No tremors were detected but the compressed air caused a decrement in mental efficiency with an increase in the alpha (8-13 Hz), beta 1 (13-20 Hz) and beta 2 (20-30 Hz) activities at 300 feet (Fig. 7). Oxygen had no effect.

Similar changes have also been reported by Roger et al. (74) and Zaltsman (87, 88), and the cause is generally attributed to an activation of the reticular activating system, seen in Stage 1 anesthesia, which is usually associated with euphoria as found in nitrogen narcosis at some 300 feet. The more potent xenon also produces an increase in EEG frequency (63) accompanied by a fall in amplitude. As the narcosis and eventually anesthesia become deeper, the increase is replaced by a slowing in EEG frequency. Confirmation of this has been obtained in animals which will be discussed later.

Little additional human work has been done on adaptation to narcosis since the experiments of Kiessling and Maag (53) showed no adaptation during 36 minutes at 100 feet. Bennett and Towse (25) have measured the effects of compressed air at 180 feet, 200 feet,

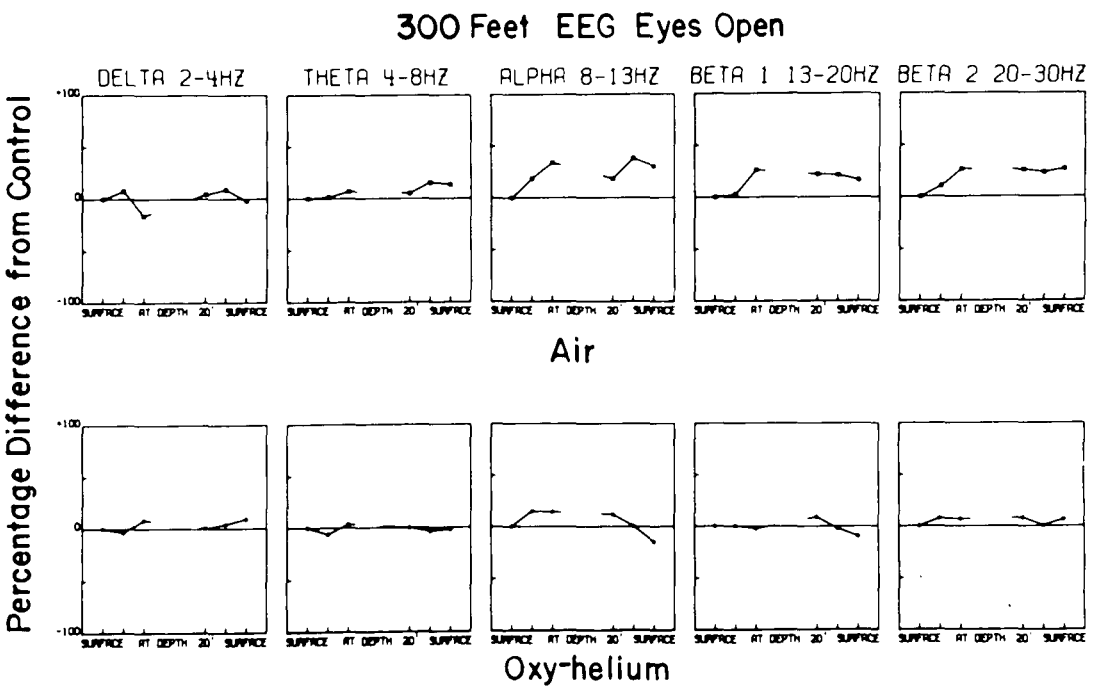

FIG. 7. Mean changes in the EEG activity of four subjects exposed to 300 feet either breathing compressed air or 20-80% oxygen-helium. The alpha, beta 1 and beta 2 activities are increased in the subjects breathing compressed air (24).

and 220 feet for 1 hour using the Ball Bearing Test, Touch Test, Purdue Peg Board, Wechsler Belle-Vue Digit Symbol Test and Arithmetic Multiplication. No adaptation was observed during this period. In connection with the use of Tektite II, subjects were exposed to 100 feet for 14 days (37). Short-term memory was tested with the Ames Crew Evaluator. A decrement occurred at depth, but after 5 days adaptation had begun and by the eighth or ninth day the results were no different from the controls. This is another area which merits further study.

Measurement in Animals

Evoked potentials have been used also to quantify narcosis in animals. In 1964, Bennett (7) studied the depression of the AER elicited in the ascending reticular formation and the cortical mantle of chloralosed cats exposed to inert gases at increased pressure. More recently Larson et al. (57) examined the effect on the VER when chloralosed cats were compressed to 100, 300 or 500 feet with oxygen at either 200 mm Hg or 1000 mm Hg. The results confirmed the earlier studies, in that at 500 feet there was a reduction of some 80% in the amplitude of the positive and negative wave of the VER. However, as with some of the human work, variability of the amplitude of the evoked response, especially in the case of the higher oxygen partial pressure, often caused difficulties in obtaining reproducible data.

The spontaneous EEG has been studied extensively since Marshall (59) reported a reversible abolition of the EEG in frogs exposed to inert gases, and Pittinger et al. (70) and Morris et al. (63) noted that during xenon anesthesia at atmospheric pressure, the EEG showed a decrease in all frequencies without the augmentation reported for many other inert gases. Similarly, Criscuoli and Albano (38) reported their studies of EEG activity in white mice, and Zaltsman (87, 88) has made an extensive qualitative study of the effects of inert gases on the spontaneous EEG.

Modern techniques of frequency analysis now permit more quantitative on-line analysis. Using a Nihon Kohden frequency analyser, Bennett and Dossett (19) compared the effects in rats of compressed air and argon-oxygen, helium-oxygen and nitrogen-oxygen with an oxygen partial pressure of 2 ata throughout. Compressed air, argon-oxygen and oxygen alone, on compression to 700 feet at 100 feet/minute, caused a reduction in all activity bands as may be seen, for example, in the case of alpha activity in Fig. 8.

Helium-oxygen and nitrogen-oxygen, on the other hand, showed little difference from the slightly depressed controls except for a marked rise in delta wave activity with helium. At 100 feet activities were augmented except for argon-oxygen and oxygen alone. Zaltsman and colleagues (87, 88) observed similar changes due to nitrogen and argon with a depression of cortical activity followed by a dominance of theta waves in the subcortical areas, leading to generalised delta activity. With helium, the theta activity becomes more generalised, often without prior suppression of cortical activity.

The lack of augmentation of the EEG with the strong narcotic argon would seem to correlate with the action of the potent narcotic xenon in animals (70). Similarly, the action of oxygen supports recent indications of narcotic, as well as convulsant properties for oxygen (13). Paton (65) has suggested that, based on lipid solubility, the anesthetic pressure of oxygen for mice should be 11 ata (i.e., close to argon) compared with 29 ata for nitrogen; there are many similarities of impaired physiological function in the presence of nitrogen

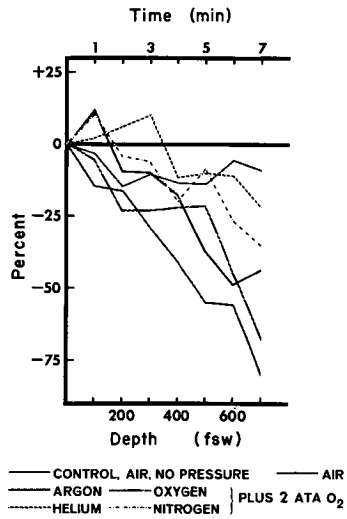

FIG. 8. Mean changes of EEG alpha activity in rats exposed to various gas mixtures and compressed at 100 feet/minute to a maximum of 700 feet. The argon-oxygen, oxygen and compressed air mixtures show a marked depression of alpha activity whereas helium-oxygen and nitrogen-oxygen are little different from controls at atmospheric pressure, except for a slight initial increase of activity (19).

and oxygen at high pressures, such as nerve transmission (36, 59, 69), action of drugs which prevent or enhance nitrogen narcosis or oxygen convulsions (6, 17, 18), adsorption to a lipid monolayer (15), increased permeability of electrolytes across cell membranes (21, 51) and depression of evoked cortical potentials (14, 57).

During submarine escape experiments by Bennett (11) with rats compressed in 32 seconds to 1000 feet, 15 seconds at depth and decompression at 500 feet/minute, further indication of the anesthetic properties of the non-inert gas oxygen was obtained. Compression with pure oxygen caused unconsciousness at 1000 feet in a few seconds, with recovery at 700 feet during decompression. Argon in the presence of 2 ata oxygen also caused unconsciousness, but neither helium nor nitrogen had any effect. Compressed air caused unsteadiness.

Studies were also made with helium at a much slower rate of compression of 10 feet/minute to 4000 feet, and a similar reduction of EEG activity was seen (Fig. 9), presumably due to pressure, since neither helium nor neon appears to be narcotic as other inert gases are (3, 17, 76). Thus, in experiments involving study of inert gas narcosis, it is necessary always to incorporate helium into the dive protocol, so that the role of pressure may be separated from the pharmacological action of nitrogen. Similarly oxygen should also be examined at the partial pressure at which it is breathed in the mixtures under study, for it has both narcotic and convulsant properties.

The alpha activity of the EEG in fact provides a useful index of narcosis in animals and in general usually correlates well with the mean of the changes in the five frequency bands: delta, theta, alpha, beta 1 and beta 2. The mean EEG of four rats exposed to 700 feet at 100 feet/minute indicate, however, that at 100 feet, 200 feet, 300 feet, and 400 feet, the results are not significantly different between argon and nitrogen although at 500 feet, 600 feet, and 700 feet, the difference is significant (Fig. 10). Conversely, in the case of evoked

potentials, VERs at 100 feet and 200 feet show a significant difference between argon and nitrogen but not at 300 feet and deeper (Fig. 11).

A similar situation may prevail in man. It is possible that at depths less than the 100 feet used by Ackles and Fowler (1) a differential effect may be seen between argon and nitrogen. Thus the evoked response may be regarded as a very sensitive indicator of the presence or absence of narcosis. A more quantitative evaluation of narcotic potency may be afforded by frequency analysis, but so far as man is concerned the depths at which this is likely to be effective, such as 400 feet, cause severe signs and symptoms of narcosis or even loss of consciousness (2).

Other methods used in animals include critical flicker frequency (CFF) (32) and a force discrimination task (78). Thus Burns found no difference in the CFF of monkeys between the control, nitrogen and argon, but 2 ata oxygen caused a dose-related depression, whereas the response rate (i.e., lever pressing) revealed a dose-related depression in the presence of nitrogen and argon but not oxygen. This was interpreted as indicating that narcosis is primarily of psychomotor origin, and that most of the tests used in the past, which show a decrement, have motor components. This is disputable since, for example, such tests as arithmetic can show narcosis when psychomotor tests indicate no decrement, and mental arithmetic and elapsed time measurements are affected without motor decrement. The CFF depression with hyperbaric oxygen is probably due to the vasoconstrictive properties of oxygen on the retinal vessels rather than to a direct pharmacological effect on the central nervous system, but this needs further verification.

The force discrimination task, with food reinforcement (78), required the monkey to maintain 285–400 grams for 2.9 seconds. Performance decrements were related to depth and the gas breathed. Task failure occurred at 400–500 feet with argon-oxygen and 500–700 feet with air. Adaptation to intermediate pressures was exhibited by recovery of effectiveness in performing the force discrimination task.

Similar operant conditioning techniques with pigeons and rats have been used by Thomas and Bachrach (79) and Thomas et al. (80) to study adaptation to inert gas narcosis. Timing

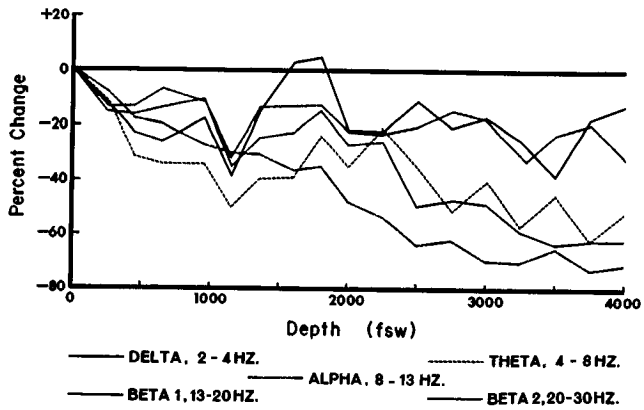

FIG. 9. Mean percentage change of the spontaneous EEG activities of two rats compressed with helium on 2 ata oxygen at a rate of 10 feet/minute to 4000 feet (19).

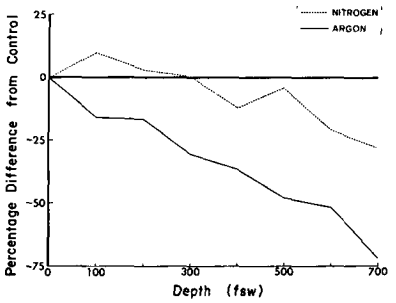

FIG. 10. Percentage change in mean EEG activity of rats exposed at 100 feet/minute to 700 feet breathing either nitrogen-oxygen or argon-oxygen. The difference between animals breathing argon or nitrogen is significant only at 100 feet and 200 feet.

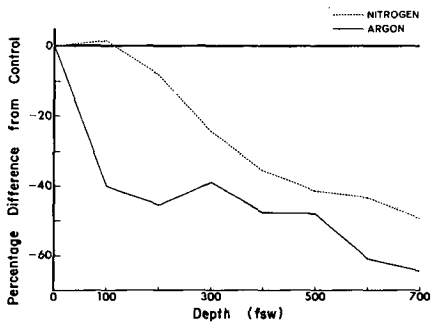

FIG. 11. Change in visual evoked potentials in rats exposed at 100 feet/minute to 700 feet breathing either nitrogen-oxygen or argon-oxygen. The difference between animals breathing argon or nitrogen is significant only at 100 feet and 200 feet.

behaviour in albino rats was found to be disrupted in an initial exposure to 200 feet of air (84), but subsequent exposures revealed a gradual adaptation to the chamber conditions with a return to levels of performance close to surface controls (Fig. 12). However, although the effects are less than with compressed air, this technique also produces performance decrements in rats and pigeons with oxygen-helium mixtures at 200 feet and 300 feet. Since helium is not narcotic, the role of factors such as the disturbances of compression, temperature and role of oxygen needs identification to clarify the role of narcosis in causing the decrement in performance with this method.

In concluding this section on measurement, clearly there still remains, after much research, the need for a simple, objective, perhaps neurophysiological test of narcosis which could be used both in animals and in man by the majority of workers in this area to facilitate comparison of results and provide effective quantification of narcosis.

CAUSE OF NARCOSIS

It has been suggested that inert gas narcosis results primarily from the pharmacological action of the inert gas molecules with the lipid constitutent of neurones (9, 10, 17). This is

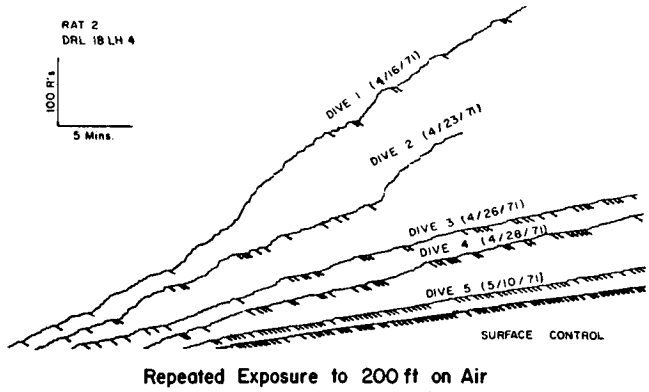

FIG. 12. Differential reinforcement of low rates (DRL) with a limited hold specification of 6 seconds involving each rat pressing a bar for food reinforcement in which an animal was reinforced for a single response which followed the preceding response by at least 18 seconds but not more than 24 seconds. Initial exposure to 200 feet compressed air causes marked disruption of temporal discrimination with gradual adaptation on repeated exposure until a dive to 200 feet has no effect (84).

in keeping with the well-known Meyer-Overton hypothesis in anesthesia (64) which implies preferential adsorption of the anesthetic (or, in this case, inert gas) to lipid in contradiction to the alternative hydrate theories (62, 66) which propose involvement of the aqueous phase, with the formation of clathrates or ordered water. The use of fluorinated gases (46, 61) has emphasised, however, the superior correlation of the narcotic potency of anesthetics and inert gases to lipid solubility.

In the early 1960s, the inert gas theory was questioned by a number of workers (5, 31, 77) who considered that impaired ventilation, resulting from the increased gas density of the gases at increased pressures, was sufficient to warrant carbon dioxide intoxication as the cause of the signs and symptoms of narcosis seen in diving. Experiments in man by Rashbass (73) and Cabarrou (33), involving measurement of alveolar carbon dioxide, and by Bennett in chloralosed cats (8) involving in vivo measurement of cortical cerebral carbon dioxide and oxygen tensions, failed to support the carbon dioxide theory except in regard to a possible synergistic role.

Recently, Vail (82) has resurrected the controversy by suggesting that narcosis is due to the molecular density of the breathing mixture which causes airway collapse, resulting in cerebral hypoxia complicated by carbon dioxide retention. It is not proposed at this stage to review the many factors discussed elsewhere (9, 10) which refute the direct involvement of either carbon dioxide or hypoxia as the cause of the narcosis. Only a few of the more important points will be considered.

Thus, there is no doubt that airway collapse and respiratory embarrassment do occur during the exposure of man to increased pressures (55, 60, 85, 86) but not to the extent suggested by Vail, whose calculations permitted him to comment that the amount of work required to breathe helium-oxygen increases by 50% at 1000 feet and "new concepts and designs will be required to sustain a diver at 1000 feet." Men have been to 1500 feet, 2000 feet, and so far as respiratory parameters are concerned, to 5000 feet, without undue respiratory embarrassment or indication of hypoxia, hypercapnia or mental impairment.

Vail suggests also that xenon causes anesthesia at atmospheric pressure by hypoxia. Now the density of a 20–80% oxygen-xenon mixture at 1 ata is 111.44, whereas compressed air at 300 feet is more than twice as dense at 288. On the basis of density, men at 300 feet therefore should be in deep surgical plane anesthesia; however, they are only euphoric. Furthermore, arterial and venous oxygen and carbon dioxide measurements in patients before and during xenon anesthesia (63) showed no significant changes.

More recently, Hesser et al. (49) have assessed the role of carbon dioxide and nitrogen in compressed air narcosis at 6 ata by relating performance to changes in alveolar nitrogen and carbon dioxide tension when, at a constant oxygen partial pressure of 1.3 ata, both the inspired nitrogen and carbon dioxide pressures were varied simultaneously, and when one or the other was varied separately. The tests of performance were the Moede two-hand position tracking task of perceptual-motor ability and the Stroop colour word test of stress sensitivity in decision-making. The results showed that the effect of carbon dioxide on narcosis is negligible at alveolar tensions below 40 mm Hg, and that high alveolar nitrogen and carbon dioxide pressures are simply additive in their effect on performance. However, if the nitrogen and carbon dioxide pressures are raised simultaneously, the changes in performance are greater than the arithmetic sum of the changes induced by either gas alone, implying a synergistic potentiation.

Mechanism and Prevention of Narcosis

It has been established that raised pressures of oxygen, carbon dioxide and the inert gases argon and nitrogen, will adsorp to a model membrane of egg phospholipid (10, 15). However, helium and neon do not adsorp, and it was proposed, therefore, that neither could cause inert gas narcosis. This has been confirmed in recent deep oxygen-helium (23) and oxygen-neon (76) exposures. Such an adsorption will expand the membrane and change the dielectric constant to permit an increased ion permeability across the membrane, and this may exceed the capability of the energy driven pumps to maintain equilibrium. The fact that such adsorption does cause an increased permeability of ions is supported for nitrogen and argon by measurements of Na^+, K^+ and Cl^- in the cerebrospinal fluid of chloralosed cats exposed to raised pressures of argon, nitrogen and helium (21). Similar permeability changes have been noted in blood (4), urine (71), and during general anesthesia and alcoholic intoxication (67, 68). Thus a mechanism of narcosis was proposed involving an electrostatic adsorption of inert gas molecules to charged sites on neurone lipid membranes which causes an increased permeability of ions such as Na^+ and Cl^- to invoke a maintained depolarisation, and thereby signs and symptoms of narcosis.

Now CCl_4 poisoning results in liver necrosis due to accumulation of intracellular Na^+, Cl^-, Ca^{2+} and water and loss of K^+ and cellular proteins (3). Simple long-chain cationic detergents prevent such liver necrosis in the rat by stopping these permeability changes. These cationic compounds are believed to be surface-active and form complexes at lipid-water interfaces, especially if the former are negatively charged. Anionic detergents, for example, prevent neither the permeability changes nor liver necrosis.

On the basis of the adsorption hypothesis for inert gas narcosis, it might be expected that such cationic detergents would also stabilise membrane and prevent or ameliorate inert gas narcosis. Experiments, therefore, were carried out to study this with rats exposed to 0.9

TABLE III

EFFECT OF SURFACE ACTIVE ANIONIC OR CATIONIC DRUGS ON INERT GAS NARCOSIS IN RATS AT 0.9 O_2-6.41 N_2 ata[a]

Drugs	Dose	Mean Percent Change in Evoked Response	Number of Rats	Serum β-Glucuronidase Units, in Rat Liver 4 Hrs. After CCL$_4$	Significance
No drug, no pressure	—	+ 4 ±4.66	10	98	—
No drug, at pressure	—	−29.8±3.87	10	—	(1-2) $P < 0.001$
		Anionic Drugs			
Sodium hexadecyl sulphate	70 μM/kg	−34 ±3.69	10	78	(2-3) not significant (1-3) $P < 0.001$
Sodium dodecyl sulphate	80 μM/kg	−20 ±4.08	10	82	(2-4) not significant (1-4) $P < 0.001$
		Cationic Drugs			
Stearylamine	55 μM/kg	− 7 ±3.81	10	25	(2-5) $P < 0.001$ (1-5) not significant
Cetyl trimethyl ammonium bromide	14 μM/kg	− 6.5±4.14	10	22	(2-6) $P < 0.001$ (1-6) not significant
		Anti-inflammatory Drug			
Acetylsalicylic acid	50 mg/kg	− 1 ±3.68	10	—	(2-7) $P < 0.001$ (1-7) not significant

[a]From Bennett and Dossett (17).

oxygen-6.41 nitrogen ata, while the presence of narcosis was determined by visual evoked responses. Anionic compounds, such as sodium hexadecyl sulphate and sodium dodecyl sulphate, had not effect, but the cationic drugs, stearylamine and cetyl trimethyl ammonium bromide, significantly ($P < 0.001$) prevented the depression of evoked potentials (Table III) in the animals compressed without medication (12, 17). In addition, since nonsteroidal anti-inflammatory drugs protect erythrocytes against lysis, acetylsalicylic acid was also studied and, as reported elsewhere using a different technique (6, .16), was similarly effective in preventing the electrophysiological indication of narcosis.

These cationic detergents cause adverse side effects in the animals at the doses used, but the experiments do indicate that compounds able to stabilise membrane may prevent inert gas narcosis. The effect of oral aspirin at a dose of 25 mg/kg was examined in men at 300 feet carrying out performance tests. No effect was seen but this dose—half that effective in rats—was also ineffective in the animals.

Due to the fact that oxygen also adsorps to membrane and has many physiological effects similar to nitrogen, except for enzyme inhibition (12), the same compounds were studied in reference to prevention of oxygen convulsions. Again, the anionic compounds were ineffective whereas the cationic detergents increased the time to convulsions (Table IV). In the case of cetyl trimethyl ammonium bromide, the time was doubled (18).

More recently, other surface active compounds have been examined, notably lithium carbonate and alpha tocopherol (vitamin E). It was found that 500 mg/kg lithium carbonate

TABLE IV

EFFECT ON CONVULSION TIME OF RATS EXPOSED TO 6.44 ATA OXYGEN

	Dose	Percent Change in Convulsion Time	Significance
	Cationic Compounds		
Stearylamine	55 μM/kg	+18.3	$P < 0.01$
Cetyl trimethyl ammonium bromide	14 μM/kg	+75.5	$P < 0.001$
	Anti-inflammatory Compound		
Acetylsalicylic acid	50 mg/kg	+ 1.3	—
	100 mg/kg	+52.9	$P < 0.001$

given intraperitoneally in rats exposed to compressed air at 300 feet, converted the −30% decrement in visual evoked potentials to surface values of only −1%. Similarly, oxygen convulsion times at 7 ata increased by 40%, which is in agreement with the earlier work of Radomski and Wood (72). Lithium ion, a proven psychopharmacological agent in the treatment of manic depressive disorders, schizophrenia and a number of other psychiatric conditions, is also believed to produce its effect either by stabilizing neural cell membranes or active displacement of intracellular sodium.

Lower doses of lithium, such as 300 mg/kg, were not as effective. Similarly, the action of vitamin E was critical as regards dose. Thus 400 mg/kg caused lethargy and did not affect the electrophysiological signs of narcosis, but at 200 mg/kg the evoked potential at 300 feet was reduced by only 8.5% instead of 30% in the nonmedicated animals. Convulsion times in oxygen increased by 20%. This work is still at an early stage and considerable further experimentation is required before a change in evoked cortical potentials may be correlated with behavioural measurements of narcosis. However, these compounds could well provide the much needed tools to unravel the mechanism of action of inert gases at the membrane level and indeed not only the mechanism of inert gas narcosis but many anesthetics as well.

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PHARMACOLOGICAL EFFECTS OF HELIUM, NEON, HYDROGEN AND NITROUS OXIDE

D. W. Kent, M. J. Halsey and E. I. Eger II

In 1885, Regnard (10) reported observing agitation of small aquatic animals—*Cyclops* and *Daphnia*—at hydrostatic pressures just below 100 ata, and their death at higher pressures. Hyperexcitability has also been observed in mice breathing high pressures of either helium–oxygen gas mixtures or fluorinated hydrocarbon liquids (6–8). Such pressure effects have been called high pressure hyperexcitability or nervous syndrome (HPNS). These hyperexcitability effects may be occurring at the present limit of man's dives and, therefore, to be able to reverse or antagonize the effect—if this could be done without impairing the diver's ability to function—is desirable. Brauer (2) noted that in the presence of certain gases such as nitrogen or hydrogen, which are now recognized to have narcotic properties, the syndrome of hyperexcitability with tremors and convulsions is minimized or postponed to higher pressures.

The antagonism of narcosis by pressure was demonstrated by Johnson and his co-workers in 1951 (5). In a pressure chamber they applied over 100 ata of hydrostatic pressure to tadpoles narcotized with ethanol. This increase in pressure rapidly caused the tadpoles to wake up and to resume normal swimming—until the pressure was released, at which time the tadpoles again became narcotized. The antagonism was rapidly reversible in either direction by alternate rapid compressions and decompressions.

These recognized effects of pressure on narcosis and of narcotizing agents on the pressure syndrome need to be quantitated in a gas-breathing mammal if such drugs are to be used to increase possible useful diving depths.

Methods

Mice were studied initially, in a 20-litre capacity high pressure chamber using helium (a gas suggested to have no narcotic effect), so that the equivalent of a pure hydrostatic effect of pressure in gas-breathing mammals might be examined. The narcotic potency of nitrous oxide in 20–30 gram male Swiss-Webster mice at various elevated chamber pressures was determined. In each run, the eight mice to be exposed to a given pressure level were placed in separate compartments of a cylindrical cage, which could be rotated at 4 rpm, to assess

their righting reflex. The internal chamber temperatures and rectal temperatures of three additional mice were monitored with small thermistor probes.

Helium has been reported as not having narcotic properties since pressurization with helium–oxygen gas mixtures causes hyperexcitability rather than narcosis. Doubting a narcotic effect of helium, we wished to verify our pressure versus narcotizing agent antagonism with another supposed nonnarcotic gas, neon, in place of the helium. Studies were also done replacing the helium with a known narcotizing agent, hydrogen which is estimated by Brauer (1) to cause loss of righting reflex in mice at about 130 ata.

Since pure neon is very expensive a small 1-litre pressure chamber of Plexiglas was made in which to perform these studies. This small chamber was designed for a working pressure of 10 ata and was placed within the large chamber which was concomitantly pressurized with helium to prevent the Plexiglas chamber from having to withstand a high pressure gradient. Gas and electrical fittings connected to the outside of the larger pressure chamber. The actual pressure gradient was monitored by a pressure gauge not in the small chamber but within the larger chamber. Final pressures were measured on an external gauge, calibrated with a dead-weight tester. This small chamber also contained a circulating fan with carbon dioxide and water absorber. For each study in this chamber, four experimental mice were placed in separate cages which could be intermittently rotated at 4 rpm, and two mice with rectal temperature probes were also placed in the chamber. Two additional temperature probes measured environmental temperatures within the small chamber, with another probe measuring the helium gas temperature surrounding the Plexiglas chamber.

With neon the protocol used for helium was repeated, maintaining mouse rectal temperatures at 37 °C. With hydrogen, the mice were first pressurized in the small chamber to about 30 ata with helium and then the helium–oxygen mixture was exchanged for 2% oxygen in hydrogen from a pre-mixed gas cylinder. Higher pressures were then achieved by the addition of either pure hydrogen or the 2% oxygen in hydrogen pre-mixed gas. With this pressurization schedule, an inflammable mixture of hydrogen–oxygen was theoretically prevented from ever being present under pressure. Throughout these studies the outer chamber was filled with helium maintained at the same pressure as the inner chamber. The individual gas partial pressures were determined from their volume concentration, measured directly by gas chromatography, and the total pressure was measured on a calibrated Bourdon gauge.

There was a complex relationship between environmental and mouse rectal temperatures which varied with the total pressure and the particular gas being studied. In all studies the mouse rectal temperatures were maintained at 37 °C. However, the gas temperature in the chamber necessary to do this varied considerably. With the helium studies in the large chamber, gas temperatures near 34 °C were needed at 10 ata, increasing to temperatures near 36 °C at 100 ata (Fig. 1). With the neon studies in the small chamber, gas temperatures near 34.5 °C were needed at 40 ata to maintain mouse rectal temperatures at 37 °C, while at 100 ata the neon needed to be at about 35 °C (Fig. 2). With hydrogen, gas temperatures near 36 °C were needed from 20 ata to 100 ata (Fig. 3).

These results can be interpreted in terms of the heat capacity, density and thermal conductivity of the gases. However, the use of rectal probes involved some immobilization of the mice, which may be important in the case of small animals. It is also known that narcosis affects the temperature regulating mechanism. Rectal temperatures do not neces-

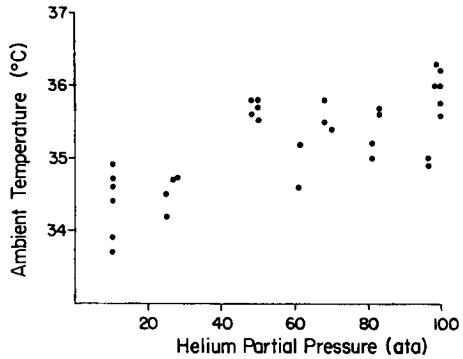

FIG. 1. The ambient temperatures of the large chamber necessary to maintain the mouse rectal temperatures at 37°C at different pressures of helium.

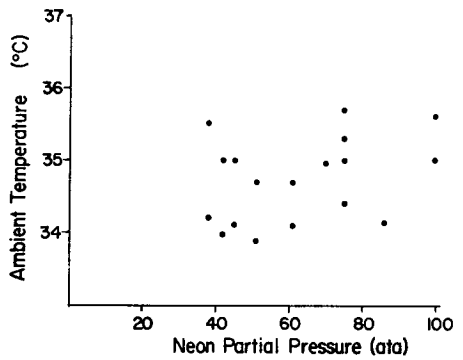

FIG. 2. The ambient temperatures of the small chamber necessary to maintain the mouse rectal temperatures at 37°C at different pressures of neon.

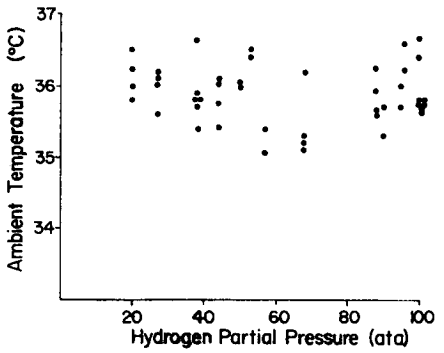

FIG. 3. The ambient temperature of the small chamber necessary to maintain the mouse rectal temperatures at 37°C at different pressures of hydrogen.

sarily reflect core temperatures but were satisfactory in these experiments under steady-state conditions.

The importance of constant normal body temperatures lies in the fact that changes in body temperatures alter narcotic potency (9). Estimates in this study of the narcotic potencies of gases at high pressures may differ from the results of others because of differences in body temperatures of the animals.

The results of the nitrous oxide potency measurements in helium suggest a linear increase in the narcotizing dose of nitrous oxide (ED_{50}) required to cause loss of righting reflex with increasing ambient pressures up to 140 ata (3,4).

Experiments in the small chamber showed control values at 2 ata to be essentially identical with the controls in the helium experiments done in the larger chamber. In pressurizing with neon it was found that instead of an apparent pressure vs. narcotic antagonism, as seen with helium, the mice maintained a normal responsiveness to exposure to nitrous

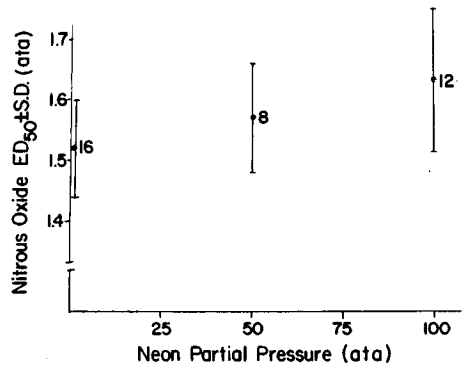

FIG. 4. The variation in nitrous oxide potency at different pressures of neon. The ED_{50}s are plotted ± 1 standard deviation and the figures at each point refer to the number of mice used in each experiment.

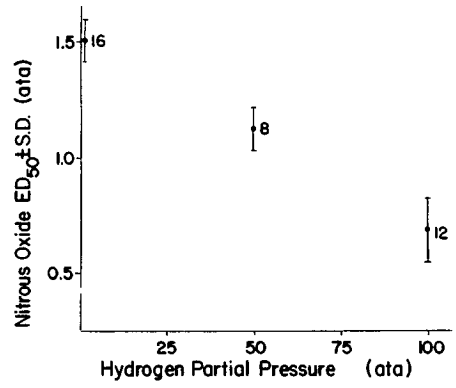

FIG. 5. The variation in nitrous oxide potency at different pressures of hydrogen. The ED_{50}s are plotted ± 1 standard deviaton and the figures near each point refer to the number of mice used in each experiment.

oxide—with no change in the partial pressure of nitrous oxide needed to cause loss of righting reflex. The preliminary results shown in Fig. 4 suggest a slight increase in nitrous oxide requirement or decrease in nitrous oxide potency—but statistical evaluation suggests that there is no significant difference.

With hydrogen, known to have a narcotic effect, the nitrous oxide required to cause loss of righting reflex decreased, apparently linearly, from the ED_{50} at 2 ata to the ED_{50} at 100 ata (Fig. 5). This indicates that hydrogen and nitrous oxide were additive in their narcotic effects. The extrapolation of this data would indicate loss of righting reflex at about 180 ata of hydrogen without nitrous oxide.

Discussion

With all of these gases—helium, neon and hydrogen—the high pressure syndrome should be independent of the means by which the pressure was attained. However, the presence of the agent supplying the pressure (water or fluorinated hydrocarbon as used by others and helium, neon or hydrogen as used by us) may modify the pressure syndrome. It is already known that the anaesthetics, nitrous oxide and nitrogen, antagonize the pressure syndrome, enabling survival to higher pressures. Bearing this in mind, it is hypothesized that both helium and neon have narcotic properties, with neon's narcotic effect almost exactly counterbalanced by the hydrostatic pressure exerted by itself, while hydrogen is a stronger narcotic and helium a weaker one. These results can be interpreted in terms of a simple model of the molecular sites of action of the various effects, as already proposed by several workers (7,8,11).

First, consider the critical site for production of hyperexcitability. Applying hydrostatic pressure will compress this site, altering its structure and, thereby function, so that the animal undergoes CNS excitation leading to death in convulsions. Adding a narcotic agent at this site, by interacting with the site, would expand the site restoring its normal size and thereby reversing the high pressure hyperexcitability.

Next, the same model can be used to represent the critical site of narcotic action. The narcotizing agent interacting at this initial site also causes an expansion, presumably stabilizing a critical site—perhaps a membrane in the CNS—reducing nervous transmission and thereby causing a narcotic effect. This can also be antagonized by applying hydrostatic pressure and returning the site to its normal shape. Increasing the narcotic dose above the amount needed to overcome this pressure effect will again cause narcosis.

The observed narcotic partial pressures must consist of two factors. First the partial pressure necessary to overcome the pressure antagonism, or to return to normal sensitivity, and second, the additional narcotic partial pressure needed to actually reach narcosis from that intermediate step. It is this intermediate step that is the limit of the dose of narcotizing agent that can be given without actually causing narcosis, and it is around this dose that the 270 ata helium-oxygen nitrous oxide exposure with mice was achieved.

Using these models, an explanation of how the pressure of an agent generally assumed to cause narcosis could safely be used to extend the pressure limits of deep dives can be given—an appropriate amount of narcotic agent may reverse the hyperexcitability effect of pressure while the pressure at the same time prevents the narcotizing agent from causing a narcotic effect.

One narcotizing agent may confer superior resistance to hyperexcitability than does another at the same narcotic dose since the two effects of narcosis and hyperexcitability may be occurring at two separate critical sites.

Obviously these studies need to be done very carefully in man, since other effects of pressure that had been masked by the high pressure hyperexcitability phenomenon may become predominant. It should be noted that all of the mice were dead at pressures of over 270 ata in spite of the presence of nitrous oxide.

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THE CHANGES IN SMOOTH MUSCLE RECEPTOR COUPLING OF ACETYLCHOLINE AND NOREPINEPHRINE AT HIGH PRESSURE

T. K. Akers and L. C. Carlson

It is well known that acetylcholine (ACh) is the transmitter substance found in the parasympathetic pre- and postganglionic synapses and the sympathetic preganglionic synapses, as well as the myoneural junction. Since the cholinergic receptor is important for the ACh action at all of these sites, it would be of interest to know whether the receptor itself is affected by increased hydrostatic pressure and/or by varying oxygen levels. Recently our laboratory has shown that pressure has an effect on adrenergic receptor activity in the smooth muscle of the gut. This effect is characterized by increased drug latency which is linearly related to increased pressure. Optimal blockade by a beta blocker (propranolol) occurs between 20 and 30 ata (1).

The effects of high pressure on smooth muscle have been investigated in the past, and it has been shown that the contractile process is affected. Small strips from the pyloric end of the stomach of the painted terrapin were subjected to pressure by Edwards (5). He noted a 54% increase in the tension developed in response to a 10-second faradic stimulus when under pressures ranging from 200 to 1500 p.s.i. This effect was reversible. Cattell (4) applied 1200 to 1300 p.s.i. to duodenal strips and noted a complete cessation of spontaneous activity and an increased tonus. This increased tension was about half that at the peak of the previous rhythmic response.

Various levels of oxygen under pressure have also been shown to be involved in smooth muscle contraction. Bean and Bohr (3) and Riggs (11) have shown that oxygen at 7 ata caused a sudden decrease in tension of the smooth muscle preparation. They completely reversed this effect by returning the preparation to O_2 at 1 ata. They state that these phenomena suggest the possibility that high pressure oxygenation may affect the contractile proteins themselves, or possibly the release or uptake of calcium by cellular elements.

Koehler and Gottlieb (8) recently did work on Na-K-Mg ATPase from rat intestine and found that it is a pressure-sensitive enzyme. They noted that O_2 inhibits the ATPase activity in the 90 to 135 p.s.i. range and enhances the ATPase activity in the 150 to 240 p.s.i. range. They also found that N_2 activates the ATPase in the 30 to 80 p.s.i. range, and they noted a progressive increase in the degree of inhibition of ATPase in the 120 to 285 p.s.i. range.

The purpose of the present study is to determine some of the effects of increased hydrostatic pressure and of varying levels of O_2 on the activity of the cholinergic receptors.

Materials and Methods

New Zealand white male rabbits, 2 to 3 kg, were sacrificed and 2-cm longitudinal strips of small intestine duodenum were excised, placed in Tyrode solution, and maintained at 37° ± 2°C. These strips of duodenum were placed in a tissue bath, which was then placed in a pressure vessel, along with the associated linear motion transducers and solenoids for the drug and washing systems.

Acetylcholine was introduced into the tissue bath upon signal from a conventional Cassella Drug Assay apparatus. Two electromagnetically controlled stopcocks regulated the release of the drug and wash solution. The tissue was washed with Tyrode solution following each addition of the drug. The drug was allowed to remain in contact with the tissue for at least 1 minute, but no longer than 3 minutes. It was then washed with Tyrode solution until the tissue relaxed to the control baseline and resumed a pretreatment contraction force.

Experiments were divided into two sets. In the first set, four concentrations of ACh were used and the P_{O_2} was kept at a constant value of 155 ± 10 mm Hg. The chamber was pressurized with He to 10, 20, and 30 ata. These pressure levels were reached in random order in both sets of experiments. Measurements of drug latencies and magnitudes were made at each pressure level. Two kinds of latencies were measured in the first set of experiments: the time it took for the tissue to make its initial response to the drug, and the time it took for the tissue to respond or contract maximally to the drug. Both measurements were taken from the time of introduction of the drug.

In the second set of experiments, the smooth muscle preparation was subjected to various P_{O_2} levels and to pressure changes. An optimal ACh concentration (4.8×10^{-6} *M*) was used. The P_{O_2} levels used were 155, 300, 400, 500, 600, and 746 mm Hg. Again, these were run at 1, 10, 20, and 30 ata, and the diluent gas was He.

Results

In the first series of experiments, using four levels of ACh concentration (4.8×10^{-7} *M*, 2.4×10^{-6} *M*, 4.8×10^{-6} *M*, 2.4×10^{-5} *M*), no significant change in the initial response latencies was found (Table 1). The maximum response latencies, however, showed significant changes, and it is this type of latency that will be referred to throughout the remainder of the paper.

Using 4.8×10^{-7} *M* ACh, the latency at 20 ata was lengthened slightly when compared with 1 ata. There was no significant change of latency at 10 or 30 ata. No significant change of drug latencies occurred at any of the pressures tested with 2.4×10^{-6} *M* ACh. The 4.8×10^{-6} *M* ACh elicited some significant changes. Latency was lengthened significantly as pressures increased from 1 to 10 to 20 to 30 ata. Using the highest dose (2.4×10^{-5} *M* ACh) significantly increased the latency only at the 30 ata level.

Table II compares the latency of each specific pressure level to the value obtained at 1 ata after flushing at each level of P_{O_2}. In 17 of the 18 levels, a significant lengthening of drug latencies was noted. In only one case (155 mm Hg O_2) was there a change in latency after replacing room air with 1 ata He. The Student "t" test was used to analyze the effect of oxygen at specific pressure levels. Almost all significant differences occurred at the 10 ata

TABLE I

PRESSURE VS. MEAN DRUG LATENCY AT VARIOUS CONCENTRATIONS OF ACETYLCHOLINE

Dose ($\times 10^{-6} M$ ACh)	Pressure (ata)	Latency (sec) ± S.D.	
		Initial Response	Maximum Response
0.48	1	6.50 ± 2.17	18.90 ± 2.70
	10	4.90 ± 0.12	18.60 ± 3.93
	20	5.70 ± 1.46	22.67 ± 3.52[a]
	30	6.54 ± 1.34	22.69 ± 1.34
2.4	1	4.86 ± 1.46	21.29 ± 5.89
	10	4.60 ± 1.35	17.80 ± 3.31
	20	5.33 ± 0.96	22.00 ± 7.40
	30	6.50 ± 1.89	25.17 ± 5.43
4.8	1	4.92 ± 1.14	16.42 ± 3.61
	10	6.31 ± 2.41	18.75 ± 4.57[a]
	20	6.34 ± 2.55	25.26 ± 6.71[b]
	30	7.41 ± 2.44	33.82 ± 9.93[c]
24.0	1	4.25 ± 0.83	17.75 ± 2.86
	10	5.25 ± 0.43	21.25 ± 3.96
	20	4.40 ± 0.80	18.60 ± 4.88
	30	9.17 ± 1.06	28.17 ± 5.04[b]

level. At 400 mm Hg O_2, the latency was 19.72 ± 5.79 seconds, as compared with 28.70 ± 3.23 seconds at 155 mm Hg O_2 ($P = 0.01$). Correlation coefficients were calculated for the latency versus the P_{O_2}, and these can be seen in Fig. 1. Positive correlations were obtained for the comparisons at 1, 20, and 30 ata, with the steepest slope at 30 ata and the most gradual slope at 20 ata. The comparison of 1 with 10 ata gave a negative slope. It was also noted that by increasing the pressure, the ACh-stimulated contraction developed greater tension than at control pressures. Under pressure, the smooth muscle preparation did not

TABLE II

MAXIMUM LATENCY OF $4.8 \times 10^{-6} M$ ACh EFFECTS AT EACH PRESSURE COMPARED AT EACH OXYGEN LEVEL (HELIUM DILUENT GAS)

O_2 Level (mm Hg)	1 ata	10 ata	20 ata	30 ata
155	12.63 ± 3.23	28.70 ± 3.23	26.00 ± 4.45	25.07 ± 5.06
300	13.86 ± 3.22	19.54 ± 3.88	23.16 ± 4.58	25.80 ± 8.12
400	13.42 ± 4.12	19.72 ± 5.79	26.61 ± 3.84	26.42 ± 5.66
500	13.71 ± 4.02	22.94 ± 6.09	24.95 ± 6.38	27.27 ± 8.09
600	11.85 ± 3.17	17.12 ± 4.95	20.51 ± 5.15	25.16 ± 7.43
746	16.88 ± 4.58	17.38 ± 3.80*	27.94 ± 8.90	26.64 ± 4.38

*Not significantly different from control.

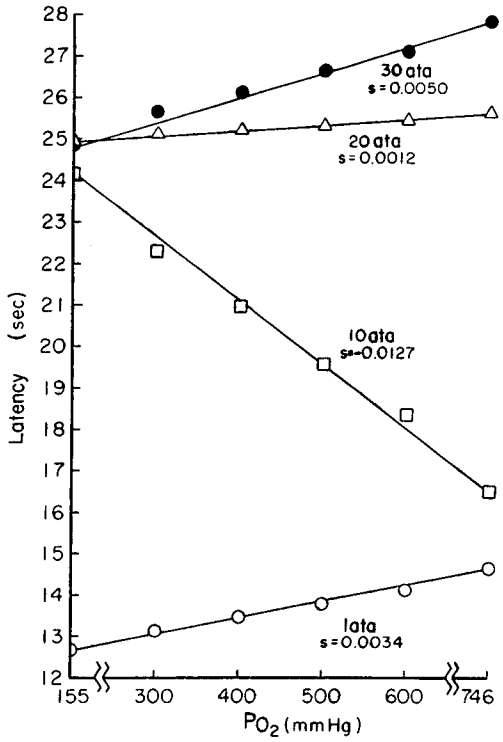

FIG. 1. Comparison of varying O_2 levels upon the latency of 4.8×10^{-6} *M* ACh obtained at 1, 10, 20, and 30 ata He-O_2 pressure. S = slope.

relax as rapidly as it did at 1 ata. These phenomena also appear to be related to the P_{O_2} changes. Reverse "creep" has been known to occur, and it was greatest at 300 mm Hg P_{O_2}. Some of the responses to ACh did not reach a maximum point of contraction within 2 minutes after stimulation with the drug. Therefore, it was impossible to read a latency time. These responses occurred only at 300 mm Hg and 746 mm Hg O_2.

Discussion

In order to explain the data obtained in this study, five basic areas should be examined. It is essential, first of all, to rule out any nonphysiological factors or experimental techniques which might account for the data obtained. Secondly, it is desirable to determine if the changes were due to pressure per se on the drug receptor interaction. The third consideration involves the transport system. Fourth, pressure may be altering excitation-contraction coupling. Lastly, the contractile process itself may be affected by pressure.

If the liquid in which the tissue is suspended is compressed by pressure, the latency might be lengthened at greater pressures because of a slower rate of diffusion of ACh into the tissue. Cattell (4) has noted that water undergoes a 20% volume reduction when subjected to 12,000 ata. According to these figures, 30 ata would decrease the volume of water by

only 0.05%. Cattell states that liquid becomes less compressible as temperature increases. In light of these facts, it is assumed that the compression of the liquid is negligible, and therefore the diffusion of the drug in the liquid is not altered by pressure. It has also been assumed that the gas content of the liquid is in equilibrium with the chamber atmosphere. The assumption is based on Henry's law, which states that "at a constant temperature, the solubility of a gas and a liquid is directly proportional to the pressure of the gas above the liquid." Maron and Prutton (10) also state that "the solubility of each gas from a mixture of gases is directly proportional to the partial pressure of that gas in the mixture."

In order to explain the results of the first set of experiments, the molecular interaction of the drug and receptor must be examined. In this first set of experiments, the effect of pressure was greatest at one of the four ACh concentrations (4.8×10^{-6} *M*). Ariens (2) states that there is an optimal drug concentration in which all of the receptors are occupied. Because only one drug molecule can occupy one receptor at one time, the receptors would gradually be filled as the concentration of drug increased until all the receptors were occupied. This would be the optimal concentration for the drug. It should also be added that the drug and the receptor must interact in an effective way to produce a response. The fact that most consistent changes in latency occurred with 4.8×10^{-6} *M* ACh suggests that this is the optimal drug concentration. At the lower concentrations, all of the receptors probably were not occupied, and therefore the responses elicited were not consistent. At the higher concentration, there was significant change noted in latency, but the significance was not as great as at the optimal dose.

It was also noted in the first set of experiments that there was no significant change in the time it took to note an initial response. The drug probably diffused at a constant rate to the tissue and reacted with a limited number of receptors in a manner sufficient to cause an increase and a contraction of the tissue. These initial responses were in no way equal to the maximum concentration noted later. This reaction was sufficient to at least initiate the contractile process.

It was noted in both sets of experiments that an increased pressure led to an increase in the time it took for the tissue to reach the point of maximum contraction. Numerous references have been made in the literature to the fact that the cholinergic receptors are located on the cell membrane. Landau (9) showed that pressure has an effect on membrane shape. He noted that human amnion cells rounded up when subjected to high pressure. He also stated that the rigidity of the cell membrane is decreased by 25% for each 67 atmospheres of increased pressure.

The present experiments suggest that the shape of the membrane is changed by pressure, with the greatest change occurring at the highest pressure level. The shape is changed more at 30 ata then at 20 or 10 ata. As the membrane shape is changed, the receptor shape will also change and therefore take longer for the drug to interact and elicit a maximum contraction from smooth muscle preparation.

Increased pressure might also alter the structure of proteins. Featherstone et al. (6) noted that an increased pressure of gases can change the function of proteins. They state that the extent and nature of alteration depend on the physico-chemical properties of the gas, rather than on pressure per se. Their data show that He is one of the gases that alters protein function under pressure. This change in protein function could cause a delay in drug receptor interaction since the concentration of the He molecules increases as pressure in-

creases. A greater alteration should be noted at higher pressures. The present experiments show a greater lengthening of drug latency as the pressure is increased. An alteration of protein function could contribute to the latency changes that were observed.

The Na transport system should be considered as a site of possible alteration by pressure and O_2. Koehler and Gottlieb (8) have noted such changes in the activity of the Na-K-Mg ATPase of the rat intestine. However, the changes which they observed do not appear to be significant enough, at the pressure levels of this experiment, to account for the changes noted.

It does not appear probable that the initial excitation-contraction coupling process is affected by pressure increases. Hurwitz and Joiner (7) state that Ca ions which initiate smooth muscle contraction are in some intracellular biophase. These Ca ions are not bound to the cell membrane. They state that there is another store of Ca ions bound on the inner surface of the cell membrane. If these bound ions reach the state of equilibrium with dissolved Ca in the biophase and with the extracellular Ca, they postulate that the rate at which Ca ions are translocated from the membrane depot to the biophase state is determined by "conditions and drugs which affect the permeability, or polarization, or both, of the excitable membranes." They suggest that these latter factors do not alter the mobilization of activator ions simply by altering the rate of diffusion, but that a more complex saturable transport system is affected. They did not postulate the nature of this transport system. From this information, it would appear that smooth muscle contraction could be affected by pressure. As the Ca in the biophase is bound by contractile protein, the mechanism of release of Ca by the membrane-bound depot could be altered. It was noted that at 10 ata, the latency decreases as the P_{O_2} level increases. The combination of an increasing P_{O_2} level and 10 ata pressure could in some way stimulate a release of an increasingly greater amount of Ca from the inner membrane depot. There is not enough known at present about the effects of pressure and O_2 to postulate a mechanism through which this could happen.

The reverse "creep" phenomenon was also noted at 300 mm Hg O_2, and to a lesser extent at 746 mm Hg O_2. These particular combinations of O_2 and pressure could also cause sustained increasing contraction. The sliding filament mechanism could be continually stimulated by an abnormally high rate of Ca release from the membrane into the biophase and thus to the contractile protein. Again, this is just a possibility, and a more elaborate and specific description of the mechanism cannot be made at this time.

From this study it can be concluded that increasing pressure alters the spatial configuration of the receptor complex. The pressure might also alter the protein or Ca bindings involved in the complex. The contractile mechanism may thus be involved, with certain combinations of pressure and oxygen being especially effective in inducing changes.

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SOMATIC AND AUDITORY-EVOKED BRAIN RESPONSES IN MAN BREATHING MIXTURES OF NORMOXIC HELIUM, NITROGEN AND NEON AT PRESSURES TO 37 ATMOSPHERES ABSOLUTE

T. D. Langley

This study was part of the neurophysiologic investigation performed during a stepwise series of exposures to increasing pressures to 37 ata in Predictive Studies III (8). Auditory and somatosensory-evoked brain responses (EBR) were used to assess brain function of two subjects. This technique has been widely used at 1 atmosphere in many disciplines such as audiometry, anesthesia, and vigilance monitoring and was introduced to diving research by Bennett, who studied auditory-evoked brain responses in both animals and man (2,3).

Methods

The method involves providing a mild but distinct stimulus to the subject, then recording the time course of resulting electrical activity on the surface of the brain or scalp. Response to a single stimulus will be lost in the composite signal of the ongoing EEG, but a time-correlated average of 50 or 100 responses results in a distinct wave form (5). A typical auditory EBR which has been processed by a signal averager is shown in Fig. 1. The N_1P_2 component consists of the deflection from the first large negative peak to the second positive one. This is considered a long-latency response, since the significant signal begins approximately 100 milliseconds after the stimulus. This sort of response is usually induced by a short beep or click into the earphones worn by the subject.

Another similar response can be evoked by nonauditory stimuli. For the hyperbaric situation this avoids the uncertainties of sound transmission, hearing and earphone performance introduced by different pressures and gas properties. A typical averaged somatic-evoked brain response is shown in Fig. 2. This response represents the general body sense and includes responses to cutaneous pressure and pain. The N_1P_2 component here may be considered analogous to the N_1P_2 in the auditory system.

The somatic responses were evoked by square-wave electrical shocks to the median nerve at the wrist, with the intensity being just suprathreshold for twitches of the fingers. Stimulus intensity (current) was monitored continuously on an oscilloscope. Stimuli were administered through skin electrodes placed on locations previously determined and marked.

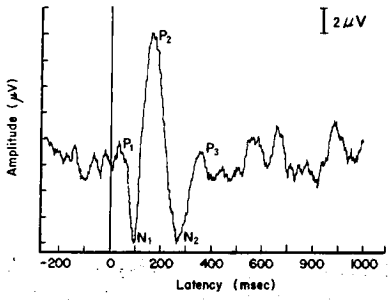

FIG. 1. Auditory-evoked response.

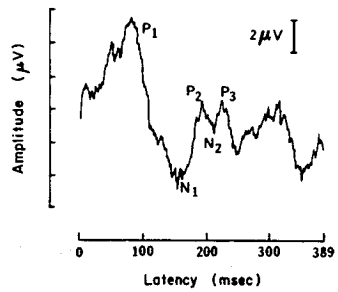

FIG. 2. Somatic-evoked response. Late components.

The somatosensory EBRs were recorded with scalp electrodes over the postcentral gyrus, at the area designated as C_2P (7), the primary cortical projection area for the hand.

The auditory stimuli were beeps delivered through binaural earphones and had rise and decay times of 6 milliseconds with a 17-millisecond plateau. The recording electrode was at the vertex. These points are illustrated in Fig. 3.

The experimental protocol consisted of an initial 9-minute gas wash-in and habituation period, during which the subject breathed the normoxic gas mixture through an oronasal mask (Fig. 4). Data were then collected during an additional 13 minutes, which began with median nerve stimulation, followed by the auditory stimuli. In order to determine somatic thresholds at the beginning of each experimental session, the stimulus intensity to the median nerve was gradually increased. This report deals with single somatic and auditory stimuli delivered at the rate of one per second.

Results and Discussion

The sensory threshold was considered to be the point at which the subject first felt the shocks in his hand. The motor threshold was the point at which his fingers just began to twitch with each shock. Figure 5 shows that, while there was no consistent change in motor thresholds, the sensory thresholds increased 25 to 35 percent over the course of the experiment. The motor thresholds could be considered to represent a relatively simple neural circuit, perhaps even an in vivo nerve-muscle preparation. Therefore, one would expect them to be quite resistant to environmental changes. The sensory thresholds, however, involve a multisynaptic circuit, including the thalamus and cerebral cortex, and would therefore be expected to be more vulnerable to outside influences. The data support this analysis.

Auditory EBRs with percent decrement from sea level are plotted against increasing chamber pressure, from 1 to 37 atmospheres in Fig. 6. The curves represent decreases in the N_1P_2 component for both subjects. In a sense, they could be considered dose-response curves. With helium, the auditory responses generally decreased. Similar decreases also occurred with neon. The lowest curve shows a greater depressant effect of various "doses" of nitrogen. It has been demonstrated that hyperbaric conditions cause apparent decrements in hearing acuity (6,9). Decrements in hearing cause reduction of the evoked response (11). These facts, coupled with the difficulties of calibration of the signal actually picked up by the auditory mechanism, cast doubts on any interpretation of the auditory EBR under varied hyperbaric conditions (1). Consequently, attention was concentrated on the somatic EBR.

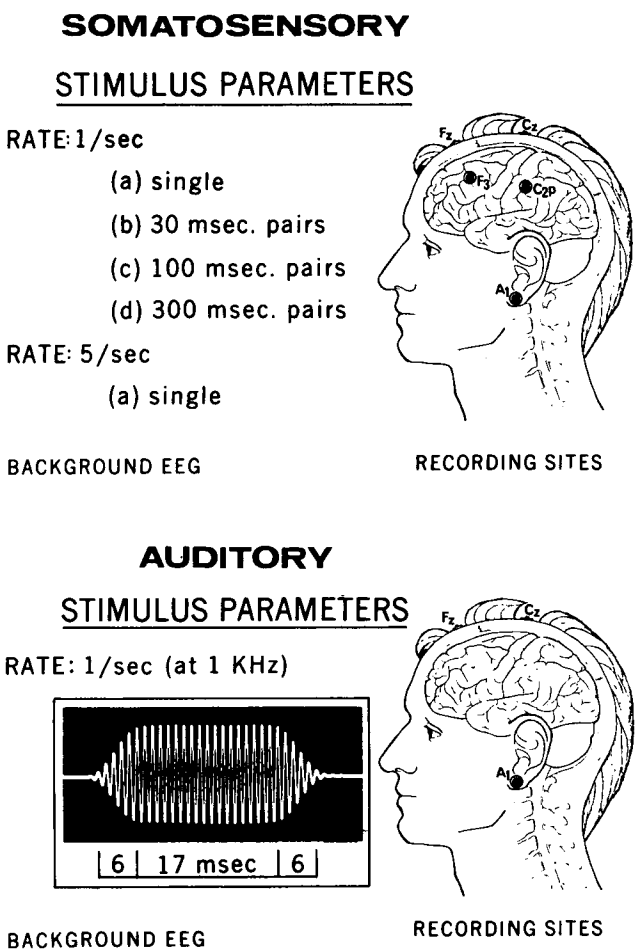

FIG. 3. Stimuli and recording sites.

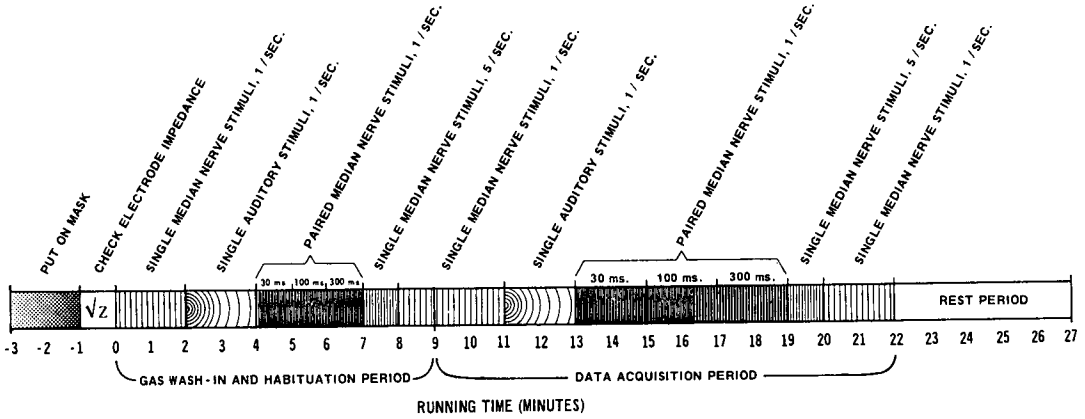

FIG. 4. Experimental protocol for evoked brain responses.

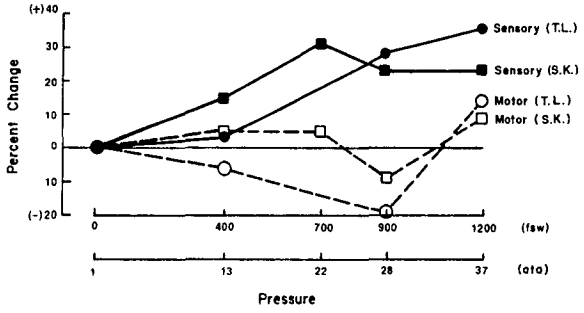

FIG. 5. The effects of normoxic helium on sensory and motor thresholds for median nerve stimulation.

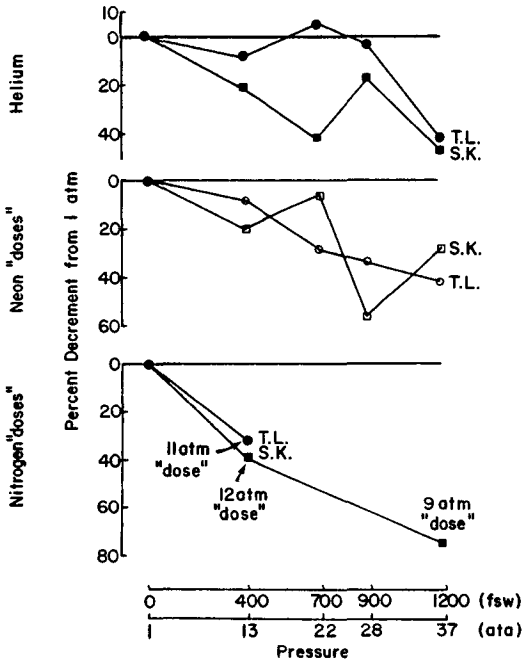

FIG. 6. The effects of normoxic helium, neon and nitrogen on amplitude of the N_1P_2 component of auditory-evoked brain responses.

In Fig. 7 averages of somatic responses are shown, taken under different conditions. The N_1P_2 component is enhanced at 37 ata of helium when compared to 1 ata, particularly for subject SK. The responses with a 24-atmosphere "dose" of neon are also enhanced, but less than with helium. For subject TL with neon, the slope of the N_1P_2 component was altered considerably. The 9-atmosphere "dose" of nitrogen which is in addition to 28 atmospheres of helium, depressed the N_1P_2 for both subjects, as well as the P_1N_1.

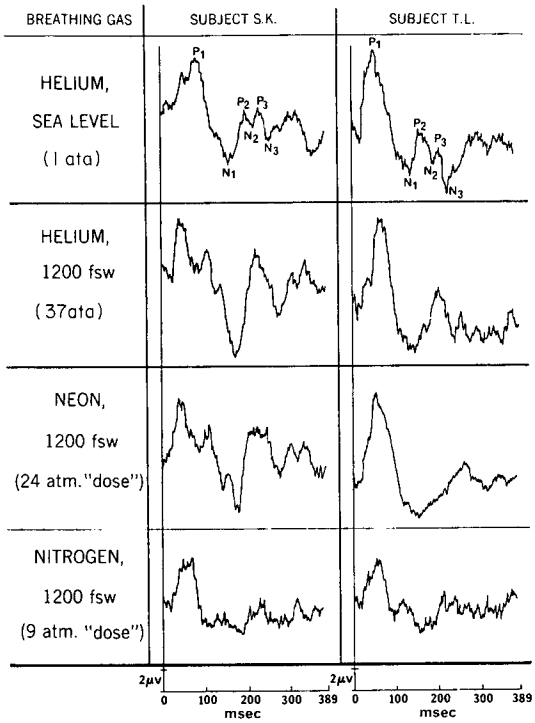

FIG. 7. Averages of somatic-evoked brain responses when breathing helium, neon and nitrogen.

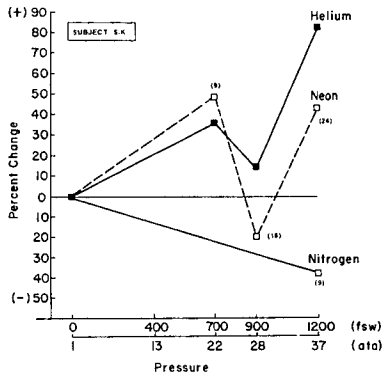

FIG. 8. The effects of normoxic helium, neon and nitrogen on amplitude of the N_1P_2 components of somatic-evoked brain responses. Subject: S.K.

The amplitude of the N_1P_2 component of somatic EBRs as a function of pressure and inert gas species is shown in Figs. 8 and 9. While nitrogen caused a decrease similar to that seen in auditory responses, helium—and to a lesser extent neon—appeared to cause a slight increase with increasing pressure.

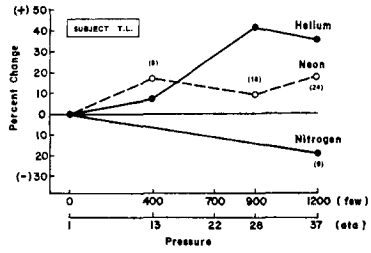

FIG. 9. The effects of normoxic helium, neon and nitrogen on amplitude of the N_1P_2 components of somatic-evoked brain responses. Subject: T.L.

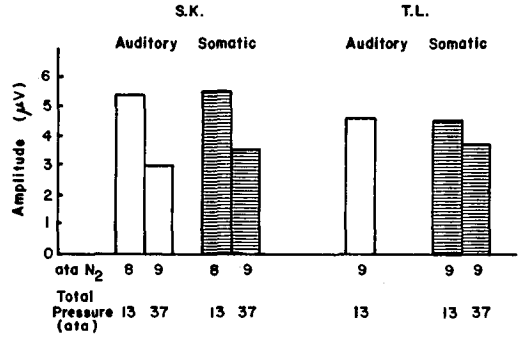

FIG. 10. The effects of various "doses" of nitrogen (in atm) on amplitude of the N_1P_2 component of auditory- and somatic-evoked brain responses.

Such increases can be caused by general hyperexcitability or, paradoxically, by simple drowsiness. Although the effects seen here may have been manifestations of the high pressure nervous syndrome, it is possible that they simply reflect drowsiness. A frequency analysis of the EEG of both subjects showed frequent drowsiness, which was increased at the higher pressures.

The responses with neon were more similar in amplitude to those at 1 atmosphere than those evoked when helium was breathed.

Figure 10 shows an analysis directed at the question of pressure reversal of narcosis. Observations in various laboratories (4,10) have shown that one of the effects of high pressures is to counteract the depression of an anesthetic. One would expect 37 ata of pressure to partially counteract the effects of a dose of nitrogen. In Fig. 10 the shaded bars represent the amplitudes of somatic EBRs with approximately equal doses of nitrogen. The left one of each pair is for chamber pressure of 13 ata, and the right one is for 37 ata. These are responses of narcotized subjects; normal responses are approximately twice as high. If the extra pressure at 37 ata did counteract the narcosis, then the right bar should be higher than the one on the left. The data do not show this.

Absence of pressure reversal of nitrogen narcosis can perhaps be explained since, in those experiments where it has been found, it is related to a recent, relatively rapid compression.

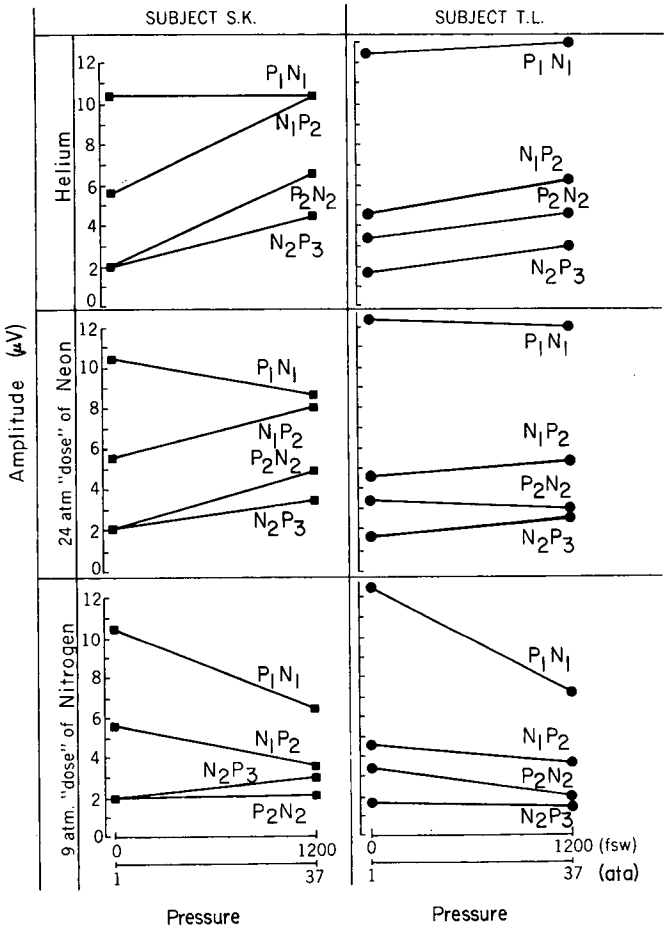

FIG. 11. The effects of normoxic helium, neon and nitrogen on amplitudes of various late components of somatic-evoked brain responses.

The subjects of the present study had been at pressure for several days, providing ample time for compensatory or adaptive processes to occur.

Amplitudes of four components of the somatic EBR were measured to see if a relationship exists between various components and susceptibility to gas or pressure influences. The rationale is that, since the site of action is likely to be at the synapse, progressively later components of the evoked response might be proportionally changed to a greater degree. For example, one might expect the P_2N_2 and N_2P_3 components to be depressed more by a "dose" of nitrogen than the earlier components, the P_1N_1 and the N_1P_2.

This is indeed what was found with the enhancing effect of helium but not with the depressant effect of nitrogen. In Fig. 11 the slopes of the amplitude curves at 1 ata are compared to those at 37 ata. For helium, there was little or no change in amplitude of the P_1N_1 component, but there were slight increases in the amplitude of later components. With the

"dose" of nitrogen, the early components were depressed, but there was little or no change of the later ones. Again, neon is intermediate between nitrogen and helium. It seems when there is facilitation, the influence is primarily on the later of these components, but when there is inhibition, the influence is primarily on the earlier ones.

From these and other observations made during the study, it is concluded that high pressures of helium raised sensory thresholds to electrical stimulation but had little effect on motor thresholds. When stimulus intensity was controlled, the EBRs were enhanced, especially the somatic EBRs. They were depressed by nitrogen. With neon, the EBRs were also enhanced, but only by about half as much as with helium. At 37 ata, the EBRs were more nearly normal during neon breathing.

In a comparison of similar doses of nitrogen at 13 and 37 ata total pressure, there appeared to be no pressure reversal of narcosis under the relatively isobaric conditions of this experiment.

The primary effect during helium breathing seems to have been on the later components of somatic EBRs, whereas nitrogen influenced primarily the earlier components.

ACKNOWLEDGMENTS

The work reported here was performed at the Institute for Environmental Medicine, University of Pennsylvania, as a component of its Predictive Studies III Program. The author acknowledges the extensive aid of the Institute staff and visiting investigators. Research support was derived from Office of Naval Research contracts N00014-69-C-0405 and N00014-67-A-0216-0026 and National Institutes of Health contract HL 08899-08 and a grant of the crude neon used by Ocean Systems, Inc.

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THE EFFECTS ON CO_2 REACTIVITY OF BREATHING CRUDE NEON, HELIUM AND NITROGEN AT HIGH PRESSURE

R. Gelfand and R. Peterson

The experiments reported here were carried out as part of the exposures in the Predictive Studies III Program (12). In this series of simulated dives to depths from 0 to 1200 feet of sea water (fsw), respiratory response to CO_2 was measured in two subjects while breathing normoxic mixtures of 1) crude neon* at 0, 400, 700, 900 and 1200 fsw; 2) helium at 0, 400 and 900 fsw; and 3) nitrogen at 0 and 400 fsw. The respiratory response to CO_2 (CO_2 reactivity) was previously studied here during a chronic exposure to normoxic nitrogen and to helium at a simulated depth of 100 fsw (14). This present study was designed to establish the dose-response characteristics of any decrement in CO_2 reactivity produced by inert gas mixtures used at increased pressures in diving. This required examining the specific possibility that a narcotic gas such as nitrogen impairs the ability of the respiratory system to respond to chemical stimuli.

Several factors can reduce a diver's respiratory response to CO_2 while in a closed habitat. Sleep by itself produces a considerable reduction in CO_2 reactivity (2). Combining this with the decrements which must occur with increasing gas density and possible narcosis by gases such as nitrogen, a circumstance may occur in which divers might suffer from insomnia induced by sleep apnea (7). An exhausted diver falling into deep sleep and breathing a high density narcotic gas, could even become sufficiently hypoxic that, in the absence of effective hypercapnic stimuli, he might lapse into hypoxic depression of CNS function and die.

There have been several prior attempts to find a relationship between inert gas narcosis and respiratory depression, involving studies of CO_2 accumulation at rest (9, 10). The results were negative in both studies, at 3.8 and 7.8 ata air, respectively. It has also been shown that the narcosis of 40% nitrous oxide at 1 ata does not significantly alter respiratory reactivity (4, 13).

In addition to the effects of increased gas density on CO_2 reactivity, the following parameters were also investigated: 1) resting end-tidal CO_2 levels; 2) tidal volume and frequency responses to increased CO_2; 3) flattening of tidal volume, frequency and minute volume responses to CO_2; 4) predicted maximum safe depth limits based on flattening of respiratory response to CO_2; and 5) extrapolation to CO_2 reactivity of liquid-breathing.

*Crude neon is an atmospheric distillate nominally containing 75% neon and 25% helium. The crude neon used in this study consisted of 76.8% neon, with the balance helium.

Methods

A closed-circuit rebreathing system was employed to use the subject's CO_2 production as the means of CO_2 administration. This was required due to the multiplicity of gas mixtures needed for the open-circuit administration of several inspired CO_2 levels at each of five depths and with three different inert gases. Due to the large number of experiments conducted in sequence (12), the experimental procedure was designed to reduce the time required to obtain CO_2 reactivity as compared to the stable-state method. The apparatus was designed to prevent contamination of the chamber atmosphere by CO_2 and the inert gases, other than helium, which were used.

The rebreathing method uses a rate of rise (ramp) of CO_2 of approximately 0.8 mm Hg/min, with the maximum elevation of end-tidal CO_2 tension ($P_{A_{CO_2}}$) limited to 10–15 mm Hg above the resting level. This method made it possible to avoid initial hyperoxia, rapidly falling inspired P_{O_2} and high levels of $P_{A_{CO_2}}$ and ventilation associated with the rebreathing method currently in wide use (17). The large volume of the rebreathing circle required to achieve a slow $P_{A_{CO_2}}$ ramp also resulted in a slowly falling inspired P_{O_2}, making it possible to maintain normoxic inspired gas mixtures even at very high pressures where the difference in percentage of O_2 between toxic and hypoxic levels becomes very small.

The volume of the rebreathing system totaled approximately 100 L (Fig. 1). The overall system includes: 1) a low-resistance bidirectional breathing valve; 2) a 100-L vinyl bag collapsed to approximately ½ volume; 3) a special pneumotachograph assembly of four Fleish No. 4 flow transducers mounted in parallel (6); 4) a CO_2 absorber consisting of two parallel Collins P-401 canisters; 5) a series valve assembly consisting of three solenoid valves (White-Rodgers Model #2509) in parallel; and 6) a shunt valve assembly consisting of four

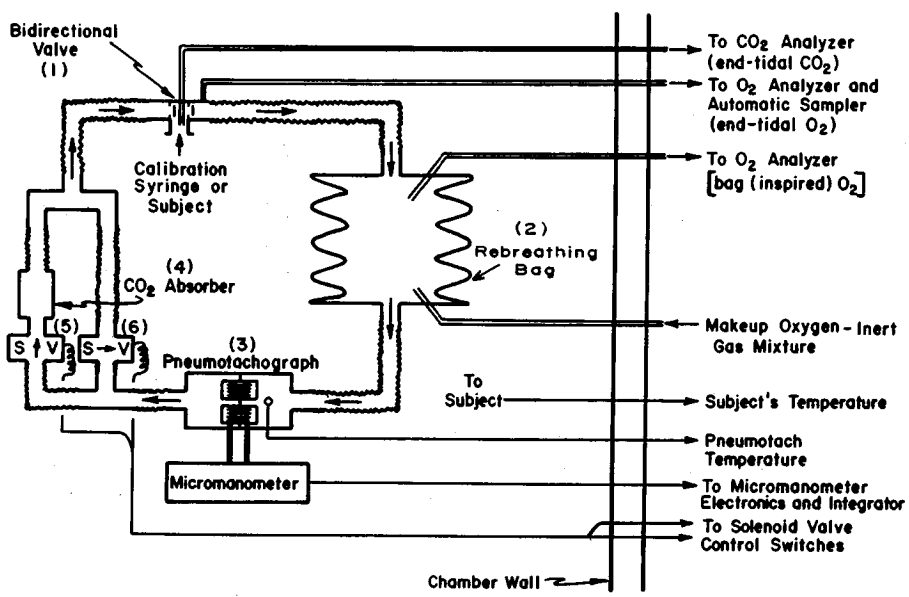

FIG. 1. Rebreathing circle employed to obtain a slow rate of rise of $P_{A_{CO_2}}$. See text for details.

of these solenoid valves in parallel. Interconnecting tubings of 1¼- and 2-inch inner diameter were employed.

The differential pressure across the pneumotachograph (calibrated with the breathing mixture actually used in each experiment) was sensed by a modified micromanometer (6) (Medistor P-10A); its output was integrated and recorded on one channel of a dual channel recorder (Brush 280). End-tidal carbon dioxide concentration (Beckman LB-1 CO_2 analyzer) was recorded on the second channel of this recorder, and 1-minute averages of inspired minute ventilation ($\dot{V}_I$), tidal volume (V_T), respiratory frequency (f) and $P_{A_{CO_2}}$ were subsequently obtained from these strip-chart records. Both alveolar and bag (inspired) oxygen concentrations (modified Servomex OA-150 analyzers) were monitored and recorded on an additional recorder. Appropriate O_2–inert gas mixtures were continuously added to the bag to maintain a normoxic P_{O_2}.

The rebreathing system operates in the following way: expired gas flows into the bag, expanding it. There is no flow through the pneumotachograph during expiration, due to closure of the inspiratory side of the bidirectional breathing valve. During inspiration gas is drawn out of the bag through the pneumotachograph. With this placement of the pneumotachograph, the gas passing through it is at or close to room temperature throughout the respiratory cycle, and condensation of water in the flow transducers is not a problem.

Experiment Procedures

Each experiment lasted 30–40 minutes. The seated subject prebreathed the test mixture for 10 minutes to eliminate helium from the lungs, circulation and CNS while the rebreathing system was flushed with the same test gas mixture. Due to possible incomplete flushing, continued elimination of helium from the subject's body stores, and possible diffusion of helium into the rebreathing system from the chamber atmosphere, N_2 and Ne were contaminated by He when employed as experiment gases. The actual gas mixture of the rebreathing system was analyzed to ascertain the degree of contamination. Table I shows the relationship of chamber simulated depth to actual inspired inert gas partial pressures, densities and density/viscosity ratios.

At the conclusion of the prebreathing interval the subject commenced rebreathing. He inspired CO_2-free gas for 10 minutes (inspired gas passing through the CO_2 absorber); the series solenoid valves were then closed while the bypass valves were opened, allowing $P_{A_{CO_2}}$ to rise approximately 10 to 15 mm Hg above the control level during the final 20 minutes of the experiment. While CO_2 reactivity was studied with crude neon at five different depths, it was possible to schedule helium experiments at only three depths and nitrogen at two depths.

Results

Twenty experiments on two subjects were completed and analyzed. Subject RB became nauseated during his experiment breathing nitrogen at 400 fsw and subsequently vomited. The data obtained in the unstable conditions of this experiment are not used here.

Table II gives the calculated values of CO_2 reactivity, which decrease markedly as gas density increases. Figure 2 shows a family of curves for the crude neon experiments constructed from the observed data. Table III gives resting values of $P_{A_{CO_2}}$ and ventilatory

TABLE I

ACTUAL INSPIRED GAS DENSITIES (ϱ), DENSITY/VISCOSITY RATIOS (ϱ/μ), AND INSPIRED INERT GAS PARTIAL PRESSURES (P_i) RELATED TO ACTUAL CHAMBER PRESSURE AND SIMULATED DEPTH[a]

Gas Under Study	Chamber Parameters		Actual Inspired Gas Parameters					
			Subject RB			Subject TC		
	Pressure (ata)	Simulated Depth (fsw)	ϱ (g/L)	ϱ/μ (sec/cm²)	P_i (ata)	ϱ (g/L)	ϱ/μ (sec/cm²)	P_i (ata)
Neon	1.0	0	0.93	3.0	0.6	0.89	2.9	0.6
	13.0	400	8.92	29.1	8.7	8.94	29.1	8.7
	22.2	700	14.43	47.0	14.1	13.20	43.0	12.3
	28.2	900	18.07	58.9	17.6	19.89	64.8	20.2
	37.5	1200	23.65	77.0	22.9	25.47	83.0	25.5
Helium	1.0	0	0.44	2.2	0.8	0.46	2.3	0.8
	13.0	400	2.56	12.7	12.8	2.61	13.0	12.8
	28.2	900	5.26	26.2	27.8	5.25	26.1	27.8
Nitrogen	1.0	0	1.29	7.1	0.8	1.29	7.1	0.8
	13.0	400	14.43	79.2	10.9	11.10	61.0	8.6

[a]Gas densities are calculated from chamber absolute pressure and the percent composition of each gas component in the rebreathing bag, as determined by gas chromatograph or mass spectrometer. Densities and viscosities employed in the calculations are as follows for neon, helium and nitrogen, respectively: density—0.900, 0.179, 1.251 g/L; viscosity—307, 201, 182 micropoise.

TABLE II

RESPIRATORY REACTIVITY ($\Delta\dot{V}_I/\Delta P_{A_{CO_2}}$) RELATED TO INSPIRED GAS DENSITY

Normoxic Inert Gas	Subject RB		Subject TC	
	Inspired Gas Density (g/L)	$\Delta\dot{V}_I/\Delta P_{A_{CO_2}}$ (L/min/mm Hg BTPS)	Inspired Gas Density (g/L)	$\Delta\dot{V}_I/\Delta P_{A_{CO_2}}$ (L/min/mm Hg BTPS)
Crude neon	0.93	4.4	0.89	6.5
	8.92	2.1	8.94	3.8
	14.43	1.3	13.20	2.4
	18.07	0.8	19.89	1.9
	23.65	0.6	25.47	1.0
Helium	0.44	4.1	0.46	5.8
	2.56	3.5	2.61	3.4
	5.26	2.6	5.25	3.5
Nitrogen	1.29	4.2	1.29	6.6
	14.43	—	11.10	2.4

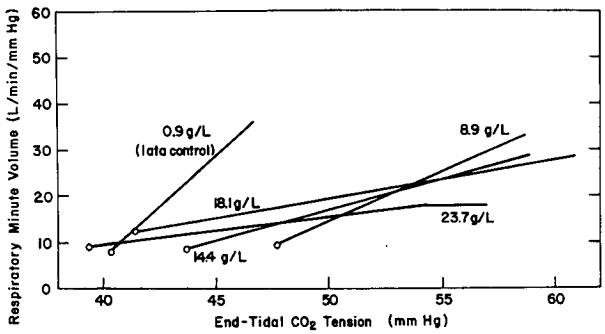

FIG. 2. Ventilatory responses to $P_{A_{CO_2}}$ increase at five pressures, for subject RB breathing normoxic crude neon. Each line represents the best fit calculated for a straight line through the $\dot{V}_I$ and $P_{A_{CO_2}}$ data points obtained during the time the ramp $P_{A_{CO_2}}$ produced a ramp $\dot{V}_I$ response. Each line so calculated is passed through the resting, stable-state, zero-inspired CO_2 "control" point (small circle) to give an estimate of the actual stable-state respiratory CO_2 reactivity. Flattening of a $\dot{V}_I$ response indicates that both f and $\dot{V}_T$ have reached upper limits. Inspired gas density is shown with each ventilatory response line. Results for subject TC are similar.

parameters. Inspired oxygen tension averaged approximately 195 mm Hg throughout the experiment procedures while alveolar oxygen tension averaged approximately 173 mm Hg. The latter was stabilized by the opposing actions of an increasing ventilation tending to raise it and a decreasing inspired oxygen tension tending to lower it. Rectal temperatures, which did not vary systematically with gas density, averaged 36.8 °C and 36.7 °C for the two subjects.

TABLE III

RESTING VALUES OF END-TIDAL P_{CO_2} AND VENTILATORY PARAMETERS RELATED TO INSPIRED GAS DENSITY

	Subject RB					Subject TC				
Normoxic Inert Gas	Inspired Gas Density (g/L)	$P_{A_{CO_2}}$ (mm Hg)	f (breaths/ min)	V_T (L BTPS)	$\dot{V}_I$ (L/min BTPS)	Inspired Gas Density (g/L)	$P_{A_{CO_2}}$ (mm Hg)	f (breaths/ min)	V_T (L BTPS)	$\dot{V}_I$ (L/min BTPS)
Crude neon	0.93	40.4	14	0.60	8.2	0.89	38.4	6	1.05	6.6
	8.92	47.7	15	0.66	9.7	8.94	47.2	9	0.78	6.8
	14.43	43.7	12	0.73	8.6	13.20	44.8	10	1.02	10.5
	18.07	41.4	15	0.86	12.5	19.89	44.6	7	1.34	9.4
	23.65	39.4	14	0.66	8.9	25.47	45.4	7	1.05	6.9
Helium	0.44	39.1	13	0.80	10.4	0.46	41.2	10	1.06	10.2
	2.56	44.9	13	0.67	8.9	2.61	48.6	10	0.65	6.5
	5.26	42.2	15	0.66	9.9	5.25	48.2	9	0.87	7.4
Nitrogen	1.29	42.6	14	0.62	8.4	1.29	40.1	9	1.37	12.0
	14.43	36.3	17	0.66	11.4	11.10	43.4	10	0.74	7.4

Discussion

Major objectives of these experiments were to obtain dose-response CO_2 reactivity curves for crude neon, helium and nitrogen over a wide range of gas densities. The effect of gas density increase on the ability of the respiratory system to respond to increased levels of CO_2 had never previously been studied for neon and very little was known even for helium or nitrogen. Furthermore, with the evidence that neon has a low narcotic potency comparable to that of helium at least to 1200 fsw (8), the results obtained in the present study can be applied to predict CO_2 reactivity decrements while breathing helium itself at equivalent gas densities (greater depths). In addition, it was anticipated that any narcotic effect of nitrogen on respiratory control could be detected by comparison of CO_2 reactivities while breathing nitrogen and crude neon at comparable densities.

Factors Influencing CO_2 Reactivity

The slope and position of the respiratory response to CO_2 are functions of many variables. In addition to breathing resistance and wakefulness, other factors include individual differences (11), arterial and perhaps brain O_2 concentration (5), and body temperature (19).

Since the subject population under these unusual circumstances could consist practically of only two individuals, the concern is not with a population average or distribution but with dose-response effects of density on individual subjects. Neither O_2 nor deep body temperature changes were great enough to introduce major complications. Otherwise the conditions of these experiments differed considerably from the typical arrangements for measuring the respiratory response to CO_2 at sea level. These differences relate principally to the subjects' environment and the activities required of them. Possibly due to the high thermal conductivity of the helium within the pressure chamber and to its increase with chamber depth, the subjects' caloric intake and resting mean skin temperature increased progressively with chamber depth. Therefore, their "resting" states are not constant from one experiment (depth) to another.

In addition, the high level of activity of the subjects, while preparing for and performing these and other experiments (12), together with the relatively short rest periods before these experiments began, meant that they were not in a basal metabolic state during the experiments. On the other hand, their high level of familiarity and experience with the experimental situation eliminated psychically-activating responses, such as fear or nervousness.

Observation: CO_2 Reactivity as a Function of Pressure

When the slopes of the respiratory responses to CO_2 inhalation ($\Delta\dot{V}_I/\Delta P_{ACO_2}$) are plotted against inert gas pressure, there is a marked decrement in CO_2 reactivity which is a curvilinear function of depth, dropping most rapidly at shallow depths. As expected, the CO_2 reactivity decreases in the order of gas density at equal pressures. Since the neon curves visually resembled exponentially-decaying functions, the data were tested to determine how they would fit simple exponential and power functions. It was found for both subjects that the data best fit an exponential function. Although no theoretical basis for this fit is known, a useful feature of the log-linear graphs, shown in Fig. 3, relates to the fact that the CO_2 reactivity plots for all gases must converge to the same reactivity at zero pressure, since

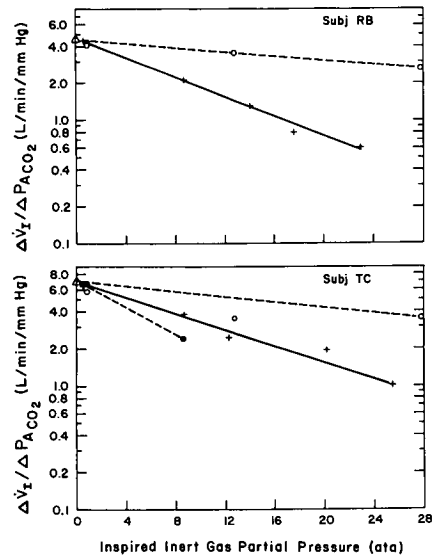

FIG. 3. Logarithm of CO_2 reactivity plotted against inspired inert gas partial pressure. The solid straight lines through the crude neon points are linear regressions on the semilogarithmic axes; dotted lines are visual fits to the data passed through the intercept of the neon regression at zero pressure. Symbols: +, crude neon; o, helium; ●, nitrogen; Δ, intercept of crude neon regression at zero pressure.

there the work of breathing is solely that of moving the chest wall. This convergence can conveniently be used on the semilogarithmic graphs as a test for the consistency of data. By this test it is clear that TC's helium point at 12.8 ata is too low. Assuming exponential models, the crude neon data can be described by Eqs. (1) and (2):

$$\Delta \dot{V}/\Delta P_{CO_2} = 4.6\, e^{-P_i/11} \quad \text{(for subject RB)} \qquad \text{(Eq. 1)}$$

$$\Delta \dot{V}/\Delta P_{CO_2} = 7.0\, e^{-P_i/13.2} \quad \text{(for subject TC)} \qquad \text{(Eq. 2)}$$

where:
P_i is the inspired inert gas partial pressure in ata.

Effects of Density and Viscosity

If gas density alone is responsible for the differences between the CO_2 reactivity curves for helium, crude neon and nitrogen in Fig. 3, then re-plotting CO_2 reactivity as a function of density, as in Fig. 4, should eliminate these differences. In this figure, the data of subjects RB and TC have been pooled by normalization of CO_2 reactivities to a percentage scale. The CO_2 reactivities at zero density obtained by extrapolation of the neon data are taken at 100%. Also shown in this figure are two nitrogen data points obtained from an earlier study (14). These represent the average data of six subjects. It can be concluded by inspection of this figure that CO_2 reactivity is a function of density alone, and that nitrogen narcosis has no influence on respiratory control in awake man. The data of Fig. 4 can be described by Eq. (3):

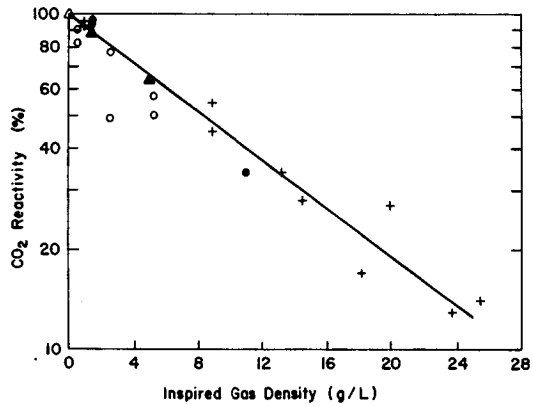

FIG. 4. Logarithm of CO_2 reactivity plotted against inspired gas density (combined data of both subjects). The solid line is the linear regression based on the 10 crude neon data points. Symbols are the same as for Fig. 3 except: ▲, for nitrogen data from a previous study (14).

$$\Delta\dot{V}/\Delta P_{CO_2} = 100e^{-\varrho/12.1} \quad \text{(Eq. 3)}$$

where:

$\Delta\dot{V}/\Delta P_{CO_2}$ is in % of the $\varrho = 0$ value.

However, there are several anomalies apparent in Fig. 4. All the helium points lie below the line of regression through the neon data points. Furthermore, the density of water (1000 g/L) lies far to the right on the horizontal axis of this figure, so the predicted CO_2 reactivity during water breathing would be zero. These anomalies are resolved by plotting CO_2 reactivity against the ratio of density to viscosity (ϱ/μ), as in Fig. 5. The helium points now scatter above and below the line of regression through the crude neon points. In addition, both H_2O and the fluorocarbon FC-80, frequently used in liquid-breathing experiments, with ϱ/μ ratios of 144 and 124 sec/cm^2 respectively, now appear to be small but finite. However, the nitrogen data points now lie above the crude neon line of regression. A possible explanation is that nitrogen narcosis produces an excitatory effect on respiratory control in awake man, analogous to the excitation of light anesthesia. Further studies are required to resolve this issue. The data for crude neon and helium of Fig. 5 can be described by Eq. (4):

$$\Delta\dot{V}/\Delta P_{CO_2} = 100\, e^{-\varrho/38.9\mu} \quad \text{(Eq. 4)}$$

where:

$\Delta\dot{V}/\Delta P_{CO_2}$ is in % of the $\varrho = 0$ value.

Other methods, which may reflect more directly the actual neural output of the respiratory center when variation in mechanical loading of the pulmonary system is an experimental condition, could be useful in further exploring this question. It was anticipated that the

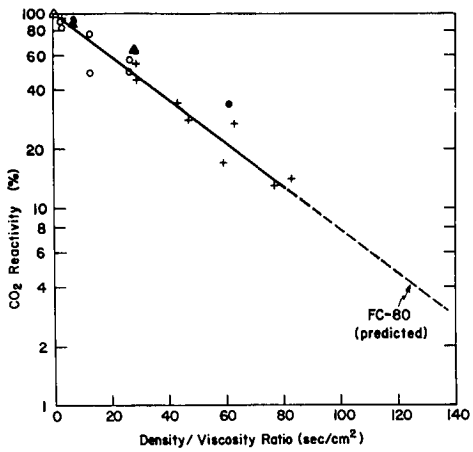

FIG. 5. Logarithm of CO_2 reactivity plotted against the density/viscosity ratio (combined data of both subjects). The solid line is the linear regression based on the 10 crude neon data points. Symbols are the same as for Fig. 4.

inspiratory work of breathing might be such an index (16). Esophageal pressure and inspiratory flow were measured and recorded in addition to the variables described previously. It was not possible to obtain lung volumes or the relaxation pressure curve of the chest wall. The nonelastic inspiratory work of breathing (W_i) was calculated and the slopes of W_i vs $P_{A_{CO_2}}$ were plotted both against ϱ and ϱ/μ. In the absence of narcosis, the slope of the neural output of the respiratory center vs $P_{A_{CO_2}}$ should be constant, independent of ϱ. If W_i were directly proportional to neural output, the W_i vs $P_{A_{CO_2}}$ slopes should also be independent of ϱ. This was not found to be the case here, although the range of variation appears less than for $\Delta\dot{V}_I/\Delta P_{A_{CO_2}}$.

RESTING VALUES OF $P_{A_{CO_2}}$

In the absence of compensatory factors, an increasing respiratory resistance ought to result in a progressive decrease in resting ventilation accompanied by CO_2 retention. Figure 2 and Table III show that both subjects exhibit a large $P_{A_{CO_2}}$ increase when breathing normoxic crude neon at a simulated depth of 400 fsw relative to the sea level values. However, as the pressure and density of the breathing mixture increase further, $P_{A_{CO_2}}$ returns toward the sea level values. If this were not so, failure of respiratory control would constitute a serious limitation to respiration of dense gases.

While it appears natural to associate increased CO_2 retention with gas density increase, Saltzman et al. have suggested that even at relatively low pressures, hydrostatic pressure per se affects respiratory regulation (18). They reach this conclusion apparently by utilizing statistical inferences based on an assumption of a linear relationship between resting arterial P_{CO_2} and pressure or density. Figure 6 shows resting $P_{A_{CO_2}}$ plotted against the density/viscosity ratio for subject RB (the results appear most consistent when the viscosity of each gas is taken into account). This relationship is clearly not linear over the range of gas densities employed here. The cause of the reversal of the increase in resting $P_{A_{CO_2}}$ as gas density increases is not known. Analysis of the data of Saltzman et al. suggests that their conclusion

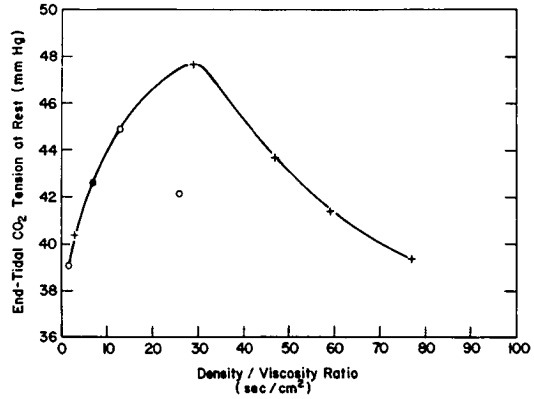

FIG. 6. End-tidal P_{CO_2} at rest for subject RB, plotted as a function of the density/viscosity ratio. The line is a visual fit to indicate the trend of the experimental data. Symbols are the same as for Fig. 3. The results are similar for subject TC.

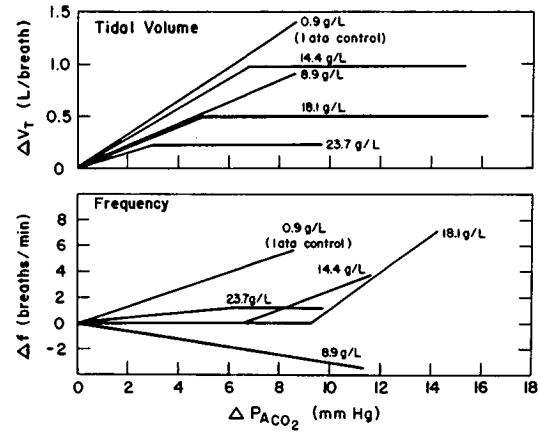

FIG. 7. Changes in tidal volume and respiratory frequency as functions of change in P_{ACO_2} and inspired gas density for subject RB. The slopes of the lines are regressions through the appropriate V_T and f data points obtained from the corresponding responses to the ramp P_{ACO_2}. Flattening of a V_T or f response shows that the corresponding variable has reached an upper limit.

that hydrostatic pressure affects respiratory control at less than 10 atmospheres is probably an artifact due to the assumption of linearity and averaging of disparate data.

EFFECTS OF GAS DENSITY ON RESPIRATORY FREQUENCY AND TIDAL VOLUME RESPONSES TO CO_2

It has been observed that an increase in airway resistance, imposed by either mechanical obstruction (1) or by increase in respired gas density (9), decreases f and may increase V_T when measured in the resting subject. To explore these effects further, both V_T and f have been graphed as functions of P_{ACO_2} and inspired normoxic crude neon gas density. Figure 7 shows that the usual sea level relationships between V_T, f and P_{ACO_2} are disrupted for subject

RB. At sea level (0.9 g/L) both V_T and f increase with P_{ACO_2}. At increasing densities the V_T vs P_{ACO_2} slope decreases and V_T reaches upper-limit values. Simultaneously f first shows a negative slope, then a zero slope at the lower values of P_{ACO_2} until V_T reaches upper-limit values when it shows a positive slope again. Finally at 23.7 g/L and at elevated values of P_{ACO_2}, both V_T and f have zero slopes (limiting values) so that $\dot{V}_I$ has also reached a limiting value (Fig. 2). The graphs are similar for subject TC.

This effect of gas density on f appears to be consistent with the analysis by Clark and von Euler of the regulation of depth and rate of breathing (3). They have concluded that the rapidity with which the lungs inflate is a determinant of both f and V_T. Ordinarily this rate of inflation is largely a function of the chemical respiratory drive. However, when gas density is increased, as in these experiments, an additional factor is introduced. Since the lungs and airways comprise a compliant chamber with a series resistance, the rate of lung inflation in response to a given inspiratory drive is a function of the flow resistance and is decreased as gas density increases.

Limits of V_T, f and $\dot{V}_I$ Increase: Flattening of CO_2 Response Curves

When air is breathed at sea level, ventilation starts to reach limits gradualy at a P_{ACO_2} of approximately 65–70 mm Hg corresponding to ventilations of approximately 55–70 L/min (11). It is apparent from Figs. 2 and 7 that, at high density, V_T, f and $\dot{V}_I$ tend to reach maxima at P_{ACO_2}s well below 65 mm Hg. The suppression of the f increase at a low P_{ACO_2}, together with its subsequent increase at a higher P_{ACO_2} (appearing to "compensate" for the failure of V_T to increase further), means that $\dot{V}_I$ will not reach its limit until both V_T and f reach theirs. $\dot{V}_I$ was actually observed to stop increasing at the inspired normoxic crude Ne densities of 23.7 and 25.5 g/L, at ventilations of approximately 18 and 30 L/min for subjects RB and TC, respectively. This occurred at actual P_{ACO_2}s of approximately 50 and 57 mm Hg. It constitutes a reduction in the *linear range* of response of the respiratory system to CO_2 accumulation as gas density increases; just as the linear range of pneumotachographic flow transducers decreases with gas density increase (6).

Predicted Maximum Density and Depth Limits, Based on Decreased Slope of CO_2 Response Curves

It is difficult to select criteria, based on CO_2 reactivity alone, to predict the maximum useful depth at which a particular gas mixture can be used in diving. Other experiments, such as exercise tolerance and pulmonary function tests, can be applied more directly to this purpose (15, 20). However in the absence of the extra stimuli of exercise and the short-term voluntary efforts of pulmonary function testing, a diver must retain the ability to respond to increased levels of inspired CO_2 due to possible CO_2 accumulation in closed habitats. When ventilation ceases to increase further in response to elevation of inspired (habitat) CO_2 level, only passive buffering by body fluids remains to slow the inevitable increase of CO_2 tension in body tissues. Thus it would appear inadvisable to permit P_{ACO_2} to exceed approximately 50 mm Hg in man when at rest during chronic exposure to normoxic mixtures of crude neon with a density of 25 g/L. This limiting density obtained from the crude neon data corresponds to depth limits of approximately 1100 fsw for normoxic crude neon, 4500 fsw for normoxic helium and 600 fsw for normoxic nitrogen. These limits assume there is

no viscosity effect during quiet breathing. If the viscosity differences of helium and nitrogen as compared to neon are taken into account, the corresponding limits become approximately 2800 fsw for normoxic helium and 360 fsw for normoxic nitrogen.

ACKNOWLEDGMENTS

The work reported here was performed at the Institute for Environmental Medicine, University of Pennsylvania, as a component of its Predictive Studies III Program. The authors are indebted to Ms. L. Giordano and Mr. J. Rosowski for their intelligent assistance during the experiments in data acquisition and recording, and subsequently in data processing. This study was supported by contract HL 08899-08 from the National Institutes of Health, and contract N00014-67-A-0216-0026 with the Office of Naval Research.

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THE EFFECTS OF NITROUS OXIDE NARCOSIS ON THE PHYSIOLOGIC AND PSYCHOLOGIC PERFORMANCE OF MAN AT REST AND DURING EXERCISE

M. E. Bradley and J. G. Dickson, Jr.

Most previous studies of the effects of inert gas narcosis have examined subjects who were at rest. This study investigates the effects of narcosis during exercise.

Working divers who are breathing compressed air at depth hypoventilate and retain CO_2 (20). Because nitrogen narcosis is potentiated by CO_2 retention, nitrogen narcosis was simulated by the use of low concentrations of N_2O at sea level. Nitrous oxide has been shown to have a narcotic potency and biological properties which are similar to those of xenon (13), which in turn is thought to act in the same manner as nitrogen at high pressure.

Methods

The physiologic and psychomotor performance of six male athletes were studied. Each spent 20–30 hours of training with the experimental conditions and testing procedures in order to achieve a stable level of psychomotor performance. The subjects were studied at rest and while exercising at 400 kg-m/min and 800 kg-m/min. The gas mixtures breathed in each of these activity states were air, 15% N_2O, and 30% N_2O. These concentrations of N_2O were selected on the basis of subjective analysis by several members of the laboratory staff who had breathed compressed air at ambient pressures up to 10 ata. Oxygen concentration of the N_2O mixtures was fixed at 21%. The balance of the gas mixtures was nitrogen.

Subjects exercised in the supine position by pedaling a bicycle ergometer at a constant frequency in time with a metronome. A harness stabilized the subject's position and left his hands free to operate the psychomotor test switches. Resting conditions breathing air and all exercise states were of 15 minutes' duration. Resting conditions for N_2O breathing lasted 25 minutes to insure adequate uptake of N_2O. All measurements reported were obtained during the final 5 minutes of these steady-state periods.

Gas mixtures were breathed through a low resistance, exercise breathing valve. Volume of expired gas was measured by a rotary, wet-test gas meter. At ventilations of 70 L/min, inspiratory resistance was 1.8 cm H_2O and during expiration, 2.3 cm H_2O. Mixed expired O_2, CO_2 and N_2O were continuously sampled from the gas meter. Oxygen concentration

was measured with a paramagnetic oxygen analyzer. The CO_2 content of the expired gas was determined with an infrared CO_2 analyzer. Spectral overlap by N_2O was avoided by keeping the head of the analyzer filled with 100% N_2O (7). The collision broadening effect of N_2O was avoided by using CO_2 calibration gases which contained either 15% or 30% N_2O together with oxygen and nitrogen. Nitrous oxide was measured with an infrared N_2O analyzer, the head of which was filled with 100% CO_2. This instrument was calibrated for N_2O with gas mixtures of which the background gases were composed of nitrogen, oxygen and low concentrations of carbon dioxide. Heart rate and rectal temperature were also continuously monitored and recorded.

Visual four-choice and simple reaction time tests were used to evaluate psychomotor performance. Red, green and yellow light signals were used as stimuli. This testing method was designed to duplicate as closely as possible the technique used previously by Frankenhaeuser et al. (15). The subject responded to the choice reaction test by depressing either the left-hand or the right-hand microswitches which were mounted on the handlebars of the bicycle ergometer. In the simple reaction time test the subject depressed only one switch. The reaction time for each light stimulus was measured and recorded on a digital printer. The number of correct responses to the choice reaction test was counted and recorded on an electromechanical counter.

Results and Discussion

Those changes which are statistically significant ($P < 0.05$) are noted in the tables.

Metabolism (Table I)

During both rest and exercise, oxygen consumption was less when N_2O was breathed than when air was breathed (Fig. 1). However, a concomitant decrease in CO_2 production was not observed.

The decreases in O_2 consumption in this study were small and fell within the range of experimental error for the method used. They are, however, quite consistent and a systematic error seems unlikely. This finding is at variance with that of Webber (26) who noted a trend of increased O_2 consumption and CO_2 production in subjects who were exercising and breathing 30% nitrous oxide. His changes were not consistent, and it is likely that Webber obtained his measurements during a time period when his subjects were not at a steady-state level.

The O_2 consumption of resting, narcotized subjects may reasonably be assumed to be diminished. They are semisomnolent and certainly have decreased tissue O_2 needs. It is difficult to explain, however, why the O_2 utilization of subjects who are working and breathing N_2O would be less than when air is breathed. There is no evidence that N_2O modifies normal pathways of aerobic metabolism or serves as a metabolic oxidant. Few studies (10,21,23) have examined the effects of nitrogen narcosis upon metabolism and their results are conflicting.

TABLE I

MEAN O_2 CONSUMPTION, CO_2 PRODUCTION, RESPIRATORY EXCHANGE QUOTIENT, HEART RATE, AND RECTAL TEMPERATURE OF SIX SUBJECTS BREATHING AIR, 15% AND 30% N_2O WHILE AT REST AND WHILE PERFORMING MODERATE AND HEAVY WORK

Mean Values	Rest			400 kg-m/min			800 kg-m/min		
	Air	15% N_2O	30% N_2O	Air	15% N_2O	30% N_2O	Air	15% N_2O	30% N_2O
O_2 consumption (L/min)	0.281	0.271	0.251	1.280	1.221[a]	1.216[a]	2.208	2.207	2.172
CO_2 production (L/min)	0.223	0.216	0.216	1.114	1.119	1.120	2.072	2.094	2.059
Respiratory exchange quotient	0.79	0.80	0.86	0.87	0.92	0.92	0.94	0.95	0.95
Heart rate (beats/min)	58.2	53.0	54.8	106.5	105.4	102.9	141.5	137.8	136.2
Rectal temperature (°C)	+0.02	+0.04	+0.01	+0.06	+0.09	+0.05	+0.16	+0.14	+0.14

[a]Significant ($P < 0.05$) effect of N_2O.

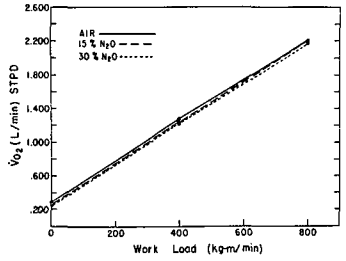

FIG. 1. Oxygen uptake of six subjects at rest and exercising at 400 and 800 kg-m/min while breathing air, 15% and 30% N_2O.

HEART RATE AND TEMPERATURE (TABLE I)

Nitrous oxide did not appear to have any effect on deep body temperature. However, during both rest and exercise, heart rates were generally lower when the subjects breathed N_2O rather than air (Fig. 2). Morris et al. (22) have reported bradycardia in patients anesthetized with xenon. Bradycardia occurs in both resting and exercising subjects who are breathing helium-oxygen (8) and nitrogen-oxygen (19) mixtures at high pressures. Concurrent high inspired O_2 levels and increased hydrostatic pressure have been implicated in this phenomenon. Flynn (14) has studied divers who were exercising at ambient pressures of 4-6 atmospheres. He found that heart rates became lower when the breathing mixture was switched to nitrogen-oxygen from helium-oxygen. This, together with the finding here, suggests that narcosis per se may be partially responsible for the bradycardia observed in men breathing nitrogen-oxygen mixtures at depth.

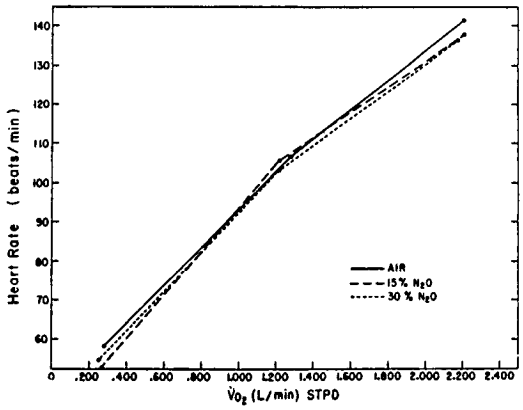

FIG. 2. Heart rate of six subjects in relation to O_2 uptake while breathing air, 15% and 30% N_2O.

VENTILATION (TABLE II)

Both resting and exercise minute volumes were increased when N_2O was breathed (Fig. 3), and calculated alveolar CO_2 tensions tended to be less during N_2O narcosis (Fig. 4). An analysis of variance showed that the combination of heavy work and inhalation of 30% N_2O significantly ($P < 0.05$) interacted to cause a greater hyperpnea and hypocapnia than would be expected to result from a simple summation of the effects of exercise and 30% N_2O.

Eckenhoff and Helrich (12) have reported that untrained, resting subjects hyperventilate and become hypocapnic while breathing a 50% nitrous oxide-50% oxygen mixture. These investigators also noted a slight increase in the respiratory sensitivity to CO_2 during N_2O inhalation. Webber (26) studied the pulmonary function of untrained subjects who were breathing a 30% N_2O mixture, the density of which was adjusted to that of air. The respiratory minute volume ($\dot{V}_E$) of his subjects exercising at 600 kg-m/min and breathing this gas mixture was 17% greater than when air was breathed. At 1200 kg-m/min $\dot{V}_E$ was increased 24%. Concurrently, he noted small decreases in calculated alveolar CO_2 tension ($P_{A_{CO_2}}$).

The assessment of changes in alveolar ventilation and $P_{A_{CO_2}}$ in this study depends largely upon the values assumed for respiratory dead space volume (3). Accepting these approximations, the calculated values for $\dot{V}_A$ increased with N_2O during exercise. The assumption of somewhat larger values would have completely abolished this increase. For this reason the findings here do not warrant the conclusion that N_2O inhalation produced significant alveolar hyperventilation. However, using reasonable values for dead space, these findings do indicate that N_2O narcosis does not depress ventilation.

The most prominent effect of N_2O upon respiration was tachypnea. When N_2O was inhaled, respiratory frequency was consistently increased (Fig. 5) and the magnitude of tidal volume diminished (Fig. 6). Webber (26) also observed this phenomenon. Almost all inhalational anesthetics increase respiratory rate, often to a striking extent (24). Several mechanisms have been proposed to explain this. The most likely explanations include sensitization of pulmonary stretch receptors and alterations in the mechanical properties of the respiratory

TABLE II

MEAN $\dot{V}_E$, RESPIRATORY FREQUENCY, V_T AND P_{ACO_2} TENSIONS OF SIX SUBJECTS BREATHING AIR, 15% AND 30% N_2O WHILE AT REST AND WHILE PERFORMING MODERATE AND HEAVY WORK

Mean Values	Rest			400 kg-m/min			800 kg-m/min		
	Air	15% N_2O	30% N_2O	Air	15% N_2O	30% N_2O	Air	15% N_2O	30% N_2O
$\dot{V}_E$ (L/min) BTPS	6.79	6.81	7.02	30.08	30.28	31.64	55.39	57.33[a]	59.45[a,b]
Respiratory frequency (#/min)	10.8	12.5	13.8[a]	25.5	26.4	30.0[a]	33.6	35.6	39.1[a]
V_T (liters) BTPS	0.662	0.557	0.511[a]	1.226	1.212	1.072[a]	1.763	1.701	1.572[a]
P_{ACO_2} (mmHg)	39.4	39.8	39.3	39.2	39.3	38.0	39.1	38.1	35.4[a,b]

[a]Significant ($P < 0.05$) effect of N_2O.
[b]Significant ($P < 0.05$) interaction between N_2O and work.

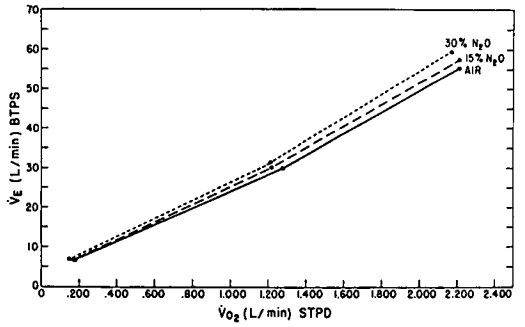

FIG. 3. Pulmonary minute ventilation of six subjects in relation to O_2 consumption while breathing air, 15% and 30% N_2O.

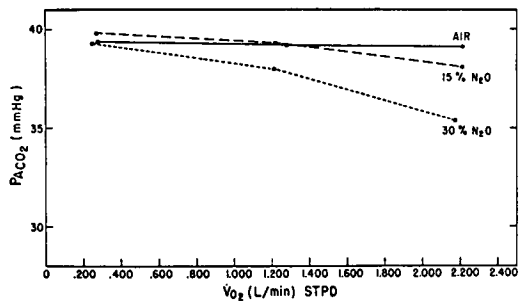

FIG. 4. Alveolar CO_2 tensions of six subjects at rest and during exercise in relation to O_2 uptake while breathing air, 15% and 30% N_2O.

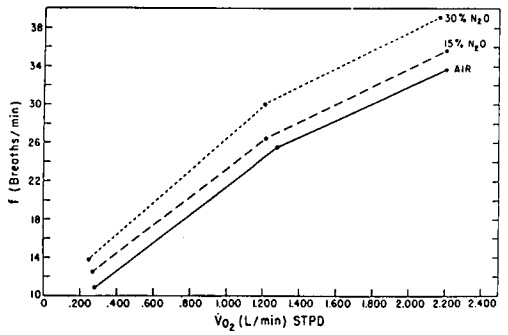

FIG. 5. Respiratory frequency in relation to O_2 uptake while breathing air, 15% and 30% N_2O.

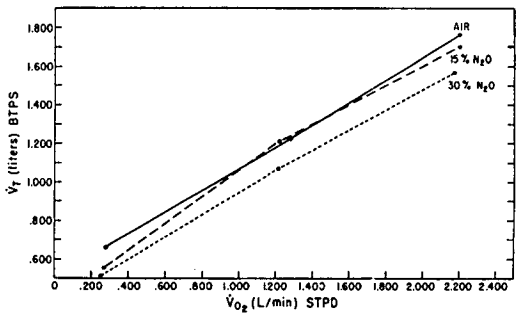

FIG. 6. Tidal volume in relation to O_2 uptake at rest and during exercise while breathing air, 15% and 30% N_2O.

system. Whitteridge and Bulbring (27) found increased discharge frequencies of inflation and deflation receptors in cats inhaling anesthetic gases, including N_2O. These investigators suggested that N_2O stimulates inflation and deflation receptors. This sensitization shortens the inspiratory and expiratory half-times, thereby decreasing tidal volume and increasing respiratory rate.

Decreases in compliance have been reported in subjects breathing N_2O (11). Under experimental conditions simulating a decrease in compliance, subjects respond by modifying their breathing pattern to one of increased rate and smaller tidal volumes (16).

The effects of N_2O on respiration are quite dissimilar from the changes observed in men breathing nitrogen-oxygen mixtures at high pressures. At depth, exercising men who are breathing air often exhibit marked degrees of hypoventilation and CO_2 retention (20). The breathing pattern of these individuals is one of lowered frequency and larger tidal volumes (19,21). Both the hypoventilation and change in breathing pattern are thought to result primarily from the increased density of gas and consequent increase in gas flow resistance. Findings in this study do not support the hypothesis that CO_2 retention in divers is a result of a depressant effect of inert gas narcosis upon respiration.

Subjective Effects

The subjective responses to N_2O narcosis were most prominent when the 30% N_2O mixture was breathed. Some subjects were profoundly affected, others minimally so. The more mature and stoical subjects reported fewer symptoms and appeared less affected. All subjects reported mild to severe paresthesias. Other symptoms noted were lightheadedness, a sensation of being drunk, sleepiness and impairment of concentration. Auditory effects, usually an increased sensitivity to low tones and a sense of being cut off from the environment, were also experienced.

The subjective effects of N_2O narcosis were greatest when the subjects rested with their eyes closed. All of the subjects reported that exercise seemed to lessen the degree of narcosis. They also stated that N_2O inhalation seemed to make the heavy work less fatiguing and easier to perform which suggests that N_2O was providing some analgesia. With repetitive exposures to N_2O, the subjects reported fewer subjective effects and a lesser degree of intoxication.

The sensations of breathing a 30% N_2O mixture seem to be quite similar to the sensations which divers experience while breathing air at 7–10 atmospheres absolute. Wide variation in the susceptibility of divers to nitrogen narcosis has often been noted (6). The fact that the more emotionally stable diver experiences a lesser level of intoxication led some early investigators to conclude that nitrogen narcosis had a psychological basis. Sensations of drunkenness, lightheadedness, paresthesias and impairment of coordination have been reported in divers who are narcotized by breathing air at high pressures (1,4–6).

Increased sensitivity in hearing certain sounds has been noted by divers at depths of 250 feet and greater (1). Case and Haldane (9) reported development of some degree of tolerance to the effects of nitrogen narcosis. Their findings, as well as those here, lend some credence to the common wisdom of the diving community which asserts that tolerance can be developed to N_2 narcosis as well as to other substances—which are able to induce narcosis—such as ethanol.

Psychomotor Performance (Table III)

As the inspired N_2O concentration was increased, the subjects' ability to perform the visual reaction tests deteriorated. Accuracy in the choice reaction test was minimally affected by breathing N_2O except while exercising and breathing 30% N_2O. As the N_2O dose was increased, both choice (Fig. 7) and simple reaction times (Fig. 8) became progressively slower.

Exercise, per se, speeded up reaction time, regardless of the gas being breathed. Choice reaction times were faster during moderate work than during rest, and faster during heavy work than during moderate work. This phenomenon was not as prominent with the simple reaction test, occuring only when 30% N_2O was breathed.

The degree of deterioration in the psychomotor performance of resting subjects breathing 15% N_2O is similar to that observed in subjects breathing air at 5.0 (15) and 5.5 (25) ata. Since reaction time testing has not been done in exercising subjects or at depths greater than 150 feet, further quantitative comparisons with these results cannot be made. In contrast, Adolfson (1) and Albano et al. (2) have reported that the psychological performance of subjects breathing air at 8–10 ata deteriorates when they exercise. This deterioration is

TABLE III

MEAN CHOICE (CRT) AND SIMPLE REACTION TIMES (SRT) AND PERCENTS OF PERFORMANCE ACCURACY OF SIX SUBJECTS BREATHING AIR, 15% AND 30% N_2O WHILE AT REST AND WHILE PERFORMING MODERATE AND HEAVY WORK

Mean Values	Rest			400 kg-m/min			800 kg-m/min		
	Air	15% N_2O	30% N_2O	Air	15% N_2O	30% N_2O	Air	15% N_2O	30% N_2O
CRT (sec)	0.41	0.44	0.50[a]	0.39	0.43[a]	0.44[a,b]	0.37[b]	0.41[a]	0.43[a,b]
CRT (% Correct)	92.0	90.4	90.6	91.9	91.1	86.5[a]	92.3	90.2	86.5[a]
SRT (sec)	0.25	0.26	0.32[a]	0.26	0.29	0.30	0.26	0.28	0.31

[a]Significant ($P < 0.05$) effect of N_2O.
[b]Significant ($P < 0.05$) effect of work.

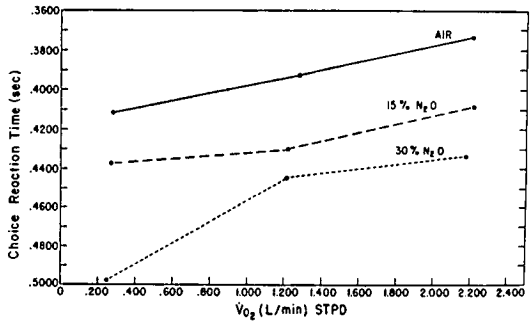

FIG. 7. Choice reaction time in relation to O_2 consumption while breathing air, 15% and 30% N_2O.

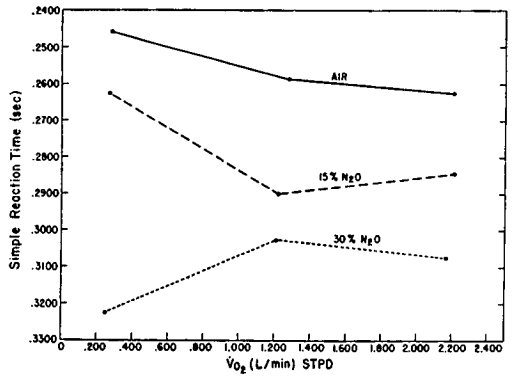

FIG. 8. Simple reaction time in relation to O_2 consumption while breathing air, 15% and 30% N_2O.

thought to be the result of the adverse effect which hypoventilation and hypercapnia have upon nitrogen narcosis (4,6,9).

That exercise improved reaction test speeds of narcotized subjects substantiates the subjects' reports of a lesser level of intoxication when they were exercising. Stimulation of the reticular activating system (RAS) may have been involved in this phenomenon. RAS activity is influenced by certain chemical and hormonal changes (such as CO_2, adrenalin and drugs) (17). The reticular formation is especially sensitive to the depressant effects of anesthetic agents (17) and increased pressures of the inert gases (6). During exercise there is increased feedback to the RAS from sensory and motor units. This feedback may stimulate the reticular formation and partially overcome the depressant effects of N_2O narcosis, thus producing arousal in the organism. An increased arousal may account for both the subjective sensation of a lesser level of intoxication and the faster reaction times of the subjects during exercise. An analogous phenomenon is seen in sports where the athlete who "warms up" has a heightened level of perception and more rapid neuromuscular response.

Summary

The respiratory effects of N_2O inhalation include tachypnea, a slight increase in minute ventilation and decreases in tidal volume and alveolar CO_2 tension. These changes are more prominent during exercise. Sensitization of pulmonary stretch receptors or changes in the mechanical properties of the lungs are thought to cause the tachypnea. Findings in this study suggest that inert gas narcosis per se does not depress respiration and contribute to the problem of CO_2 retention in divers. Nitrous oxide inhalation produced a bradycardia and slight decreases in O_2 consumption in both resting and exercising subjects.

There were profound subjective sensations, and slower and less accurate psychomotor performance when subjects breathed N_2O. These changes are qualitatively similar to those that occur with nitrogen narcosis. Exercise lessened the subjective level of narcotic intoxication and reaction times became faster. Stimulation and arousal of the reticular activating system is postulated to cause this phenomenon.

ACKNOWLEDGMENTS

The work reported here was performed at the Institute for Environmental Medicine, University of Pennsylvania. The authors acknowledge the aid of the Institute staff. Research support was derived from Office of Naval Research contract N00014-67-A-0216-0026 and National Institutes of Health contract HL 08899-08.

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PART VIII. INERT GASES AND HYDROGEN*

DISCUSSION

H. R. Schreiner, Chairman

Dr. Schreiner: I would like to turn the discussion to the uptake of oxygen in the presence of other gases.

Dr. Saltzman: There have been several studies of arterial oxygenation performed at pressures equivalent to depths to 1000 feet, at rest and during exercise. They all failed to demonstrate hypoxia in arterial blood as a result of pressure. However, most of these studies occurred under circumstances in which narcosis was not present, since helium was the breathing gas. In another study to a pressure-depth of 250 feet, steady state measurements were made of gas exchange between the environment and arterial blood, including breathing normoxic nitrogen at 200 feet. These studies also failed to demonstrate hypoxia. Indeed there was no significant change in exchange between the surface and the most dense gas breathed. They also showed only a small increase of P_{CO_2}. At the same time a study of psychomotor performance revealed quantitative evidence of narcosis.

These studies rule out either arteriohypoxemia or respiratory gas transport impairment as a phenomenon associated with inert gas narcosis. What they do not bear on, however, is whether the presence of inert gases or solubility changes, such as elucidated in this very paper, do ultimately affect the prevailing levels of oxygen or carbon dioxide within the cell. And I think the occurrence of membrane impediment or solubility changes leading to altered oxygen and carbon dioxide levels intracellularly clearly needs further exploration.

Dr. Lambertsen: Narcosis, oxygenation and gas exchange at high pressures must be considered in relation to the physiological state of the individual. One such circumstance is variously described as work or exercise, another is natural existence at rest. A third one, not usually mentioned, is sleep, which is itself a depressant circumstance. I would like to have some attention and discussion concerning the relative degrees and importance of the respiratory impairment which theoretically should become limiting factors at extreme pressures, during these three different forms of existence.

Mr. Gelfand: It is well known that during sleep there is a decrease in ventilation, a rise in arterial P_{CO_2} and a decreased CO_2 reactivity. This is one of the reasons why it is so important to find out whether or not inert gas narcosis is a component in the depression of respiratory responsiveness while breathing inert gases at high pressures. It is conceivable if such were the case that, in some nearly limiting situation, the respiratory reactivity would be so low when not asleep that when you did go to sleep you might more or less go over the hill in the sense that a decreasing arterial Po_2 and an increasing CO_2 tension would not be checked by a compensatory respiratory drive. Conceptually there could evolve a situation of progressive hypercapnia, hypoxia and, if not awakened, I suppose even death.

Dr. Bradley: I would like to extend Mr. Gelfand's statement just a little bit, not only to the respiratory system but also to all of the organ systems. Certainly narcosis should have a profound effect on the cardiovascular system when you are decompressing a man, and decompression tables then simply may not apply.

Dr. Gottlieb: Dr. Halsey, you seemed to say that the partial pressure of hydrogen did not reverse the partial pressure of nitrogen and of nitrous oxide anesthesia; that is, the ED_{50} of the nitrous oxide decreased with increased

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**Panelists:* T. K. Akers, P. B. Bennett, M. E. Bradley, R. Gelfand, D. W. Kent, T. D. Langley, J. Walkley

partial pressure of hydrogen. This seems to be the reyerse of the helium results. What possible explanation do you have for that?

Dr. Kent: We pointed out in the discussion that there is an apparent discrepancy between the pressure antagonism of narcotic effect with helium, no apparent effect with neon, and the opposite effect with hydrogen. This deviates from the concept of a membrane or a receptor site changing in volume that was described several years ago by Dr. Smith and Keith Miller from Oxford. Such volume expansion with narcotic interaction at the membrane site or model site was actually shown by Roth and Seeman with the red cell membrane.

The important part about this (and similar things which have been done and suggestions which have been made by other workers) is that there are probably two effects going on. There is the antagonism of pure hydrostatic pressure by anesthesia. Brauer demonstrated that animals could survive to higher pressures without eliciting HPNS by the addition of various narcotics.

The opposite effect is that of reversing the narcotizing effect by putting on pressure; both of these effects will be occurring at the same time, perhaps at different sites. The point is that hydrogen is a stronger narcotic at the same pressure than is neon. We hypothesize that neon is a stronger narcotic than helium. Therefore the pressure effect, being a physical effect, is going to remain independent of whatever agent you apply it with. You have more narcotic if you have nitrous oxide plus hydrogen. You have less narcotic in the form of neon and you have even less narcotic in the form of helium, to which you are adding nitrous oxide.

Perhaps Dr. Smith, who was one of the originators of this concept of looking at membrane expansion associated with the anesthetic effect, might like to make a comment about that.

Dr. Smith: I think Dr. Kent has explained very clearly that hydrogen, being more soluble, has a bigger expansion effect and therefore the effect of the pressure reversal is less.

I want to comment on Dr. Bennett's review. He suggested that in the increase in ion permeation, the probable mechanism is narcosis. I would like to say that the change in permeability of membranes is really rather low, independent of anesthetics, and that active transport is very resistant to narcosis. The resting potential of a nerve cell is very resistant to anesthesia. Therefore the idea that an increase in ion permeability is an obvious mechanism of narcosis or anesthesia is not quite correct. Certainly there is a lot of evidence that suggests we would have to look for other mechanisms.

Even in the actual transmission of an action potential the sodium flux may be inhibited by anesthetics rather than potentiated.

Dr. Bennett: My idea was only a working hypothesis, a model to be further tested. I recognize Dr. Smith's work in this area and know that he has not found the same amount of electrolyte perturbation, certainly not sufficient to say that this is the mechanism of narcosis. On the other hand, in the in vivo situation, if you measure electrolytes in cerebrospinal fluid, in urine or in blood, you will find a decrease in the sodium levels.

The hypothesis is that the sodium has an increased flux into the cell. It may be that the pump fails. It may be all sorts of things. But the sodium does not come out, and if you stabilize the membrane and prevent the electrolyte perturbation, then you do not get any electrophysiological signs of narcosis. If you use lithium, the lithium is substituted for the sodium, and you are able to stop the narcosis or at least the electrophysiological signs.

There may be many other ways of interaction on the membrane between the molecules of inert gas. This is just one aspect of the narcosis mechanism.

Dr. Schreiner: Dr. Bennett, would you comment on the statement you made in your review that the French could not verify hydrogen protection against convulsions? On the other hand, how would you reconcile this with the Eger group finding that the effects of hydrogen and nitrous oxide are in fact additive?

Dr. Bennett: Certainly the French work did not substantiate the effect of hydrogen in preventing HPNS. On the other hand, we have heard during the course of this week several papers which have quite definitely, from my own point of view, convinced me that they do. It is still a controversial area. How much evidence do we need to convince ourselves that there is a positive action?

Dr. Kinney: As a collaborator of Dr. Langley and the University of Pennsylvania group during its Predictive Studies Program, I measured visual-evoked responses at the same time on the same subjects when he measured some somatosensory and auditory-evoked responses. I did not find any evidence of hyperexcitability with the visual method, with any of the gases used at 1200 feet of sea water. There was virtually no change, and no difference between helium and neon. I did find the usual depression with nitrogen at pressures to 400 feet of sea water.

I cannot explain the slight discrepancies between the auditory and visual-evoked response studies. However, one can think of several ad hoc explanations. One of them is that perhaps the different senses are behaving differently under pressure to different gas mixtures. I think this most assuredly deserves more work in the future; I would like to ask individuals not to give up on the auditory-evoked response, which has problems with precise

stimulation, but rather to work out these problems and hope that we can resolve this issue in the near future.

As a visual scientist normally concerned with the surface rather than diving, I must emphasize that the use of the N_1P_2 index stems from auditory-evoked response work. Since the auditory-evoked response is a rather simple response measure, it is perfectly adequate there. However, for those who might be interested in working with visual-evoked response, it is not at all a good idea to force the visual-evoked response into this mold. The visual-evoked response is a much more complex phenomenon. Visual scientists cannot actually agree on the number of components, but there are five, seven, perhaps ten. I do commend Dr. Langley on measuring all the different components in the somatosensory-evoked response and I think this is what has to be done in vision, even on an individual basis. One must find the components for an individual and measure them pre- and postdive.

Dr. Farmer: I would like to reiterate what Dr. Langley has pointed out about the difficulties of doing auditory-evoked responses at depth. There are two mechanisms which can lead you astray. One is the reversible conductive hearing loss that does seem to be present at depth, both in air and in helium-oxygen atmospheres. Second is the change in the sensitivity of electro-acoustic transducers.

We have correction factors for these transducers up to 1000 feet of helium and air, and these are published. I would like to suggest that we all put this together. I think the auditory-evoked system should be used, but let us make sure we are putting in the same stimulus strength at all times.

Dr. Langley: Dr. Bennett has referred to data that were collected in our laboratory at Ocean Systems, Inc., using the auditory-evoked responses. In this particular series at the University of Pennsylvania our intent was to compare the effects of helium, neon and nitrogen. We wanted to bracket neon somewhere between helium, being a nonnarcotic gas, and nitrogen, being a narcotic gas. In doing this it was our intention at that time to use the auditory responses as an index of a brain function to compare to 1 atmosphere. Because of problems associated with changing depths the intent in that series of experiments eventually changed to focus upon particular depths—for example 400 feet of sea water—and use the auditory response to compare helium, neon and nitrogen at the fixed depth.

For this reason, in the data that Dr. Bennett referred to, I would say that we now use the auditory responses simply at a given pressure to compare gases, realizing that there may still be slight differences due to the different gas densities. But the major difference of pressure is controlled if you look at only one particular depth with different gases.

For reasons of difficulty in controlling auditory stimulus, in the experiments in Dr. Lambertsen's laboratory we wanted to use both auditory and somatic stimuli. The auditory stimulus was used as a link to previous work that has been done in diving with evoked brain responses, but we wanted to use the somatic stimulus as a method which is independent of pressure.

The whole basis of the technique of evoked brain responses is that you have to assume a constant stimulus. Then any changes you see are attributable to what is going on in the brain. If you do not know that your stimulus is constant, you do not know how much of the change that you see is due to something in the periphery or something in the brain.

Some of the techniques that Dr. Farmer is using to do calibrations are very germane to the topic. He and his colleagues have adopted one of the indirect methods of microphone calibration called the electrostatic actuator method. Ross Sargent, on the other hand, at New London has adopted the method of reciprocity.

Both of these are indirect methods of microphone calibration. The best method in my opinion, if you wanted to pursue with auditory-evoked responses under pressure, would be to use the subject as his own control. This has not yet been done. If it were done, you could record compound action potentials of the cochlear nerve by putting an electrode on the ear lobe and using a signal averager. In that way at 1 atmosphere it has been shown by a number of investigators that as you change stimulus intensity through the earphone, the amplitude of the compound action potential decreases, so it seems to be a way of using the person as a direct measure of hearing loss.

Dr. Farmer: The evoked response technique at 1 ata among most otologic centers is still very controversial. If you do it by conventional psycho-acoustic techniques you are going to have to accept an error of plus or minus 5 to 10 dB which is inherent in the technique itself. I hope that we can go to a more direct system.

Dr. Langley: Dr. Kinney mentioned the N_1P_2 index. These are late components, long latency components beginning at around 100 milliseconds. We also recorded the very short latency components, which are more analogous to the short latency components in the visual system, but we have not analyzed these yet. But we want to analyze the whole spectrum—the short latency as well as the long latency.

Dr. Bennett: Dr. Kinney said there is disillusionment in the use of evoked potentials as a method of quantifying narcosis. I think before we even think of improving techniques we have to come to some understanding

as to why both argon and nitrogen have exactly the same effect.

Dr. Langley: My explanation for that is the use of the N_1P_2 component. The N_1P_2 component is a long-latency, nonspecific response, and it is my opinion that looking either at the shorter latency responses or looking at processes such as recovery cycles, which we did at the University of Pennsylvania, might tend to be more sensitive indicators of brain function and give us better resolution so that we can distinguish between different levels of narcosis. N_1P_2 is a crude measure of brain function.

Dr. Lambertsen: I want to remind you that there was, in view of the interest in evoked responses at high pressure, a planned effort to get Dr. Kinney with visual-evoked potentials and Dr. Langley with somatic- and auditory-evoked potentials, to make measurements on the same subjects at essentially the same time on the same gases in a great variety of different circumstances; this was done so they could get together and see what the differences were without the variations of subjects, gas, time, fatigue or any of the other kinds of troubles that one normally gets into. Through analysis of these concurrent observations it should be possible to resolve a number of the questions raised here.

Dr. Raymond: Mr. Gelfand, in your very clear schematic of your experimental setup you showed an esophageal pressure transducer, I think in order to make the important distinction between alterations and central sensitivity and changes in ventilatory response due to the mechanical properties of the airway contents, the airway composition. You did not present results on what happened to the esophageal pressure and I wonder if you could help us draw that important distinction.

Mr. Gelfand: Actually we measured esophageal pressure and flow velocity to have a look at the work of breathing under these various circumstances and the results from that have not been analyzed completely enough to present here.

Dr. Saltzman: My colleagues and I have reported experiments in which normoxic gases were breathed at several different densities. It was possible to show no effect of these different gases of different densities upon either oxygenation of arterial blood or upon the arterial P_{CO_2}. But there was a very clear correlation between hydrostatic pressure itself and arterial P_{CO_2}, which rose between 5 and 6 mm Hg from surface measurements breathing helium to a simulated depth of 250 feet breathing normoxic helium. It seems to me that Mr. Gelfand presented data which had a greater opportunity to dissociate density and barometric pressure. Would you comment upon how hydrostatic pressure itself may be related to respiratory gas exchange?

Mr. Gelfand: We have found no such relationship, even to the extreme pressures studied.

Dr. Hamilton: I would like to add something to Dr. Bradley's work. Along with the other gases explored in the University of Pennsylvania Predictive Studies, nitrous oxide was used for both the evoked response recording and performance testing. On the basis of the comparison of N_2O and N_2 a factor of about 1 to 40 was found for the ratio of narcotic potency of nitrogen and nitrous oxide—that is, nitrous oxide is about 40 times more potent than nitrogen. This would indicate that about 20% nitrous oxide is the equivalent of 300 feet of air—or about 8 atmospheres of nitrogen. It agrees fairly well with observed subjective responses.

Part IX. PERCEPTION, PERFORMANCE, COMMUNICATION

PERFORMANCE, PERCEPTION AND COMMUNICATION UNDERWATER

J. A. Adolfson

The role of the psychologist in the field of diver research, especially in performance, perception and communication will be briefly reviewed here.

Although there are abundant resources within the sea which can be used for man's benefit, their development requires that man be able to live and work productively for extended periods of time beneath its surface.

The psychological aspects of diving research are focused toward the understanding and measurement of impairment of diver performance resulting from ambient environment exposure. The psychologist is always dealing with an end-product. A stream of stimuli from the environment reaches the organism, something happens in the organism, and the organism reacts in a special way. The output of central nervous system processes—the end-product—appears as a more or less characteristic behavioral pattern which depends not only on inherited and acquired psychological and physiological qualities and characteristics but also on the sort of stimuli and the environment itself. If the stimuli or the environment or both are changed, then the behavior will generally also be changed; this changed behavior is registered and measured by the psychologist.

Psychomotor tests are frequently used to study the effects of procedural, environmental or psychological variables on human performance. This is also true for basic information concerning man's ability to function in a hostile undersea environment. As man's role in the sea continues to expand, the need for more basic information increases, primarily to make the diver as effective as possible.

Environmental Factors Related to Performance Ability

A preliminary theory concerning the effects of task and environmental factors on human performance which can well be applied to diver performance has recently been presented by Teichner and Olson (12). Because the most frequent environmental data obtained have been concerned with physiological effects rather than effects on performance, it was found necessary to develop relationships between performance and physiological data and then to use these to predict the effects of the environment on performance for conditions where physiological, but not performance, data are available.

Some physiological effects are common to all environments, and Teichner and Olson suggest that the first step in analyzing the effects on performance of any environment must be an analysis of the compensatory physiological responses induced by the environment and of the sensory phenomena which accompany the responses. The state of the receptor with regard to its adaptation to the environmental energies must be given careful attention, and the individual's experience with the environment must be evaluated, since the activating effects will vary depending upon how accustomed the person is to it and on what his previous experience has been with it.

Teichner and Olson indicated that even though not all environments have the same critical physiological effects, they may have similar activation effects. These effects might be correlated with changes in heart rate, oxygen consumption, arterial oxygen saturation and blood flow. If this is true, then these are variables to which performance and with which central nervous system variables should probably be related.

A common experience among behavioral scientists who deal with divers at depth is the difficulty in separating psychological from physiological factors when measuring performance underwater. Such variables as heart frequency and respiration, for example, can very well increase remarkably during periods of what may be called "stress," while the performance can be rather unaffected in experienced subjects and highly affected in inexperienced subjects with equal physiological responses [Selye (11), Levi (9)]. Another difficulty is the measurement of cognitive performance (such as decision making, information retrieval and information processing) which is very often affected earlier than the physiological changes indicate. Although there are quite special stress-inducing factors underwater—such as the biological life under the surface, increased ambient pressures, special breathing gases—the stress-inducing factors in general do not differ from those in all other dangerous and troublesome situations. However, one can expect that many stress factors in normal environments are magnified underwater.

General Areas of Diver Activity

A diver must be an all-around worker: he is expected to act as an unskilled laborer as well as a skilled mechanician, a draftsman and a construction builder. Sometimes he has to perform as an artist while making underwater drawings of the damage on sunken ships or of the sea bottom, and he is always expected to be watchful and observant in order to report correctly and circumstantially to the staff on the surface. As technological advances extend the operating duration and depth of tethered or free-swimming divers, the number and complexity of tasks to be performed underwater increase rapidly. Future skill requirements are expected to include operation and maintenance of sophisticated electronic equipment, advanced life support systems and autonomous underwater power sources. When one adds to these considerations the diverse demands of normal living within a habitat, which act across the spectrum of social and psychological parameters, and the dynamic control skills required in the use of underwater vehicles and tools, it becomes clear that there is very little in the way of performance which will not be required of future aquanauts. Commercial diving is tough work, and the diver must be economically effective.

The diver is clearly one element of a system which at any point in time has well-defined objectives. The selection and design of underwater work systems is dependent on the ability of the designer to quantitatively estimate the performance of the proposed systems. Such a

system is defined by Reilly and Cameron (10) as a collection of interacting components, the organization of which is designed to achieve particular outputs or end-products in response to specific inputs. Although a system may be composed entirely of hardware, most contain one or more human operators who play vital roles as system components.

It is appropriate, therefore, in studying various aspects of diver performance, to view such activities within the context of a system. Further, a system-analytic approach lends structure and organization to the problem by delineating the various man–machine environment interfaces and loops within the total situation.

Thus, since man is the center of all these work systems or at least plays a vital role as a system component, it is necessary to analyze, empirically evaluate and quantify the capabilities of the human operator to perform applied undersea work tasks not only as a diver but also in his role as the operator of a manipulator-equipped small submersible. The well-known impairment in human performance during the course of a dive has been related to three types of factors: 1) the effect of the environment on the interrelated functions of the human body; 2) the constraints resulting from the equipment and safety requirements; and 3) problems referred to as depth narcosis or inert gas narcosis (8).

Perception Underwater

The undersea environment is dangerous, and as greater depths are reached the hazards will increase at an exponential rate (2). As man enters the undersea world the sensory information he receives is greatly altered. The physicochemical qualities of the environment act on both the signal (the stimulus energy) and the receiver (the sensory-perceptual system). As Albano wrote (1): "The moment the diver goes underwater he is in an environment that is about 60 times more viscous, more than 800 times as dense, and having a thermal conductivity 25 times higher than that to which he is accustomed."

Vision

Vision is normally man's primary source of information concerning his environment. It is possible to detect the presence of objects in the hydrosphere by means of sonic energy, but until the objects are seen, we really are not quite sure what they are.

When light enters a layer of sea water it is gradually weakened in three ways: 1) by absorption by the pure sea water; 2) by scattering by the pure sea water; and 3) by scattering, diffraction and reflection by suspended particles in the water—say, by impurity of sea water.

Briefly, what this means to sight underwater is that visual acuity is exceedingly poor for the unprotected eye. Contact lenses and face masks restore normal refraction to the eye but produce a refraction at their own outer surface. Underwater contact lenses are judged to be of value only for specialized use when a face mask would be disadvantageous.

In addition, the diver's field of view is restricted due to two factors: 1) the rubber portions of the face mask block vision and 2) the refraction of light at the water–air interface tends to totally reflect light beyond some critical angle (about 48.6°).

Third, the range of underwater visibility is greatly reduced, among other factors by the physical properties of the light in the sea and by water turbidity. Objects tend to look near at close distances in clear water, and too far away in murky water and at far distances in

clear water—divers tend to underestimate the distance of the surface more than that of the seabed. Most divers also perceive objects as enlarged underwater.

Fourth, polarized light might probably be of some help in increasing the range of visibility underwater, and predive dark adaptation of the diver might be helpful. However, hardly any investigations have been done in these fields and they are strongly recommended.

Finally, the diver's ability to discriminate colors underwater is mainly dependent upon the type of water itself.

Communication Underwater

Any system that uses man as an integral part of its design will almost necessarily include some form of communications. Several of the sensory modalities of both the sender and the receiver are often intimately involved in this transmission process and therefore can be considered as part of the communication system.

In the design or improvement of a communication system, a complete classification, census and content analysis of all messages, their origins and their intended destinations, should be carried out. Such an analysis should make clear distinctions between necessary and unnecessary communications in content and direction of messages. From this information it should be possible to produce a communication system that is versatile enough to handle all situations and yet not so complex that it exceeds the individual's span of comprehension and immediate memory span. The fewer the messages and the greater the simplicity of content, the easier, more precise and rapid can be the receiver's response.

Visual Communication

The optical transmission of water sets an unavoidable limit on the ranges over which visual methods can be used. Because the optical characteristics of sea water vary so radically, the effectiveness of distant visual communications will be very irregular. There are also many human physiological functions that will limit visual communications underwater.

The number and variety of the messages which can be transmitted without complex and bulky systems, or which require extensive training, are limited. Verplanck more than 20 years ago reported that: "A completely flexible visual communications system that does not utilize the printed word would require such a tremendously complex series of visual symbols, each with its own meaning, that only with extreme difficulty could the sender and receiver learn them" (13). Divers have used printed words to communicate between themselves and with topside personnel for many years. However, this form of communication is limited, awkward and slow and is usually used only as a last resort.

There is, however, one form of visual communications system which is commonly used by divers all over the world, and that is the hand signal. However, like all flexible visual communications systems it requires the attention and constant monitoring on the part of the receiver before he can communicate his information.

Tactile Communications

The skin is sensitive to chemical, thermal, electrical and mechanical energies, and any one of or combination of these energies could be used as a communications stimulus. Several

different approaches have been tried in the past, but the two that seem to hold the greatest promise are electrical and mechanical. Most of the research thus far has been on mechanical stimulation and falls generally into one of two categories: 1) those in which speech energy is applied directly to the skin, and 2) those in which coded energy patterns are applied to the skin.

A tactile communications system which could be used in an operational setting has been developed by Geldard and Howell, based upon the international Morse Code. Diachenko at NMRI has explored the possibility of adapting the present limited set of signals used by a diver to a vibrotactile communications system. By restricting the size of the message sent and using the already familiar diver signals, very little training would be required to establish a usable system of this kind.

Verbal Communication

It is generally accepted that one of the significant advantages which man has over other species in dealing with his environment is the ability to communicate verbally. It therefore seems obvious that providing the diver with the ability to communicate verbally would allow him improved execution of tasks underwater. Not only would a diving team be more efficient in its performance of tasks if provided with speech communication equipment, but also the manner of task performance, in terms of cooperation, would be qualitatively different.

Oral communication in underwater situations is difficult and often unsatisfactory, since speech in the underwater environment can be affected by any one or a combination of distortions. These distortions may result from: 1) electronic equipment; 2) high ambient pressures; 3) exotic gases (helium, hydrogen); 4) reverberations within a chamber or helmet; 5) inadequate talker or listener characteristics; or 6) poor word choice with respect to intelligibility.

When an acoustic signal is transmitted directly into the water for any distance, echoes from the surface, bottom or objects within the water can degrade the signal prior to its reception. The greater the distance, the larger these disturbances become. In addition to reverberation in the water, reverberation occurs within the cavities of diving helmets and in larger enclosures such as underwater habitats. Noise from the environment is a further problem.

There are three ways in which the quality of the human and vocal elements in communication can be considerably improved: 1) operators manning the more important communication posts can be selected for their inherent ability to speak intelligibly, as well as to interpret messages which have been masked by noise; 2) all operators can be trained to speak more intelligibly; and 3) all important messages can be standardized so that, without departing too far from common usage, they are short, simple, easy to say and easy to understand.

Speech

Speech in man is generated by an air stream traveling from the lungs through the vocal tract. This vocal tract acts as a continuous acoustic tube having a number of resonant chambers. The oral muzzle used in SCUBA for communication adds another resonator to

the oral tract, as closely coupled to the larynx as the mouth and, according to Coleman and Krasik (3), subsequently shifting the characteristics of speech.

Speech output from the oral muzzle is dependent upon the interrelation of the mask and the microphone used as a transducing device. The combination should be designed to produce a flat frequency response over the speech range. However, most muzzles produce resonance at certain frequencies with certain significant distortion products, thus making a flat frequency response impossible.

Hollien and Tolhurst (7) describe work done on the effects of the external cavity size on underwater speech. Data abstracted from the laws governing the resonance characteristics of such cavities and from phonetic theories of speech production should be used to provide predictions of the size and configurations of the cavities optimal for intelligible speech.

Distortion of a diver's speech is due to two factors, according to Fant and Lindqvist (4). One is the pressure, or rather density, which causes a nonlinear shift of low frequency vocal resonances, subjectively perceived as "nasality." This effect originates from the participation of vocal cavity walls in vocal resonances. The other factor is the well-known linear transposition of vocal resonances in proportion to the velocity of sound for the particular gas mixture (the "Donald Duck" effect).

It may be stated that speech distortion is due to the sound velocity characteristics of the gas. The velocity depends upon the gas density which in turn depends upon the molecular weight, the percentages of various gas constituents, and the pressure.

The increase of the level of voiced sounds should be considered when designing audio links for great diving depths. A tape-recorder adjusted for optimal input level at 1 ata may be severely overloaded at pressures greater than 10 ata. The gain in voice level might also be a point to consider in speech training since the voice effort might be reduced accordingly.

Transmission Equipment

Generally, a communication link consists of three main subsystems: the microphone (the input transducer), the transmission system and the loudspeaker or the headset (the receiver/transducer).

The transmission of acoustic energy through the ocean is subject to absorption that is strongly frequency-dependent. This frequency dependence is the dominant factor determining the usable bandwidth of an acoustic channel for any particular range in the ocean.

Hollien, Coleman and Rothman evaluated in 1970 several diver communication systems and concluded that *none* of the major systems currently available provided acceptable levels of speech intelligibility (5). Similar evaluations and findings of this same nature are also described by Hollien, Coleman, Thompson and Hunter (6).

In summary, there are many problems connected with the measurement and the analysis of diver performance, diver perception and diver communication which must be solved very soon to contribute to and keep up with the rapid technological advancement in safely and effectively putting man into the sea.

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DIFFERENTIAL BEHAVIORAL EFFECTS OF NITROGEN, HELIUM, AND NEON AT INCREASED PRESSURES

J. R. Thomas, J. M. Walsh, A. J. Bachrach and D. R. Thorne

The purpose of this report is to demonstrate the use of behavioral techniques of operant conditioning in the assessment of performance under hyperbaric pressure. The conditions under which behavior is established and maintained comprise the subject matter of this experimental analysis. Interest is focused on the interaction of ongoing behavior and its effects on the environment which reinforces or maintains the behavior. The contingent relationship between response and reinforcement constitutes what is technically called a schedule of reinforcement (1). Using schedules of reinforcement, behavior can be developed or manufactured to specification so that the performance of an individual organism is predictable and controllable over an extended period of time.

The establishment of stable behavioral baselines in organisms is time-consuming, often requiring in excess of 100 hours of training. However, the result of this conditioning is a baseline pattern of behavior which is extremely sensitive to a wide variety of variables (2). These operant techniques can be applied in the assessment of performance at different depths, breathing several gas mixtures, and in the adaptation to hyperbaric conditions.

The first example of an operant schedule which has been used to assess hyperbaric variables is a temporal discrimination task where animals must space the time between successive lever-pressing responses. Free-moving, food-deprived animals, trained to depress a lever according to the temporal schedule in order to obtain food pellets, were used in the experiment. The schedule specifies that the subject's response be reinforced only if it follows the immediately preceding response by a specified minimum length of time. In this experiment the animals were reinforced with a food pellet for a response which followed the preceding responses by at least 18 seconds. In addition, an upper limit of 24 seconds was placed on this response to increase the demand for accurate timing. Therefore, to obtain food the rat had to press the lever within the period of 18 to 24 seconds after the immediately preceding response. Responses outside this limited time period went unreinforced and served only to reset the timing period for the next response. In operant terminology this schedule which evaluates timing behavior is known as the differential reinforcement of low rates, or DRL schedule with a limited hold (3). This particular schedule generates a low, steady rate of response. The temporal discrimination manifested by an animal on this schedule can be seen by looking at the relative frequency distribution of the inter-response times which is shown in Fig. 1 (for rat D). All of these frequency distribu-

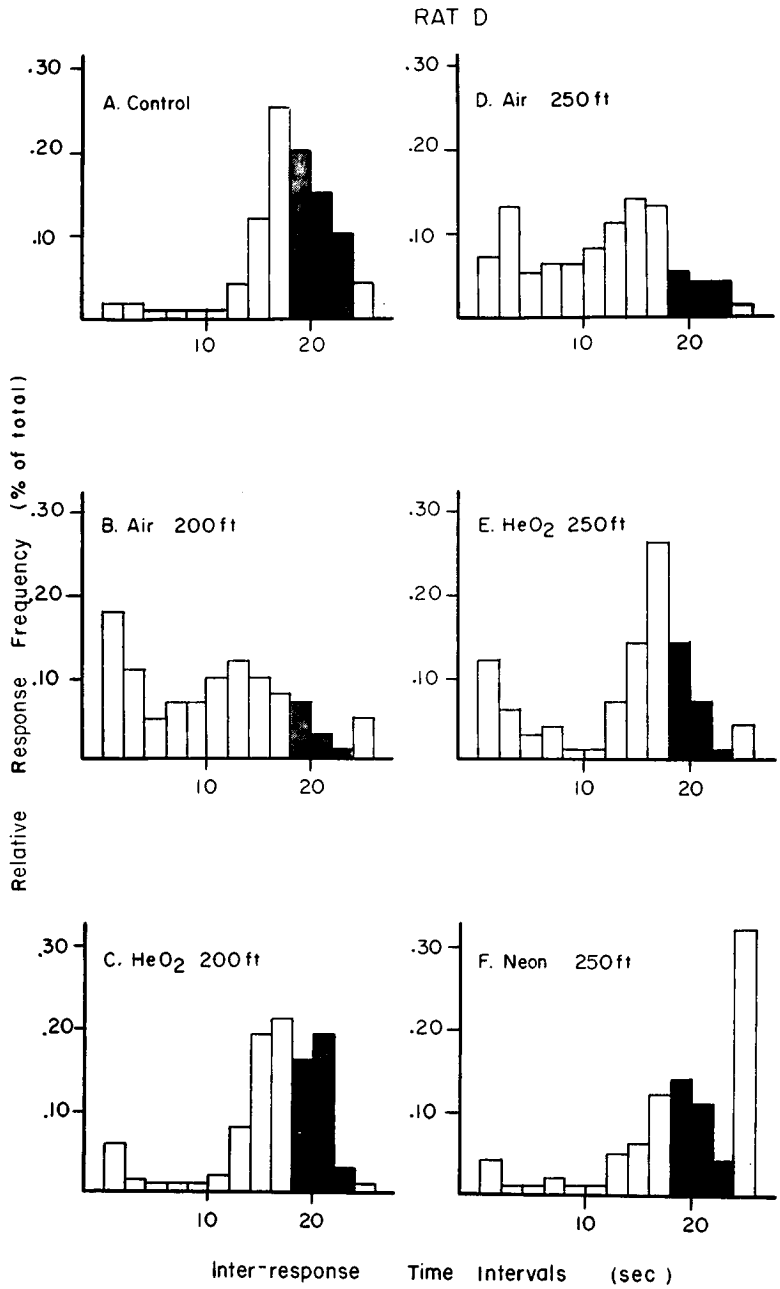

FIG. 1. Relative frequency distribution of the time intervals between successive lever responses for surface control (A); air to 200 and 250 ft (B and D); helium-oxygen at 200 and 250 ft (C and E); and crude neon at 250 ft (F). Each interresponse time interval is 2 sec wide. The last interval contains all responses on the DRL schedule that followed a preceding response by 24 sec or more. Shaded intervals indicate those responses during the limited-hold period that actually produced a reinforcment.

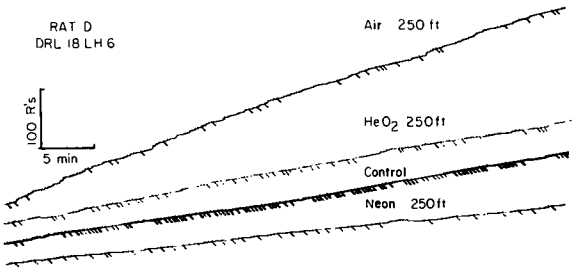

FIG. 2. Cumulative response records of surface control DRL responding and performance under air, helium-oxygen, and neon. The records are plots of cumulative responses over time. Each response steps the recording pen upward and pips indicate reinforced responses.

tions show the proportion of responses occurring within a session plotted against time since the immediately previous response. Each inter-response time interval across the abscissa of the graph is 2 seconds wide; therefore the first interval represents the proportion of responses within a session which followed a preceding response by 2 seconds or less; the second interval shows those that followed the preceding response by 2 to 4 seconds. The last interval represents all responses that occurred within the response time greater than 24 seconds—that is, beyond the limited hold period. The shaded interval on the graph indicates those responses which actually produced the food reinforcement. The graph in the upper left shows the control baseline distribution. All animals showed indication of a distinct temporal discrimination, that is, the relative frequencies of inter-response times were maximal in the area of the 18-second interval. Rat D, when subjected to pressures of 200 and 250 feet breathing compressed air, showed an increased response rate as indicated by a shift in responses to the shorter intervals. This breakdown of the temporal discrimination—both at 200 feet and 250 feet breathing air—is clearly evident in the decline of the shaded, reinforced area on both the graphs. Responses on the DRL schedule at similar depths breathing an 80-20 helium-oxygen mix resulted in near baseline performance. However, there was a small increase in rate of response, and some shifting of responses to the shorter intervals occurred; the distinct temporal discrimination, however, is still evident. In all the animal dives discussed in this report the same compression rate (10 feet/minute) was used.

When rat D was exposed to 250 feet breathing a crude neon mix, responses shifted in the direction opposite to the air and helium dives. On neon the frequency distribution indicates that the temporal discrimination was still rather well maintained; however, a large percentage of responses occurred at very long intervals following the limited-hold period.

Comparisons of neon with the air and helium mixes can be made—both in terms of actual depth and in terms of partial pressures—since the partial pressure of neon at 250 feet is approximately equivalent to that of the other two gas mixtures at 200 feet. This behavioral baseline indicates that the subtle effects of breathing neon under pressure are quite different from those of either helium or nitrogen.

Figure 2 shows the cumulative records of rat D from the same four conditions. This record is a plot of cumulative responses over time. The control record shows a low, steady rate of responding, the downward movement of the pen indicates reinforcements, the number of which is equivalent to the shaded area shown in the frequency distribution. The

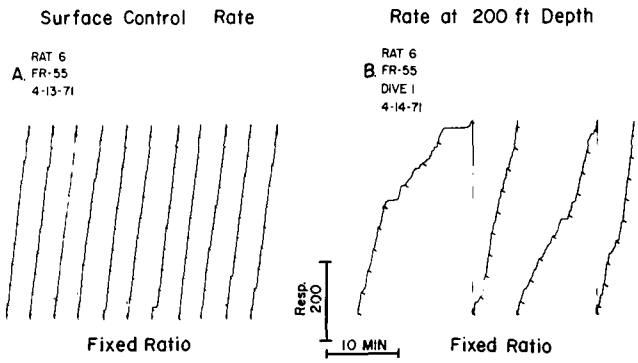

FIG. 3. Cumulative response records of performance on fixed ratio schedule at ambient surface pressure (A) and at 200 ft breathing air (B).

density of reinforcements during the control sessions is greater than during any of the other conditions. Breathing air, the low response rate is most disrupted. Breathing a helium mix under the same pressure results in elevated response rates but much more closely approximates control behavior, and the density of reinforcement is much greater than under the condition of breathing air. Neon at 250 feet shows a much slower rate of responding with occasional periods of pausing indicated by the flat points in the record. These data have shown that this low rate baseline schedule provides a method to distinguish gas mixtures at various depths.

Another example of an operant schedule used to evaluate hyperbaric performance is a counting schedule where the animal must simply press the lever a specified number of times to receive food reinforcement. This schedule, technically known as a fixed ratio (FR) schedule (1), generates high rates of responding. Figure 3 shows a comparison of the control rate of this behavior at ambient pressure and at 200 feet. The animal (rat 6) is required to press the lever a fixed ratio of 55 times in order to receive food reinforcement. The cumula-

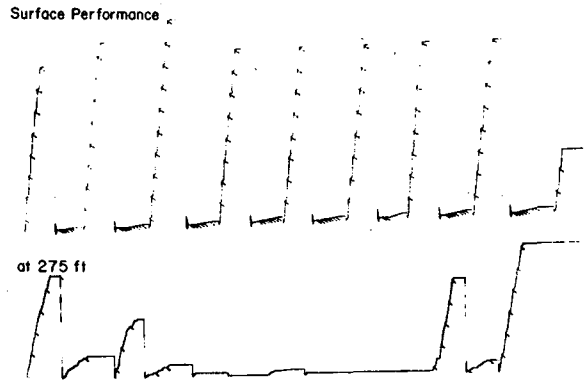

FIG. 4. Cumulative response records of performance on multiple FR-DRL schedule. The top record shows control surface baseline performance and the bottom record shows performance at 275 ft breathing air. The FR and DRL schedules alternate every 3 minutes and the recording pen resets to baselilne at the end of each schedule component.

tive records show responding at a very high rate as indicated by the slope of the record in comparison to the low rate, previously described. This animal performed at a rate of 3 responses/second over a long period of time. When this animal was subjected to pressures of 200 feet breathing air, the rate of response declined markedly and at points during the record there are long pauses where no responding is evident. It is interesting to note that air at elevated pressures produced a decrease in response on this high-rate behavior, while rate increases were observed on the low-rate schedules. This differential rate change emphasizes the importance of the nature of the particular behavior or measure being used to evaluate performance under pressure—that is, with the same performance task, differential results can be obtained as a function of the contingencies maintaining the behavior.

This fact can be more clearly demonstrated by evaluating the performance of a single individual organism utilizing *both* of these schedules within a single session. Animals have been conditioned to emit these patterns of behavior—both the high and the low rate—within the same session. The control over which schedule is in effect was programmed automatically and was indicated to the organism by the presence or absence of a stimulus light above the lever assigned to each of these two schedules. Figure 4 shows performance of an organism on this multiple schedule. The upper record shows a control baseline performance. The bottom half of this figure shows the same animal at 275 feet breathing compressed air. A comparison of the two records shows that the high-rate behavior has declined and the low-rate of responding has increased, thus replicating both conditions within a single subject within a single session. The complete cessation of responding for part of the record was due to a temporary incapacitation resulting from the fact that the animal was breathing air under nearly 10 atmospheres of pressure.

Much of the literature regarding the narcotic effects of breathing air under pressure has suggested that after multiple exposures one can develop a tolerance or adaptation to narcosis such that an individual may work with no performance decrement (4). However, there has been little or no quantitative evidence to support this assertion.

The application of behavioral techniques has also been used to quantify the effects of repeated exposures to hyperbaric pressure. Several rats were trained on the DRL timing schedule and were repeatedly exposed to 200 feet of pressure breathing compressed air.

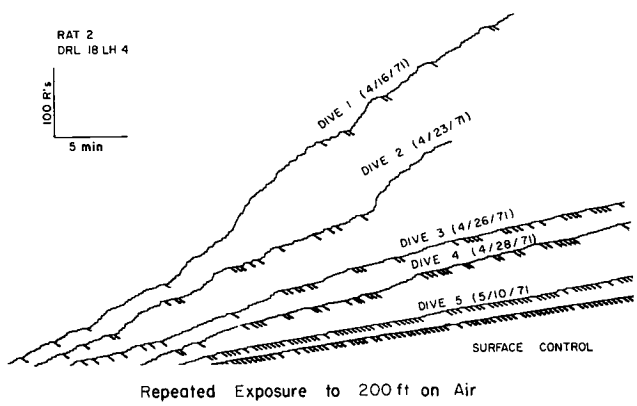

FIG. 5. Cumulative response records of performance on DRL schedule during successive exposures to 200 ft breathing air.

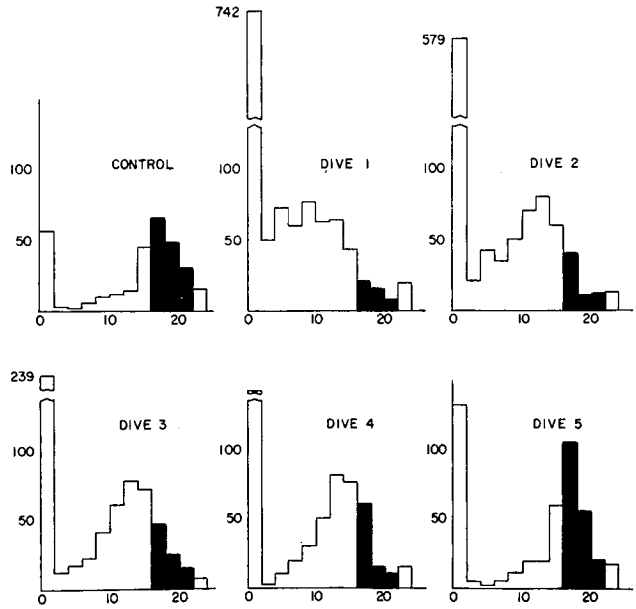

FIG. 6. Absolute frequency distribution of inter-response times on DRL schedule for control and successive exposures to 200 ft on air. Note that absolute numbers of responses are plotted rather than the relative proportion of responses as in Fig. 1. The number of responses emitted is recorded on the ordinate and the inter-response time intervals (in sec) are indicated on the abscissa.

FIG. 7. Cumulative response records of performance on DRL schedule for control session and repeated exposures to 200 ft. The initial exposure to 200 ft was on a helium-oxygen mixture (80–20) and subsequent dives were on air.

Figure 5 presents the cumulative records of a subject (rat 2) where the slope of the records indicates a substantial increase in responses being emitted on the initial exposure, relative to the control rate. With each successive exposure to the 200-foot depth, the rate gradually returned to a point where, on the fifth dive, the record indicates a rate that approximated normal surface control. Figure 6 shows the frequency distribution of the inter-response times for rat 2. On the initial dive responding increased significantly and model values shifted to the shortest intervals. The distributions for dives 2, 3, 4, and 5 reveal that with each exposure to hyperbaric pressure less disruption of the temporal discrimination occurred. With the exception of the shortest interval, the frequency distribution of the inter-response time for dive 5 shows the animal to be discriminating at 200 feet with a degree of accuracy which is comparable to his surface baseline performance.

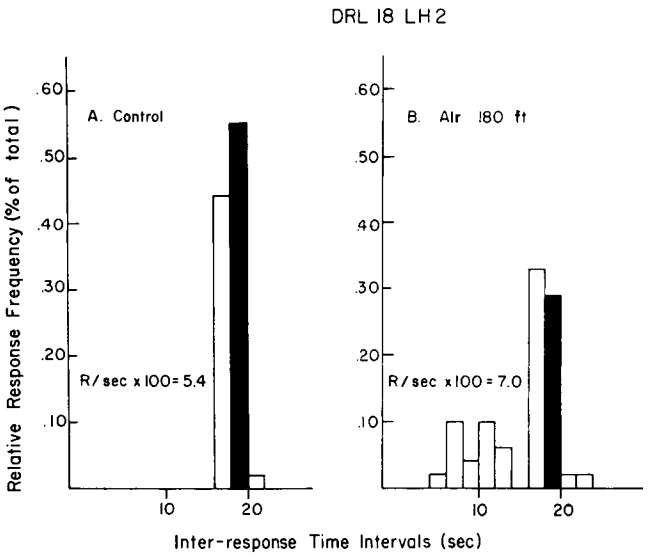

FIG. 8. Relative frequency distribution of time intervals between successive responses for human DRL performance on DRL 18 schedule with a limited hold period of 2 sec for surface control (A) and air at 180 ft (B). Each inter-response time interval is 2 sec wide. Shaded intervals indicate appropriate responses.

The results from a number of the animals in this experiment have indicated that the interval between dives may be a critical factor in the adaptation process. Figure 7 illustrates the cumulative records of another animal, trained on the DRL timing schedule, whose initial exposure to 200 feet of pressure was on an 80–20 helium-oxygen mixture, with subsequent dives on compressed air. The slope of the records does not show the large increase in response rates seen in the previous animal—however, the change in the density of reinforcements is indicative of the impairment of the temporal discrimination which occurred during the initial exposures.

After five exposures to 200 feet of pressure with intervals as long as 12 days, no behavioral adaptation was evident in this subject. In fact, performance continued to deteriorate rather than improve. That is, rate of response continued to increase across dives rather than to approximate control levels. A sixth and seventh exposure with a 1-day interval between exposures resulted in the return to baseline rate of responding. It appears that the interval between dives is an important factor in behavioral adaptation to pressure. When dives were infrequently spaced, limited adaptation was seen. But this same animal, when exposed daily, adapted in as few as three sessions. The analogy may be drawn between exposures under hyperbaric pressure and pharmacological agents. It is well-documented that repeated administration of a drug within a limited time period results in the reduced effect or physiological tolerance to that drug (5). A similar explanation may be suggested for pressure adaptation where frequent diving builds a tolerance or immunity to the adverse effects of gas and pressure. If this assumption is true, then if enough time elapsed between exposures to pressure, the adverse effects would reappear as occurs with drugs. Other studies have demonstrated that frequent diving, 2 to 3 days apart, will enable the organism to work without decrement at pressures as high as 9 ata breathing compressed

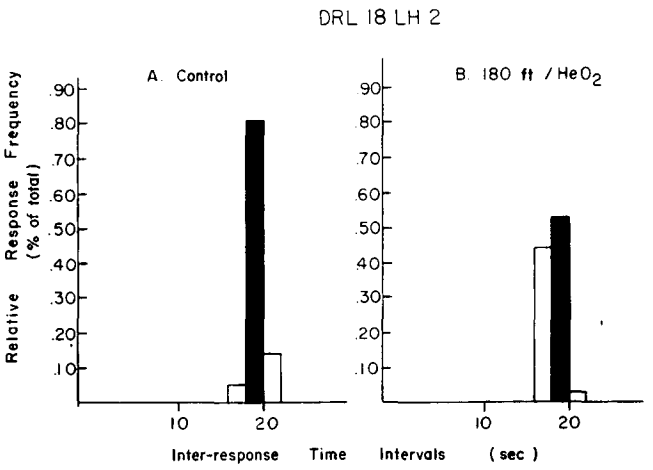

FIG. 9. Relative frequency distribution of time intervals between successive responses of human DRL performance on DRL 18 schedule with a limited-hold period of 2 sec for surface control (A) and breathing an 80–20 helium-oxygen mixture, at 180 ft (B). Each inter-response time interval is 2 sec wide. Shaded intervals indicate appropriate responses.

air; however, time lapses between dives, as short as 1 week, may result in a recurrence of detrimental effects equivalent to the initial exposures.

The application of this behavioral analysis to human performance serves to emphasize the significance of these techniques in evaluating hyperbaric behavior. Figure 8 illustrates the assessment of human performance under hyperbaric pressures. The left-hand side of the figure shows the baseline performance of a diver performing on the DRL schedule. Here the time frame for correct responding is 18–20 seconds. The diver is required to depress a button which indicates his timing response, and an appropriate response is signalled by a feedback light. The temporal discrimination evidenced by the inter-response-time distribution is basically similar to those presented earlier, although the temporal discrimination here is much sharper. In comparison, performance at 180 feet in a hyperbaric chamber breathing compressed air, shows an increase in response rate as evidenced by the shift in responses to the short intervals, with a concurrent decrement in the number of correct responses as indicated by the shaded area on the graph. Figure 9 illustrates the performance of the same diver breathing an 80–20 helium-oxygen mixture at 180 feet where there is only minimal disruption of the temporal discrimination in comparison with the air dive. These results demonstrate that when functionally equivalent procedures are applied to hyperbaric conditions, even across species, similar results can be obtained.

In summary, the results of these studies indicate that operant conditioning techniques can be extremely useful in the assessment of hyperbaric variables on performance. The basic objective behind the introduction of these particular behavioral methods and analysis into hyperbaric research is to urge the use of such technology which offers versatile methodologies, precise quantification of a wide variety of behavior phenomena, and results which are comparable across species.

ACKNOWLEDGMENTS

This research was conducted according to the principles set forth in the "Guide for Laboratory Animal Facilities and Care," prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences, National Research Council.

From the Bureau of Medicine and Surgery, Navy Department, Research Subtask *MF51.524.004.7007DX9K*. The opinions and statements contained herein are the private ones of the writer's and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

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PSYCHOMOTOR PERFORMANCE OF MEN IN NEON AND HELIUM AT 37 ATMOSPHERES

R. W. Hamilton, Jr.

One of the major objectives of the Predictive Studies III Program was to assess the effects of high pressures of inert gas on human performance (8). Of greatest interest in this category were the essentially unstudied physiological effects of neon. The biological properties of this gas have not been widely investigated. Its properties fall in a diversified way between the familiar gases helium and nitrogen. Furthermore, neon has future potential for diving, and the program permitted the breathing of that gas at pressures well beyond those at which it is likely to be used for diving. It was also possible to carry out a comprehensive series of comparative tests with helium as well as to study several "doses" of nitrogen at a single pressure, consequently independent of pressure effects.

For this report the term "human performance" is limited to cognitive and psychomotor functions—a subject's ability to observe, think, and respond, and his ability to perform meaningful coordinated movements. In general the term "performance" might also include physiological and neurological function—these were studied as part of the same program and are to be reported elsewhere.

The study was based in part on an earlier series of experiments involving rapid compression with the three inert gases, helium, neon and nitrogen, to pressures as great as 600 feet of sea water (fsw) (4, 5). In those experiments it was shown that, in the first half hour following rapid compression with mixtures of 5 percent oxygen and each of the three inert gases, the effects of helium and neon mixtures on performance were inseparable, but that the anticipated narcotic effects of nitrogen were exerted beyond about 200 fsw. Previous experiments concerned with effects of neon on performance have shown it caused no narcosis (7), and none has been seen with helium (2).

Experimental Conditions

Objective and reproducible measurement of performance is difficult even under ideal conditions; it was made considerably more difficult by the following conditions. All experiments had to: 1) be fire-safe in an atmosphere enriched in oxygen; 2) involve no electric shock hazard; 3) be operable with the recording investigator isolated from the subject; 4) be usable where communication was made difficult by helium; 5) be performed by subjects

wearing breathing masks and rubber suits; 6) be done in a limited time frame; and 7) involve a modest amount of training.

Tests were chosen to measure mental ability, reaction time, manual dexterity, gross and fine muscle coordination, muscle strength, time-estimating ability, and flicker fusion frequency. The tests used were ones in which considerable previous experience existed. Most were standard psychological tests, although some of them had been designed originally for the purpose of testing innate ability or characteristics rather than response to various environmental conditions.

The environments studied were based on pressures ranging between sea level and 37 atmospheres, with subjects breathing mixtures rich in neon, nitrogen or helium and having an oxygen partial pressure of 0.2–0.5 ata. The neon used was a mixture of neon and helium (75% neon and 25% helium) obtained from the distillation of atmospheric air. This particular product is referred to here as Neon 75, although it is also called "crude neon." This mixture apparently is usable as a deep diving inert gas and, consequently, was of particular interest in this experiment.

The two subjects (SK and TL) who carried out the performance experiments lived and worked in a separate chamber of the complex, designated "Chamber 3." This chamber, its occupants and its team of investigators were dedicated exclusively to neurophysiological and performance studies.

The basic plan was to administer the various tests several times in a uniform format, with the primary variable being the partial pressure and species of inert gas, and with all other variables changed as little as possible between successive tests. The tests were given in discrete sequences (Fig. 1). Gases were delivered by mask, with the subjects breathing by open-circuit demand mask during sea level and shallow exposures (to 400 fsw). For deep exposures subjects rebreathed from a 100-liter, vinyl Douglas bag equipped with a bank of four Baralyme canisters. The bag was supplied constantly from outside the chamber with an excess gas flow to maintain inspired P_{O_2} and to reduce changes in gas composition as a result of diffusion exchange between the bag and the chamber atmosphere. The test sets followed the gas wash-in period in the same sequence each time. After training in individual tests, the test sets were rehearsed and conducted several times at sea level, using air as the breathing gas, and were then carried out in helium and neon at 400, 700, 900 and 1200 fsw total pressure. Helium doses were essentially equivalent to the ambient chamber pressure, while the neon doses were generally about 65 percent of the total pressure, with the balance helium and a little oxygen. Control test sets were performed at sea level using both air and a $He\text{-}O_2$ mixture.

A shorter test set—the first 26 minutes of that shown in Fig. 1—was used in tests with nitrogen. A complete set of nitrogen tests was done on the same day, at 400 feet total pressure, and with nitrogen mixtures of 40, 60, 80 and 98 percent. This resulted in isobaric "doses" at 5, 8, 10.5 and 13 atmospheres of nitrogen.

In effect the scores of these tests represent a dose-response curve. This is particularly true of the nitrogen runs but less so with helium and neon, since different tests were given on different days and the results were subject to some environmental variables other than inert gas partial pressure. The data are shown in Figs. 2–8 so as to compare the total breathing mix at a given pressure, rather than just the doses of helium and neon, while Figs. 10– 12 are dose-response curves.

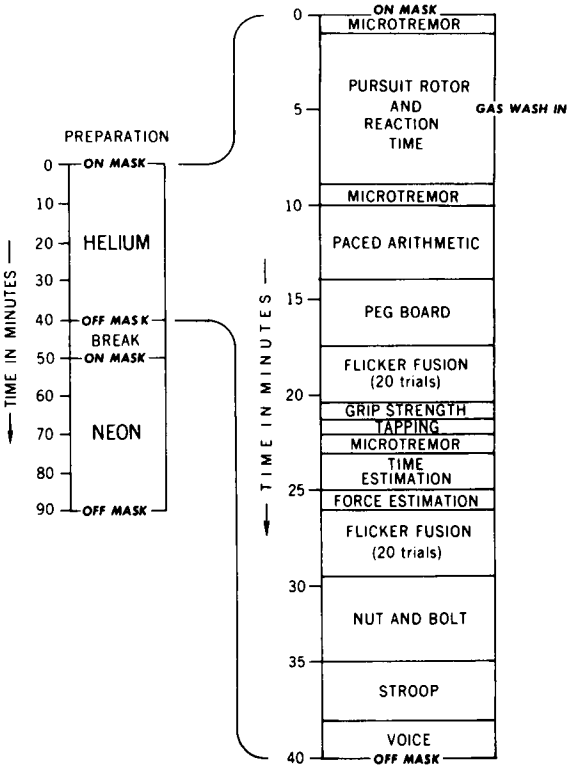

FIG. 1. Performance test set time line. Column at left shows the complete neon-helium performance test which was run several times at sea level and at 400, 700, 900 and 1200 fsw.

A set of tests providing a dose-response curve using various doses of nitrous oxide was also conducted at sea level during the training phase. A comparison of the narcotic potencies of nitrogen and nitrous oxide is the subject of another report (6).

Tests and Results

The results of most tests are displayed here in a consistent format, with the selected criterion of performance plotted on the ordinate, against a series of control (sea level) values on the left and the environmental variable on the right.

ARITHMETIC

The arithmetic test (Fig. 2) was presented with a memory drum. One-by-two-digit multiplication problems were exposed in a window for a few seconds, during which time the subject wrote his answer on the drum. There were 40 problems per set, and subjects took about 3.5 seconds per problem. The pace at which problems were presented was determined in preliminary trials to permit about 80 percent correct under normal conditions. This precludes perfect scores and avoids the dilemma of choosing between speed and accuracy ("number attempted" and "number correct," respectively).

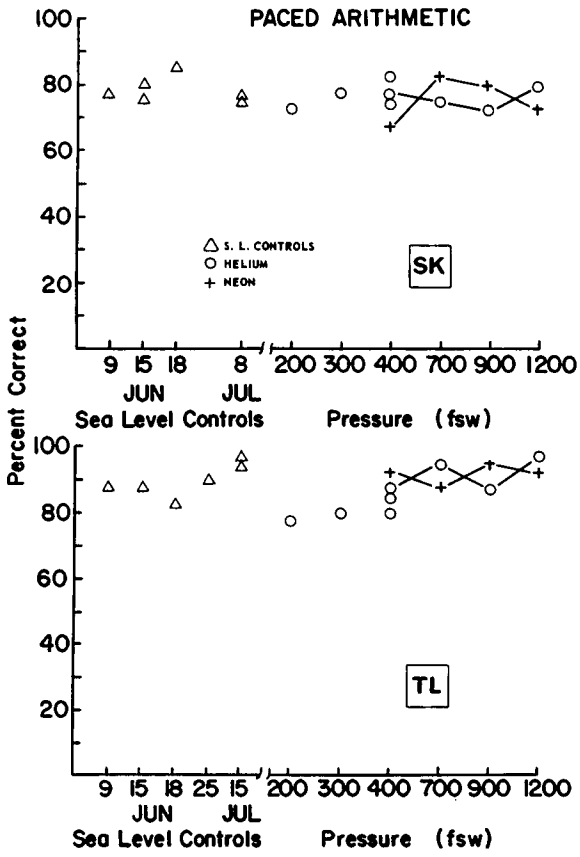

FIG. 2. Paced arithmetic test. Problems were presented at a pace determined in preliminary trials to give consistent scores well under 100% correct. Results show reproducible scores unaffected by inert gas.

The arithmetic results are typical of all the tests in that no consistent trends due to pressure or to the doses of helium or neon were seen. Scores are grouped throughout all tests given.

REACTION TIME

In the test for reaction time, the subject responded to a light by pressing a button with his thumb. The accumulated time of 16 responses during a 1-minute period was averaged to provide a score. These measurements were made in alternate minutes during the gas wash-in period of each test package for a total of at least 96 responses.

Results are given in Fig. 3; in this test a lower score indicates a better performance. The response time appears to have been slightly faster in helium than in neon in only one subject, but neither showed any difference from the control value. In addition, the scores did not change with depth (hence "dose" of inert gas).

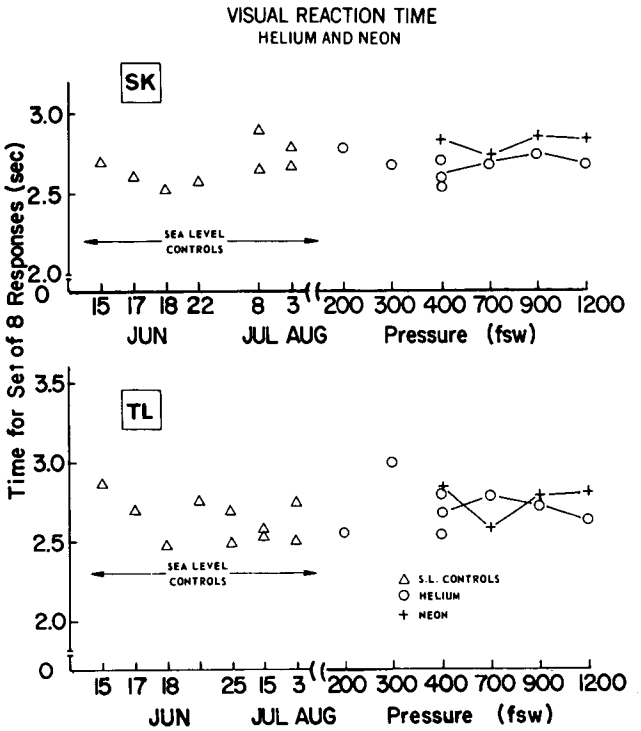

FIG. 3. Visual reaction time. The subject pressed a button with his thumb in response to a light signal. Faster response is indicated by a lower score. No depth or gas effects are seen, except a hint of a reduction in response time by SK in neon.

PURSUIT ROTOR

To test coordination, a standard pursuit rotor device was adapted for chamber use; subjects tracked a 2-cm disc rotating at 45 rpm for 20 seconds per trial. Several trials were used each time the test was given, alternating with reaction time. A high score indicated good performance. This test was alternated with reaction time during the wash-in period.

The pursuit rotor score (Fig. 4) showed a slight drop at 1200 fsw in subject SK, and in this test he scored slightly better with neon. In determining significance of these test results three things were looked for: a trend with increasing depth, a dose agreement between the two subjects, and results consistent with other tests of a similar nature. If any of these was lacking, then the results of a single test were not considered conclusive.

This was the only psychomotor test on subject SK which showed a drop in performance with helium or neon in the maximum-pressure portion of the dive.

PURDUE PEGBOARD

Manual dexterity, primarily of the fingers, was tested with the Purdue Pegboard. The test involves placing small pegs in holes, or assembling pegs, washers and collars.

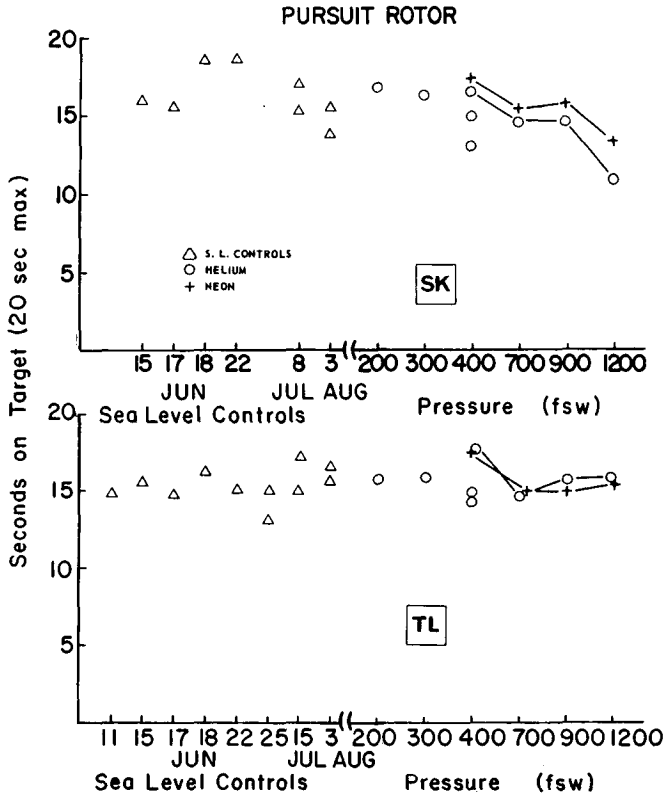

FIG. 4. Pursuit rotor. With a stylus the subject tracked a 2-cm disc rotating on a turntable at 45 rpm; score represents time on target out of 20 sec. The decrease at 1200 fsw by SK is the only such decrement.

The scores (Fig. 5) of the "both-hands" and the "assembly" test were combined into a single total, a high score indicating good performance. As before, all scores measured at pressure are in the range of the control scores and there are no trends with depth. Also, no differences between neon and helium are apparent.

NUT AND BOLT TEST

Another test of coordination and dexterity is the task referred to as the "Nut and Bolt" test.* Using tools, the subject removes nuts, bolts and washers from one panel and replaces them on another. Assembly time is the scoring criterion, so a low score indicates good performance. This is a somewhat crude test which is normally used to assess basic mechanical ability in job applicants but, because the required actions are typical of those which may be done by working divers, it is regarded as meaningful. Results of this test (Fig. 6) showed a slight but continuous improvement with practice and little evidence of environmental influences. This test is quite sensitive to the detrimental effects of narcosis; the results shown here support the notion that neither helium nor neon have narcotic properties at these depths.

* Bennett hand-tool dexterity test (1).

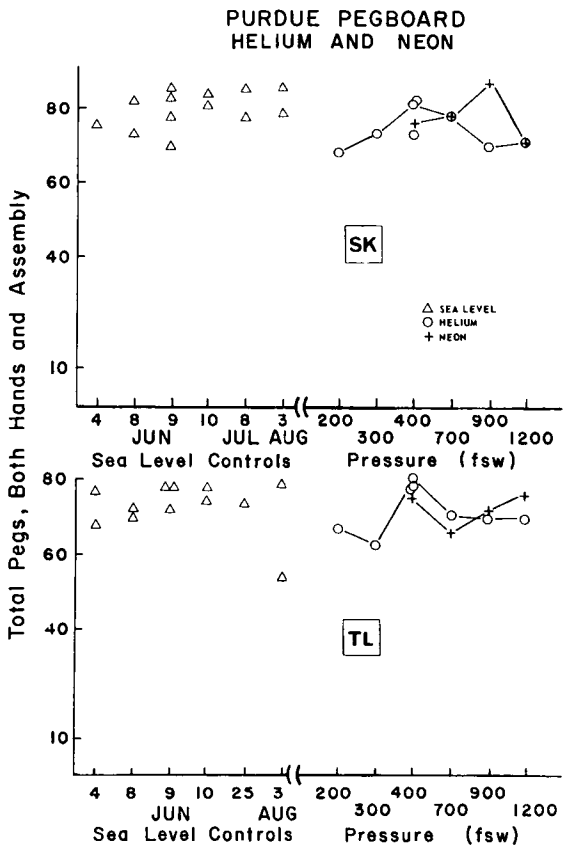

FIG. 5. Purdue Pegboard. The task was to assemble pins, washers and collars with the fingers, working for a total of 90 sec. No pressure-related effects are seen, or differences between helium and neon.

GRIP STRENGTH AND FORCE ESTIMATION

A simple grip dynamometer, adjustable for each subject's preference, was used to assess changes in muscle strength as a result of the exposure. None was found (Fig. 7). The subjects were also asked to produce a 30 kg force (which they had learned to do in previous training) with no information feedback except the previous maximum grip score. Subject SK showed a continuous tendency to underestimate when at pressure; this may have been a result of HPNS, but these results were not corroborated by subject TL.

TAPPING SPEED

Speed of tapping—pressing a button as fast as possible for 4–5 seconds—might perhaps increase in a condition of hyperexcitability of the nervous system, but no such results were seen in these experiments (Fig. 8). The connected points represent tests taken during complete performance "packages"; the other points were taken under other conditions.

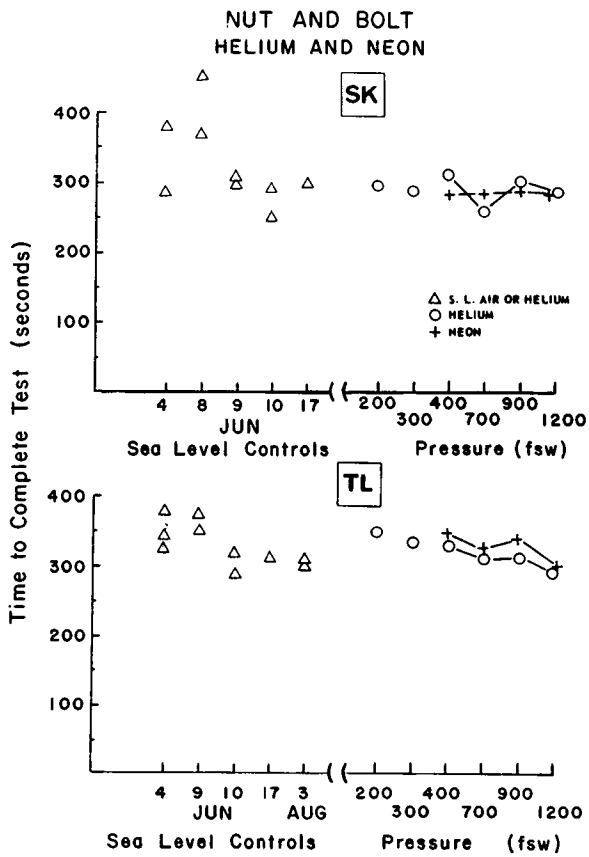

FIG. 6. Nut and bolt test. This hand-tool dexterity test resembles work often done by divers. The test required removing and replacing washers, working against time. A training effect is seen throughout the program, but no difference between gases.

TIME ESTIMATION

The ability to estimate a time interval has been shown to be affected by various influences; for example, time appears to pass rapidly under the influence of high temperatures or amphetamines and slowly under narcotics or barbiturates (3). The subjects were asked to hold down a button for each of eight intervals, ranging between 2 and 12 seconds, to provide a measure of productive time estimation. Results from one subject are shown in Fig. 9; downward lines indicate the subject responded with a shorter time than called for. This coincides with the possibility of a slight hyperactivity while at pressure, but it could also reflect impatience on the part of the subjects. Results from the other subject show the same trend.

DOSE-RESPONSE CURVES

The preceding graphs all reflect plots of scores as events in an essentially chronological sequence. The study was designed to permit evaluation of scores as points on the dose-response curves of classical pharmacology. The responses of some of the tests have been

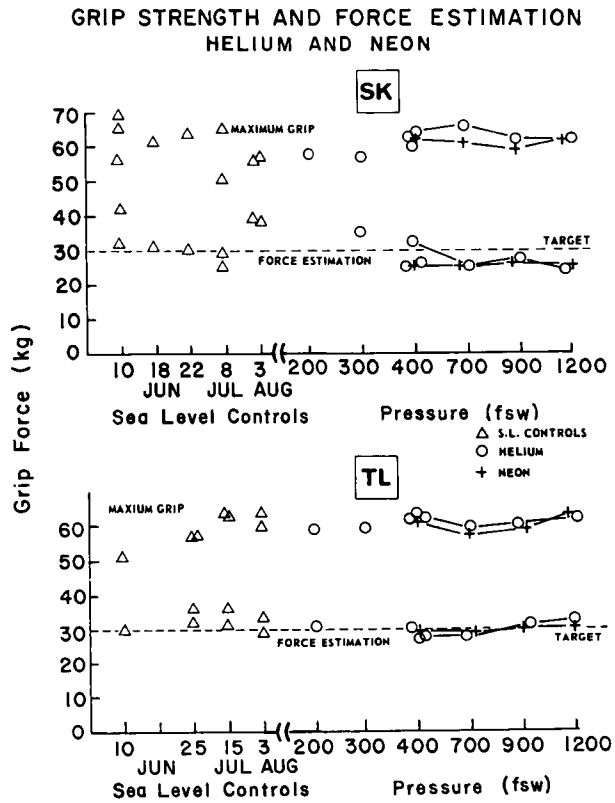

FIG. 7. Grip strength and force estimation. Using a grip device subjects produced a maximum effort, then estimated 30 kg six times, without benefit of knowing the score. One subject (SK) consistently underestimated at depth, but showed no increase with increasing pressure.

plotted against dose levels of the inert gases being studied. Figure 10 shows results of paced arithmetic tests plotted in this manner. Sensitivity of the tests used in this program is indicated by the manner in which they are affected by narcotic doses of nitrogen. Similar results are seen with the pursuit rotor (Fig. 11), and the Pegboard (Fig. 12). In judging the findings, it must be kept in mind that: 1) neon doses represent only about 65% of the total gas pressure; 2) neon and helium runs were done over a 10-day period; and 3) nitrogen scores were determined on the same day and at a constant total pressure, with helium as the background gas. It should be mentioned that 12.8 atmospheres of nitrogen are equivalent to nearly 500 fsw with air as the breathing gas. The most striking demonstration in these three figures, corroborated in all experiments, is that no difference in effect on performance could be determined between helium and neon, and that no narcosis was detected with either of these gases.

EFFECT OF HIGH PRESSURE ON NITROGEN NARCOSIS

Another series of tests was run to investigate whether hydrostatic pressure or hyperbaric helium exerted effects on nitrogen narcosis. Test scores were determined at 9 atmospheres

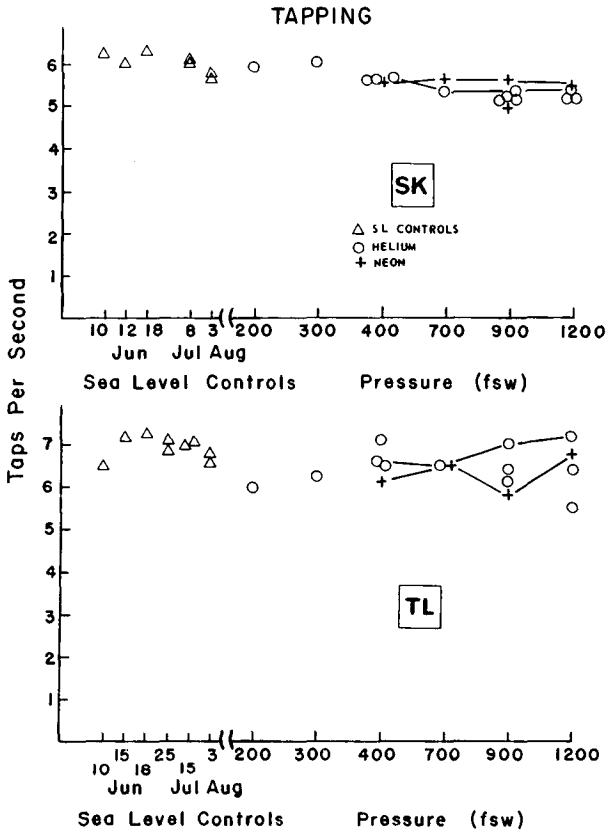

FIG. 8. Tapping. Subjects tapped a button as many times as they could over 45 sec. SK was consistent and improved with time. TL varied more. Neither showed pressure or gas effects.

of nitrogen partial pressure with the pressure due to the nitrogen alone (plus 0.2 atm oxygen), and another time with the same nitrogen and oxygen partial pressures but with the total pressure now 37 atmospheres. These results are given in Table I. All parameters measured were affected by the nitrogen except grip strength, but in no case was there a consistent difference between the effect of nitrogen with or without the higher pressure helium background. These results are not inconsistent with the concept of pressure reversal of anesthesia (9), because the subjects had been compressed so slowly and were so well-equilibrated that no real counteracting effect could be expected. It would be interesting to carry out similar experiments with subjects who have just been compressed to similar depths.

Conclusions

These experiments support the following conclusions concerning both cognitive and psychomotor performance under normal oxygen and as affected by the three principal inert gases used in diving:

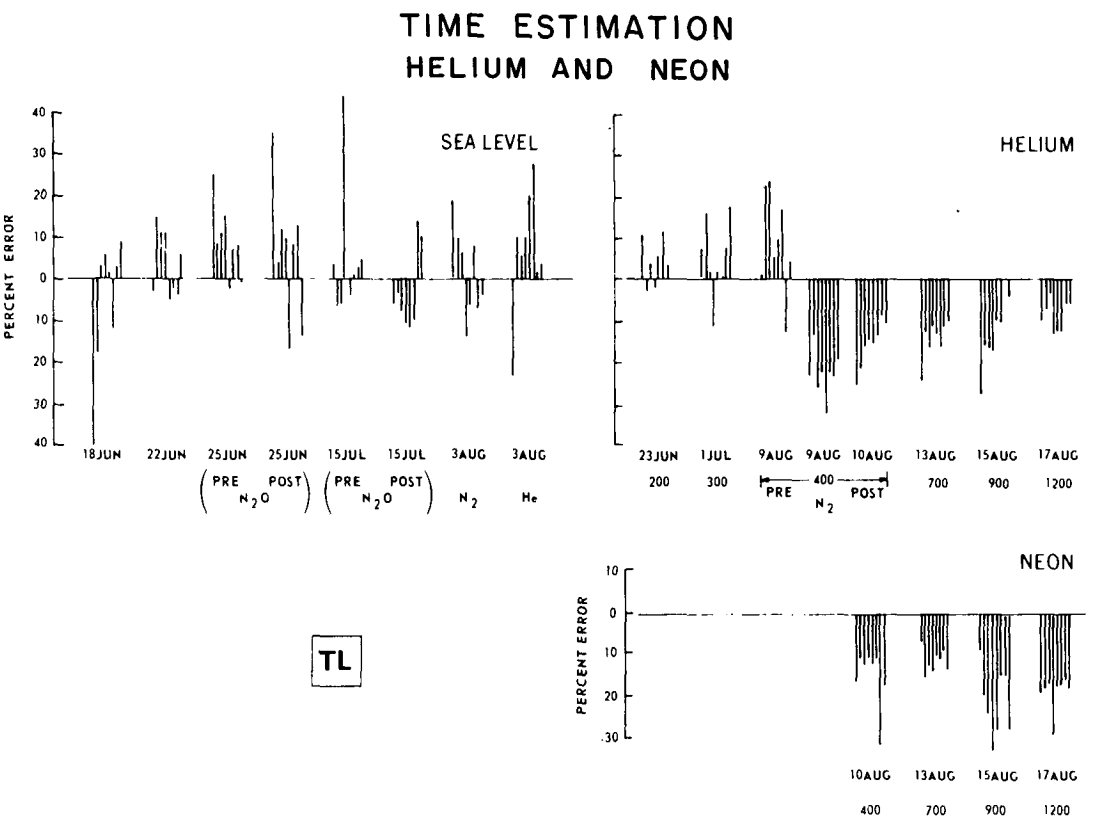

FIG. 9. Time estimation. Subjects were asked to hold a button down for 8 intervals between 2 and 12 sec, estimating the interval without feedback. Subject SK showed similar results to subject TL—a tendency to underestimate time when saturated at high pressure.

1) Little if any decrement in performance is seen in either helium or neon at pressures to 1200 fsw (37 ata).
2) No differences in performance can be detected between helium and neon at these pressures.
3) Nitrogen causes serious but by no means total debilitation at pressures equivalent to those of air at 500 fsw.
4) The narcotic effect of nitrogen follows the S-shaped dose-response curve typical of pharmacological agents.
5) Pressure reversal of nitrogen narcosis by subjects well-equilibrated at 37 ata is insignificant.
6) Neon, specifically the commercial mixture containing 25% helium, seems eminently suitable as a diving gas in the depth ranges where its density permits it to be used.

ACKNOWLEDGMENTS

The work reported here was performed at the Institute for Environmental Medicine, University of Pennsylvania, as a component of its Predictive Studies III Program, designed

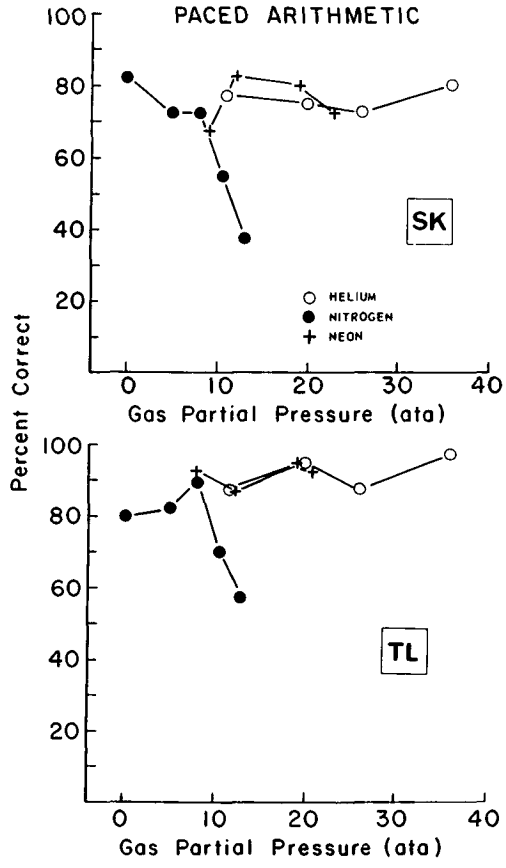

FIG. 10. Arithmetic dose-response curves. Paced arithmetic test data are plotted against partial pressure of inert gas. Prominent narcotic effect of nitrogen confirms negligible difference between neon and helium.

TABLE 1

EFFECT OF HYPERBARIC HELIUM ON NITROGEN NARCOSIS

Test	Subject	Scores		
		Typical S.L.	9 atm N_2	9 atm N_2 at 37 ata
Paced arithmetic	SK	78	64	65
(% correct)	TL	92	78	85
Pursuit rotor	SK	17.0	9.0	11.2
(sec/20 sec)	TL	16.0	10.7	9.6
Reaction time	SK	2.8	3.0	3.0
(sec/8 responses)	TL	2.7	2.8	3.1
Pegboard	SK	81	50	60
(items/90 sec)	TL	74	64	41
Tapping	SK	5.8	4.7	4.7
(taps/sec)	TL	6.7	5.7	5.2
Grip strength	SK	62	60	63
(kg)	TL	60	60	63

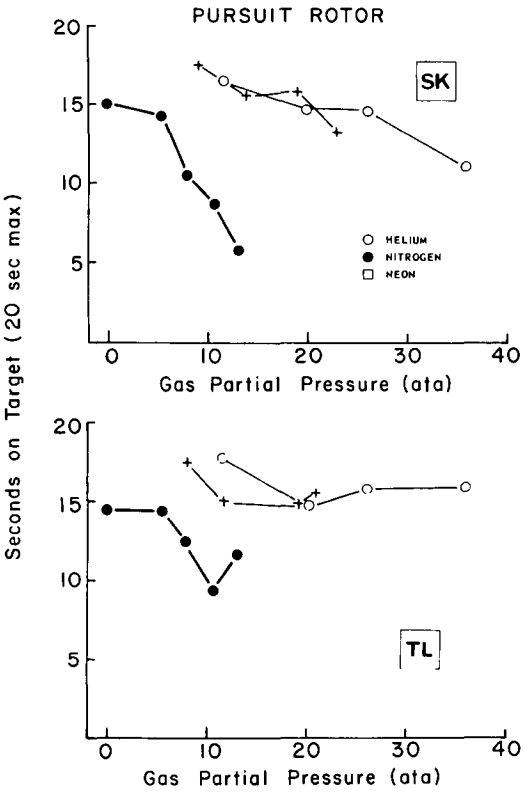

FIG. 11. Pursuit rotor dose-response curves. In this test subject SK showed decrement at maximum pressure with both neon and helium (see Fig. 3).

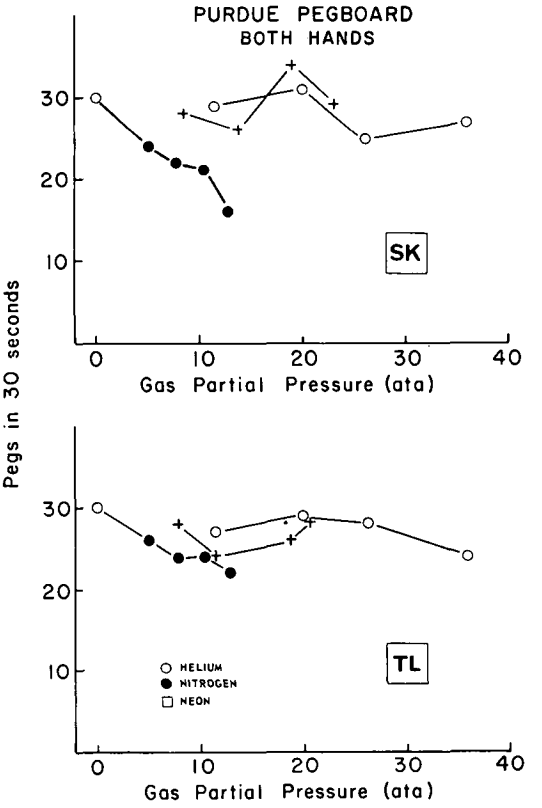

FIG. 12. Purdue Pegboard dose-response curves. Subject TL showed only slight effect of nitrogen narcosis; other results are consistent with other tests.

and led by Dr. C. J. Lambertsen. The author acknowledges the extensive aid of the Institute staff and visiting investigators, including A. Bachrach, T. Langley, D. Thorne, G. Maitland, A. Findling and J. Stortz. Research support was derived from Office of Naval Research contracts N00014-69-C-0405 and N00014-67-A-0216-0026 and National Institutes of Health contract HL 08899-08 and a grant of the crude neon used by Ocean Systems, Inc.

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THE AUDITORY SENSITIVITY OF DIVERS AT HIGH PRESSURES

H. Hollien, S. H. Feinstein, H. Rothman and P. Hollien

Auditory Sensitivity in Water

In 1943 Sivian (23) suggested that human hearing underwater would be attenuated by as much as 40 dB. He argued his position from a theoretical basis, contending that the loss would result from the impedance mismatch between the tympanic membrane (and the middle ear) and the water in the external auditory meatus. Moreover, he expected additional attenuation to result from 1) unbalanced static pressure on the eardrum and 2) ambient noise in the diver's environment. Sivian's experiments, conducted in a swimming pool with a sound source suspended over the water were rather crude by today's standards; however, they seemed to support at least some of his assumptions. Later Ide (18) also tested sensitivity and found a difference of roughly 65 to 70 dB sound pressure level (SPL) between hearing underwater and that in air for a series of frequencies between 100 Hz and 6000 Hz.

Reysenback de Haan (22) attempted to measure the underwater sensitivity of three men who had normal hearing. In one test, their external canals were filled with air and in a second test, they were filled with water. He found thresholds to be lower at 1 kHz but higher at 2, 4, 8, 12 and 16 kHz, in the latter condition. Unfortunately, no description was given either of the experimental procedures or the experimental controls. A year later, Hamilton (12) measured hearing thresholds using a modified method of limits. His subjects were tested two at a time as they sat side by side in the water; the frequencies investigated were 250, 500, 1000, 2000 and 4000 Hz. He reported that the thresholds in the water were on the order of 44 to 60 dB greater than in air. Furthermore, he suggested that underwater hearing is by "bone conduction" because when his subjects occluded one ear with a finger, sensitivity did not seem to change. In 1958, Wainwright (26) used the method of limits (with two subjects) in order to determine hearing thresholds at seven frequencies between 250 Hz and 4000 Hz. His subjects did not wear rubber diving suits and closed circuit SCUBA was utilized. Minimum audible fields were compared for the same subjects both for air and water. Wainwright states the "greatest loss in sensitivity in water occurred over the frequency range from 500 Hz to 2000 Hz and amounted to approximately 20 dB, while below 300 Hz the threshold intensity in water was lower than that in air." He reported that occluding the ears with the fingers had no effect on threshold.

Montague and Strickland (20) apparently were concerned about the lack of agreement

among the data that had been previously published. Accordingly, in 1961, they redetermined underwater hearing thresholds in an attempt to resolve the discrepancies between the data obtained by the earlier investigators. Furthermore, their subjects were tested with and without diving hoods in order to determine the amount of attenuation caused by the hood itself. Thresholds were obtained at 0.25, 0.5, 1, 1.5, 2, 3, 4 and 6 kHz using the Békésy technique. These authors reported that the SPL needed to reach threshold in water was about 40–70 dB higher than the minimum audible pressure (MAP) threshold in air; the greatest loss in acuity occurred in the region of greatest air sensitivity. In addition, at frequencies above 1000 Hz, the diver's hood yielded approximately 20 dB (or more) attenuation. While Montague and Strickland added significantly to the information concerning underwater auditory sensitivity, they did not totally resolve the problem—especially with respect to the *mechanism* by which the ear operates in this milieu.

Communication Sciences Program on the Hearing Sensitivity of Divers

It should be apparent that the reported research on underwater hearing sensitivity left many questions unanswered. First, the exact thresholds that could be expected when the head is immersed in water had not been established, nor had it been determined whether the ambient pressure and/or the water medium alone determined the sensitivity of the ear. Moreover, as has been stated, these early studies did not provide basic information on the *mechanism* of hearing underwater. Accordingly, early in 1966 a program of research designed to answer some of these questions was embarked upon. It is now felt that reasonable information on both the "how" as well as the "how much" has been obtained. More importantly, this model of underwater hearing should serve to explain why this human sensory system acts as it does in this milieu.

Experimental Environment

The site of all experiments, except those so identified, was the Bugg Springs field facility of the Naval Research Laboratory's Underwater Sound Reference Division, Orlando, Florida, located near Leesburg, Florida.

The head of the spring is an elliptical cavity, approximately 200 feet by 100 feet, submerged in a nearly circular pool about 400 feet in diameter. The side walls of the cavity drop almost vertically downward to a depth of about 175 feet. The water temperature is constant throughout the year at 22 °C. Although there is flow from the spring, there are no noticeable currents. Ambient noise is approximately that of sea-state zero and consists of wave slap, some hiss from the spring, and fish sounds. Situated over the deepest point of the spring is the USRD facility which is used in the testing of Navy underwater sound equipment. It consists of a large floating barge with two laboratory rooms situated on either side of a well, through which the Diver Communication Research System (DICORS) was lowered to the proper depth (17). The barge is kept in place by large cables extending to attachments on the surrounding shore.

Research Equipment

If precise and rigorous research on diver hearing is to be carried out successfully, the experimental procedures cannot be haphazard. For example, research on auditory function

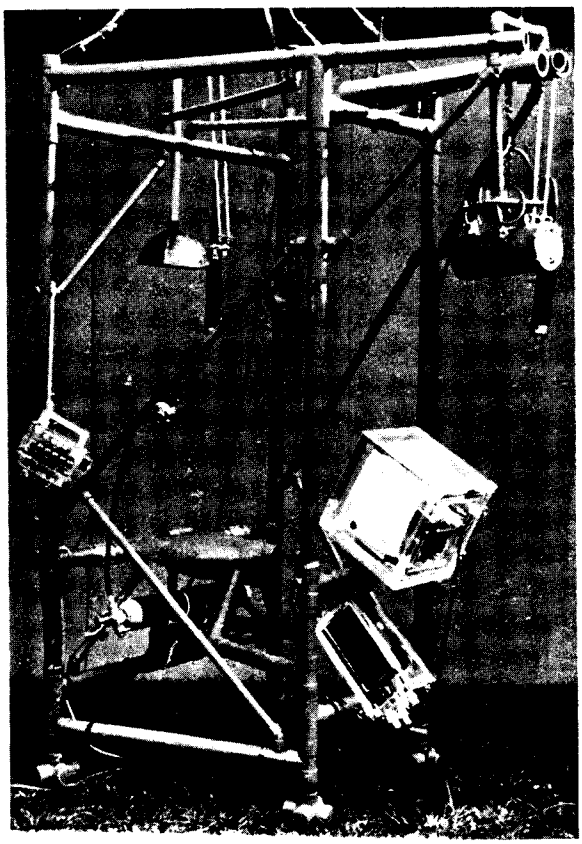

FIG. 1. Diver Communication Research System (DICORS).

in air involves a substantial variety of highly sophisticated techniques and methodologies which permit a precision and rigor in that milieu that were unattainable underwater prior to our methodological thrust in this area. Consequently, in an attempt to utilize acceptable techniques and to control as many extraneous variables as possible, an underwater system was developed which provides for experimental control of diver positioning, stimulus presentation and subject response. DICORS has been described previously (17), hence, only specific features bearing on hearing research will be discussed here.

An understanding of how DICORS is used in auditory research can be obtained from Fig. 1. First, a J-9 projector is mounted on the frontal projection of DICORS and an F-36 hydrophone on the rear frame at a distance of exactly 3 meters from the projector. The J-9 provides the sound source and the F-36 hydrophone provides the means by which calibration of the full system may be accomplished. That is, by placing the hydrophone in a position very near to where the diver's head would be during an experimental procedure, calibration of the entire system (including the diver) is possible. Of course, during an actual experiment, the calibration hydrophone is removed and the diver's head positioner is substituted. Figure 2 provides a schematic drawing of the stimulus, response and calibration system.

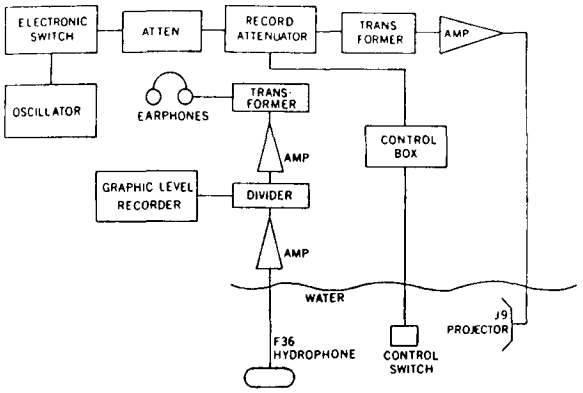

FIG. 2. A block diagram of the stimulus, response and calibration system.

The large Plexiglas box directly in front of the diver's knees (shown in Fig. 1), contains a television monitor which can visually convey information to the diver (subject). For example, for a procedure involving speech reception thresholds, the diver is presented a particular stimulus word (auditorily); immediately thereafter he observes a group of words (among them the correct item) on the monitor. He then responds by activating that switch (among those in a set) which corresponds to the particular item chosen as the word which was heard. Several available switching arrays were used, including six underwater switches placed in a Plexiglas box which in turn was held by the diver. When a given switch is activated, the response is observed and recorded topside by means of an IBM 010 key punch coupled to a bank of response lights. This overall system indicates immediately the diver's (subject's) particular response—as well as simultaneously punching the same information on an IBM data card for computer analysis at a later time.

When hearing thresholds were obtained (sinusoids), a single hand-held switch was used to control attenuation of the Békésy audiometer. In these experiments, sinusoidal test stimuli generated by a beat-frequency oscillator* were passed through an electronic switch† and associated equipment to the J-9 transducer mounted on the frame of DICORS. Test frequencies of 125, 250, 1000, 4000 and 8000 Hz were utilized; they were gated ON and OFF with a period of 500 msec, a 50% duty cycle, and 2.5 msec rise-and-decay times. The attenuation rate of the recording attenuator was 8 dB/sec. The air conduction thresholds (for comparison) were obtained by standard audiometric procedures or by using a Rudmose‡ automatic audiometer modified to allow presentation of the same frequencies as those used in the experiment.

EXPERIMENTAL PROCEDURE

All of the studies followed the same basic procedure. Only individuals who were competent divers and had experience in taking hearing tests in air and water were used as subjects. When the diver was in position and had equalized the air pressure in the middle ear against the water pressure in the external auditory meatus and was ready to begin the thres-

*General Radio, type 1304-B.
†Grason-Stadler, model 829D.
‡Model ARJ-4.

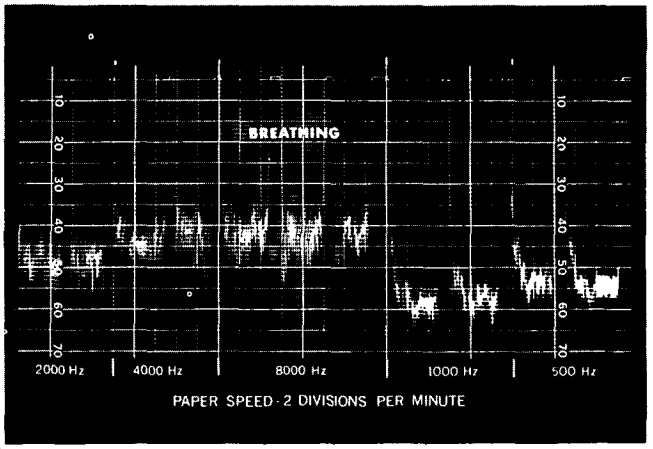

FIG. 3. A typical response trace as it appears on the recording attenuator.

hold test, he signaled the experimenter at the surface. The test stimulus was first presented at a high enough SPL to be clearly audible. The diver (listener) adjusted the SPL of the stimulus so that it shifted back and forth across the audibility threshold level by activating a hand switch connected through a control box to a recording attenuator (see Fig. 2). In all cases, the threshold measures were taken while the listener was holding his breath. This procedure was necessary because considerable noise is generated around the diver's ears when he exhales. Figure 3 provides an example of a typical response trace.

EXPERIMENTAL RESULTS: PURE TONE SENSITIVITY

The first series of projects was focused on the issue of defining exactly how much of a threshold shift occurred underwater. Accordingly, a series of experiments was carried out on a large population of divers with essentially normal hearing. Specifically, a large number of subjects was tested 1) at 35 feet only; 2) at 12, 35, 70 and 105 feet; 3) with helium introduced into middle ear; 4) for speech reception thresholds; and 5) for speech discrimination. Figure 4 provides a graphic representation of the results of the first or basic study. Moreover, the findings resulting from these several experiments may be summarized as follows:

1) Underwater thresholds are from 30 to 60 dB higher than for air conduction, the difference increasing with frequency. Specifically, underwater sensitivity varies with frequency from 67 to 80 dB SPL, a range of about 13 dB with a mean threshold of about 70 dB SPL. The pattern of the "loss" appears quite similar to that seen in patients with conductive hearing disorders (6).

2) Thresholds do not appear to vary as a function of depth. Increases in ear depth from 12 feet to 105 feet and the concomitant positive increases in water pressure (5.3, 15.6, 31.2, and 46.7 p.s.i. at 12, 35, 70, and 105 feet, respectively) or corresponding increases in atmospheric pressure of 1.4, 2.1, 3.1, and 4.2 ata, have no effect upon free-field underwater hearing thresholds in the frequency range between 125 and 8000 Hz. It should be noted also that these data confirm those obtained in the first experiment (5).

3) The effects of helium in the middle ear—and the associated modification of middle ear

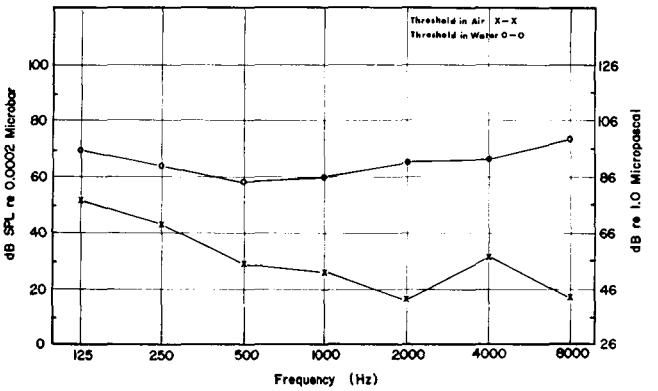

FIG. 4. A graphic representation of the results of the first or basic auditory acuity study.

impedance—appeared to have no effect or at most a minimal effect on underwater thresholds.

4) The effects on hearing appear to be conductive in nature rather than neurological. That is, when speech reception thresholds and speech discrimination scores were obtained and compared to the absolute thresholds for pure tones, it appeared that once the absolute threshold of hearing is reached for the underwater listener, normal speech reception and discrimination could be expected.

In summary, our early studies led to the conclusion that, when a diver's head is submerged, a stable and calculable shift auditory sensitivity results, apparently from a situation where the external and middle ear systems become essentially inoperable and hearing is accomplished by bone conduction.

Recently experiments which tested the bone conduction hypothesis were performed. The experimental evidence is as follows:

1) There is no difference in auditory threshold whether the meatus is filled with water or it contains a trapped air bubble. This experiment was first attempted by Reysenback de Haan (22) but no details of his procedure were given. More recently, thresholds of human hearing underwater were obtained for two conditions: a) with the external auditory meatus completely water filled and b) with a bubble of air trapped against the tympanic membrane (15). The first condition was accomplished by forcibly irrigating the external meatus underwater; the second condition was obtained by placing ear plugs in the meatus and keeping them there until the head was underwater and the test (with the plugs removed) initiated. Subjects were seven divers who were tested in DICORS at a depth of 12 feet; free-field thresholds were obtained for the frequencies 125 Hz, 250 Hz and 1, 2 and 8 kHz. Threshold shifts (in relation to air) for both conditions of underwater hearing were consistent with those previously reported. On the other hand, the thresholds for the two experimental conditions were virtually identical for all frequencies except 250 Hz, where hearing was 6 dB better for the water-filled meatus condition. Apparently, the presence or absence of air bubbles in the external meatus contributes little if anything to underwater hearing thresholds. Yet the impedance mismatch is so different for the two conditions that, if the external-middle ear system were even minimally functional, substantial differences in threshold would have been expected.

2) Prior to the second experiment in this series, Norman, Phelps and Wightman (21) reported four experimental conditions in which they measured sensitivity to auditory frequencies for a diver a) with a bare head (bare head condition); b) with only the ears covered with a set of neoprene ear patches fastened over both pinnas (ear patches condition); c) with most of the head covered, with the exception of the ears, by using a standard neoprene hood with holes cut out at the ear-locations and with the pinnas pulled through the holes (hood with open ears condition); and d) with a full hood covering the ear openings (hood condition). They found that "at high frequencies sound conduction through the ears does not appear to be important." The hood (even with open ear holes) attenuated 1 and 2 kHz stimuli by 30 to 37 dB.

Recently an experiment was performed in which the thresholds of seven listeners were obtained under three conditions: 1) wearing a full wet suit with no hood; 2) wearing a full wet suit with a hood; and 3) wearing a full wet suit and hood with ¼ inch rubber tubes passing through the hood and into the meatuses. There were no differences between conditions two and three, but threshold was significantly lower for condition one and the thresholds for condition one are similar to those we previously reported. Quite obviously, if the external and middle ears are used to any great extent in underwater hearing, a substantial difference in thresholds between these two conditions should be expected.

The approach taken by Smith (24, 25) to the question of bone conduction has been quite different from those previously mentioned. It is his feeling that one needs to know the bone conduction thresholds in air of one's subjects before it is possible to determine whether diver hearing is accomplished by such a mechanism. This position is perhaps a bit stronger than is necessary, but at the same time his results and thinking provide further evidence supporting the bone conduction hypothesis. Indeed, Smith found that depressed air conduction hearing levels were not reflected as reduced underwater sensitivity, except as the depressed air conduction hearing levels were accompanied by depressed bone conduction sensitivity. He was able to demonstrate good correspondence between bone conduction thresholds (in air) and underwater thresholds.

The question of the mode of hearing underwater is still not completely settled; Bauer (2) has suggested that the occluded bubble procedure is not a valid test of the function of the middle ear. His contention is based on his earlier work with Torick using underwater earphones (4) and on a combination of an equivalent circuit of the tympanum according to Zwislocki (27), and of the external ear according to Bauer, Rosenheck and Abbagnaro (3).

Bauer's arguments are based on the assumption that placing the sound source in front of the ears is equivalent to excitation of the middle ear. This may or may not be the case. For example, if one assumes that an earphone in close proximity to the skull acts as a bone conductor, then one might expect that the middle ear is not necessary for the excitation of the cochlea. However, he argued further that the earphone was not a bone conductor because "if reception occurred through normal auditory channels, then directional perception via the earphones would be possible, whereas, if it took place through the bony portions of the skull, then earphones could not be used for directional perceptions" (4). (He did, in fact, find directional perception with the earphones.) Bauer then went on to report that "the earphones were placed against the ears and against other portions of the head. A significantly louder sound was heard with the earphone held against the ear than for any other locations" and that "when the earphone was held at arm's length, all sense of direction ceased."

In light of the now overwhelming amount of evidence for human underwater sound localization (1, 8-10, 13, 14, 16, 18, 19), one finds it hard to account for the inability of Bauer's subject to locate a sound source at arm's length. Moreover, implicit in Bauer's own argument is the deduction that if one can localize a distant sound source which is not in front of the ears one must do so by bone conduction. Finally, since the methods he used to test the loudness of various placements of the earphones are not known, it appears pointless to try to account for his results.

In any case, it is possible to hypothesize why underwater hearing is primarily bone conduction in nature. Basically, the external and middle ears appear to be removed from the acoustic pathway in a fluid medium because of impedance mismatches which can occur as a result of inappropriate force and amplitude relationships. Sound travels through a gas in a high amplitude, low force (Af) relationship; through fluid as high force, low amplitude (aF). The external and middle ear function to increase F in sound energy from its airborne level to a level that will interface properly with the fluid contained in the cochlea. Hence, for hearing in air: Af → aF. When man is underwater, however, the process is one of aF → Af → aF and the serial impedance mismatches are so great (especially the one at the first transform) that these conditions effectively negate the external auditory mechanism. Furthermore, the acoustic impedances of the surrounding medium and the skull are so close that any mismatch can be considered to be essentially zero. Thus sound energy should flow directly into the skull as if it were a continuation of the medium.

Effects of Pressure on Auditory Sensitivity

There are at least two ways by which one can determine how pressure alone affects diver hearing. In the first procedure, hearing is tested at various depths in the water and in the second, hearing thresholds are obtained in the dry atmosphere of a hyperbaric chamber. The former procedure was previously utilized to test hearing up to 105 feet and no significant changes in sensitivity as a function of depth were found. This research was replicated to a depth of 870 feet at the Duke hyperbaric facility during January 1973.

The first hyperbaric threshold measurements were obtained by Fluur and Adolphson (11) at a simulated depth of 330 feet of sea water. They observed reversible, depth-related conductive hearing losses in 26 experienced divers; these losses approached 30 to 40 dB in the middle frequency range of hearing. They attributed the losses to impedance changes in the middle ear.

Hyperbaric observations of hearing thresholds were also made by Farmer, Thomas and Preslar (7). They obtained air conduction thresholds and sensory acuity levels (bone conduction) to a simulated depth of 600 feet on six divers at 250, 500, 1000, 2000 and 4000 kHz. They tested at five depths during descent, twice at 600 feet and at six depths during ascent. During the two tests on the bottom, frequency difference limen (the ability to discriminate between frequencies) at 1.0 kHz was obtained. These investigators reported that "a proportional and variable elevation of the air conduction thresholds appeared during compression" which "gradually decreased during decompression . . . . No significant differences in these thresholds and surface thresholds at any frequency were observed at depths of 100 feet and less." The maximum elevation in thresholds was reported to be 26 dB in the lower frequency ranges at 600 feet. Finally, they found no significant changes in bone conduction or in ability to match frequency. These findings support the conclusion that the loss in sensitivity is not sensorineural.

Attempts to do psychoacoustic measurement in hyperbaric atmospheres have generally suffered from inadequate calibration techniques. At the present time, the only attempt to properly control experiments on the effects of hyperbaric gas mixtures on auditory sensitivity are those by Farmer, Thomas and Preslar (7) of the Duke University Environmental Medicine Laboratory; they found evidence that humans with patent eustachian tubes develop a reversible conductive hearing loss possibly due to increased impedances of the middle ear plus an upward shift in the ear resonant frequency in helium–oxygen mixtures. Unfortunately, however, these experiments were not the primary mission of that dive so that the psychoacoustic experiments had to be run under less than optimum conditions.

Recently auditory threshold tests were conducted at 250, 450, 650 and 870 feet under both dry and wet chamber conditions at the Duke hyperbaric facility. In this instance the experiments were a primary part of the mission and this situation allowed a level of experimental control not previously possible to be exerted. As a result it was possible, for example: 1) to choose divers whose hearing was normal by audiometric standards; 2) to test these divers when they were reasonably rested and had no conflicting duties to perform; 3) to allow sufficient time for the psychoacoustic tests so that subjects and experimenters were not hurried; 4) to provide sufficient time to run a signal detection experiment in order to determine the degree to which threshold shifts may be a function of psychological rather than sensory variables; and 5) to compare the dry thresholds to the wet thresholds (cited above) at the various depths. It is hoped that the results of these experiments will allow postulation of a hypothesis explaining shifts in auditory sensitivity (if indeed any occur), in much the same manner as it has been possible to provide a theory that explains man's auditory sensitivity underwater.

ACKNOWLEDGMENT

Support for the research reported, as well as the preparation of the present paper, was provided by the Office of Naval Research through contract number N00014-68-0173-0008 and the National Institutes of Health through grant number NS-10121.

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AUDITORY CHANGES IN PROFESSIONAL DIVERS

D. Zannini, G. Odaglia and G. Sperati

A hearing loss among subjects exposed repeatedly to compression-decompression cycles (such as aviators, shell divers, caisson workers, helmet divers and SCUBA divers) has been pointed out by many investigators and is commonly attributed to results of repeated episodes of otic barotrauma (2, 3, 5, 7, 10, 14, 15, 17, 18, 20). Other authors (6, 19) confirmed that, excluding common causes such as noise, acoustic trauma by firearms, barotrauma, or decompression sickness, the hearing of divers should be completely normal.

On the basis of observations by Haines and Harris (9), and Alfandre (1), whose numerous subjects did not reveal audiometric changes after long exposure to high pressure, Harris concluded that "there are no suggestions that repeated compression-decompression cycles have per se any effect on auditory acuity" (11).

In the literature doubts are still expressed about this problem, cases being found of persistent or slow progressive hearing loss, even without evident previous barotraumatic episodes (14, 17). A typical syndrome of progressive perceptive hearing loss with dip on 4096 Hz in cases of divers who had not suffered evident barotrauma was observed (14, 17), similar to what Pagano noted as a consequence of barotraumatic otitis in caisson workers (15).

Hence the following problems remain unsolved:

1) whether permanent or progressive hearing losses are present with greater frequency in divers than in the general population;

2) whether hearing losses are attributable to prior evident barotrauma or unobserved barotrauma; or

3) whether other etiopathogenic mechanisms related to underwater activity must be involved.

To help clarify these problems we examined case histories of our divers, studied systematically for the past 10 years, and subjected them to repeated audiometric examination.

Methods and Results

This study, done in 1971, is based on 160 divers, 139 of them in military and 21 in industrial occupations. Ages ranged from 23 to 52 years, with an average being 33. They

TABLE I

DISTRIBUTION OF THE CASES WITH HEARING LOSS OF 40 dB OR MORE

Hearing Loss	Total Number of Cases	Cases with Loss of 40 dB or More		
		Unilateral	Bilateral	Total
Conductive	31	6	7	13
Mixed	35	10	18	28
Perceptive	57	2	9	11
Total	123	18	34	52

began diving without apparatus for fishing, sport, training purposes, or with SCUBA at ages between 13 and 35 years. The duration of their professional underwater activity at the time of this study varied between 3 and 30 years (average 10.6). Twenty-eight percent of them had some difficulties of ear equalization and 16.9% had had barotrauma of the ear (intense ache, perforation of the tympanum, barotraumatic otitis).

The dives were made for the purposes of work or practice, generally with air tanks at different depths. Most of the time the dives were carried out between 20 and 40 meters, sometimes down to 60 and 70 meters, and some subjects carried out a certain number of simulated dives down to pressures of 120–150 meters. The working activity consisted of dives for the purpose of research, recovery, operations for underwater cutting and soldering, control and maintenance of pipelines or metallic structures etc., in waters often quite cold with temperatures even below 10 °C.

Objective findings show that a normal state of the eardrum was found in only 19% of the subjects. In all the other cases, changes of varying degree and type, especially thickening, opacities and retractions, were present.

However, as indicated earlier, the audiographic changes seemed the most interesting. In fact, in 123 subjects (76.9%) a hearing loss of more than 20 dB is evident in the last examination carried out in 1971 or in 1972. This hearing loss commonly takes place on the high frequencies (4000–8000 Hz).

The audiographic findings in divers varied a great deal from one case to another. In many cases the loss was modest. However, 52 subjects had loss equal to or greater than 40 dB; in 33 cases the loss was bilateral and in 19 cases it was unilateral (see Table I). Thus, among these 52 subjects the loss in 31 was equal to or more than 50 dB. In 31 subjects the hearing loss was the conductive type; in 57 the perceptive type, and in 35 of mixed type (both conductive and perceptive type).

To compare this incidence with that of nondiver subjects, audiograms were selected of 160 male subjects from the same area, who were nondivers, not exposed to industrial noise, and who had been examined with the same instrument in the course of preventive medical visits or control visits for various reasons unrelated to hearing problems. Each nondiver was paired with a diver with respect to age in order to obtain an age frequency distribution equal to that of the divers.

The incidence of cases with loss of hearing above 20 dB was found to be 10.7% in the nondivers. The prevalence of audiographic changes in divers is thus much higher and statistically significant ($P < 0.0001$). It must be stated, however, that almost all the audio-

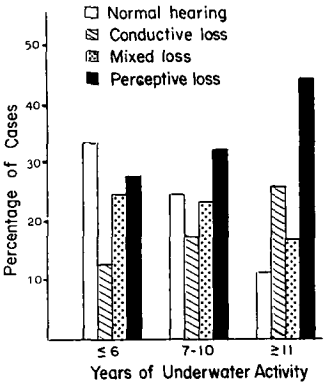


FIG. 1. Percentage distribution of audiographic findings of professional divers grouped according to years of underwater activity.

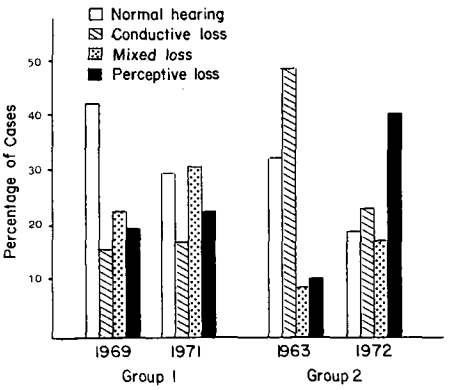


FIG. 2. Percentage distribution of audiographic findings in two groups of professional divers observed at intervals of 2 and 9 years.

grams were carried out for the first time when the diver had already some years of underwater experience.

The audiographic findings were divided according to type, age group, age of beginning diving, years of professional underwater activity and ability to equalize ear pressure. The case distribution is represented in Table II.

The statistical analysis carried out with the χ^2 has shown that distribution of hearing losses presents no significant differences as far as the age and the age of beginning diving are concerned; the difference is significant ($P < 0.05$) in relation to the years of professional activity, the number of cases of hearing loss increasing with the number of working years, especially with respect to loss of the perceptive type ($P < 0.02$) (Fig. 1). The difficulty of equalization is statistically significant only in respect to cases of perceptive type loss ($P < 0.01$). Only three subjects with normal hearing were found to have experienced difficulties of equalization.

As for the duration of the activity, the fact that the perceptive forms are more frequent among subjects with more years of activity must be emphasized, and that among 58 subjects with more than 10 years' of work experience, only seven had normal hearing.

Since audiograms were taken over the years, the variations observed in individual cases after 2 years (group 1) and after 9 years (group 2) were recorded and are reported in Tables III and IV.

In Table III, with reference to the first group, one may observe that in the space of 2 years among 30 found normal at the start there remained only 18 normal at the end, indicating that hearing losses of the conductive type can in some cases improve or pass to the mixed or perceptive type and that those losses of mixed or perceptive type do not become normal again. In Table IV it was observed that after 9 years the evolution of the audiographic pictures is even more marked, in the sense that the number of cases that pass from the normal or from changes of conductive or mixed type to changes of perceptive type is greater than in the cases observed after 2 years. Instead, the percentage of cases that pass from a normal audiogram to a changed one is the same as the first group, indicating perhaps that the initial audiographic changes occur rather early. Even more evident is the evolution of cases taken as a whole, as one can see from Table V and Fig. 2. Some patterns of audiographic changes are shown in Fig. 3*A-F*.

TABLE II

DISTRIBUTION OF AUDIOGRAPHIC CHANGES BY AGE, AGE OF BEGINNING DIVING, YEARS OF UNDERWATER ACTIVITY AND EAR PRESSURE EQUALIZATION

Change	Number of Cases	Age			Age of Beginning Diving			Years of Underwater Activity			Ear Pressure Equalization	
		≤29	30-39	≥40	≤20	21-25	≥26	≤6	7-10	≥11	Easy	Difficult
Normal hearing	37	16	16	5	13	15	9	16	14	7	34	3
Conductive loss	31	5	21	5	6	19	6	6	10	15	23	8
Mixed loss	35	14	16	5	12	15	8	12	13	10	30	5
Perceptive loss	57	16	29	12	17	21	19	13	18	26	38	19
Total	160	51	82	27	48	70	42	47	55	58	125	35

TABLE III

EVOLUTION OF AUDIOGRAPHIC FINDINGS OF 71 DIVERS (GROUP 1), EXAMINED FROM 1969 TO 1971

1969		1971			
	Number	Normal Hearing	Conductive Loss	Mixed Loss	Perceptive Loss
Normal hearing	30	18	6	5	1
Conductive loss	11	3	5[a]	1	2
Mixed loss	16	—	—	16[b]	—
Perceptive loss	14	—	1	—	13[c]
Total	71	21	12	22	16

[a] 3 unchanged, 1 improved, 1 increased.
[b] 5 unchanged, 3 improved, 8 increased.
[c] 4 unchanged, 1 increased.

TABLE IV

EVOLUTION OF AUDIOGRAPHIC FINDINGS OF 89 DIVERS (GROUP 2), EXAMINED FROM 1963 TO 1972

1963		1972			
	Number	Normal Hearing	Conductive Loss	Mixed Loss	Perceptive Loss
Normal hearing	29	16	4	5	4
Conductive loss	43	—	17[a]	5	21
Mixed loss	8	1	—	5[b]	2
Perceptive loss	9	—	—	—	9[c]
Total	89	17	21	15	36

[a] 8 unchanged and 9 increased.
[b] 1 unchanged and 4 increased.
[c] 4 unchanged and 5 increased.

TABLE V

AUDIOGRAPHIC FINDINGS OBSERVED IN TWO GROUPS OF DIVERS AFTER 2 AND 9 YEARS

Audiographic Findings	Group 1				Group 2			
	1969		1971		1963		1972	
	N°	%	N°	%	N°	%	N°	%
Normal	30	42.25	21	29.57	29	32.58	17	19.10
Conductive loss	11	15.49	12	16.90	43	48.31	21	23.59
Mixed loss	16	22.53	22	30.98	8	8.98	15	16.85
Perceptive loss	14	19.71	16	22.53	9	10.11	36	40.44

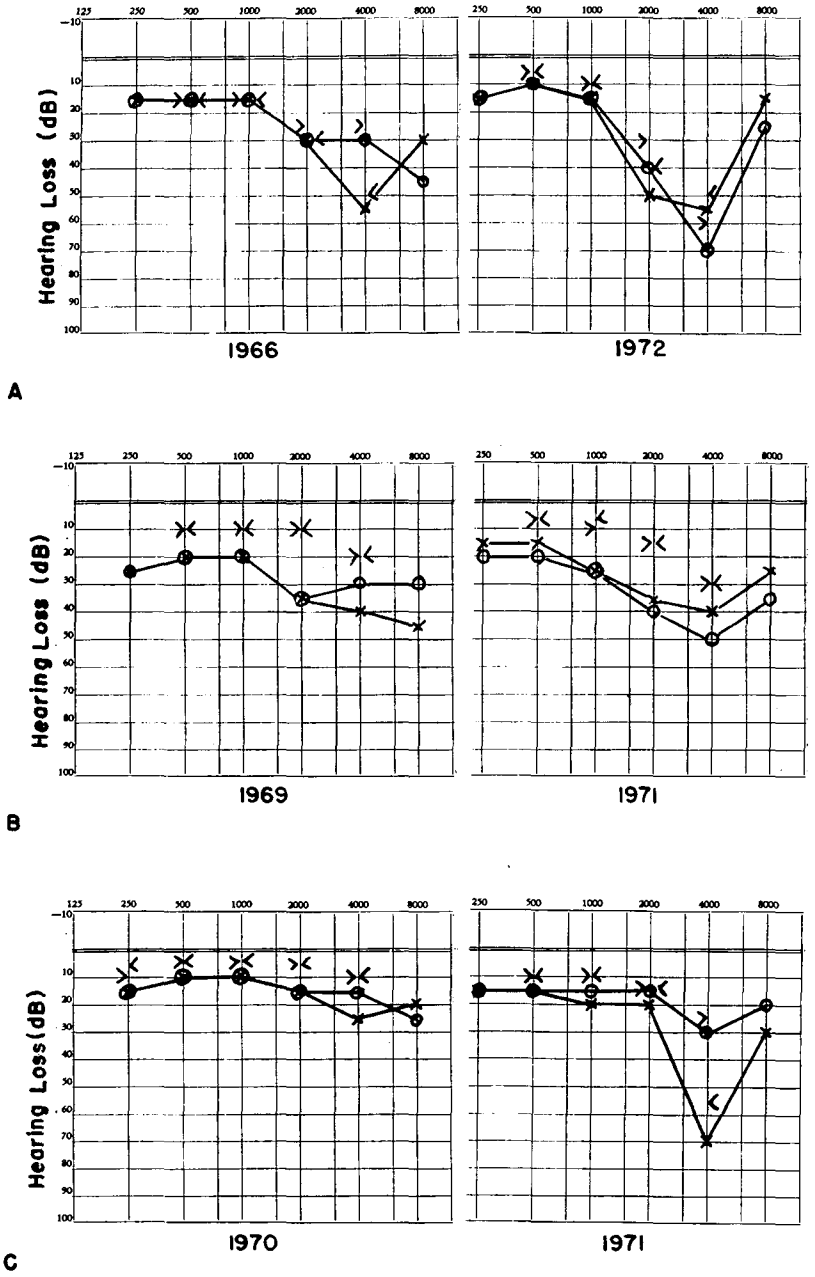
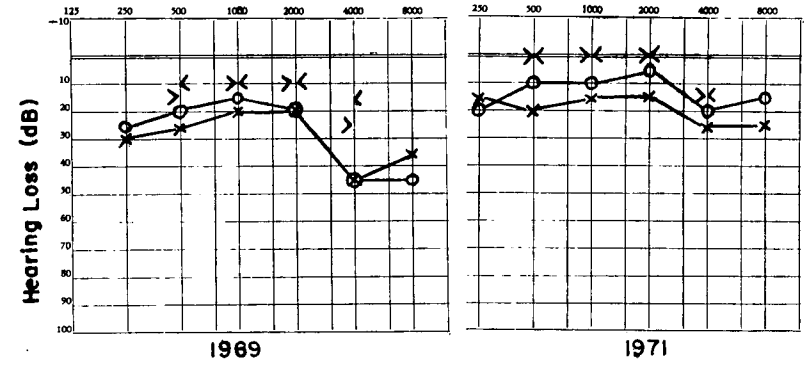
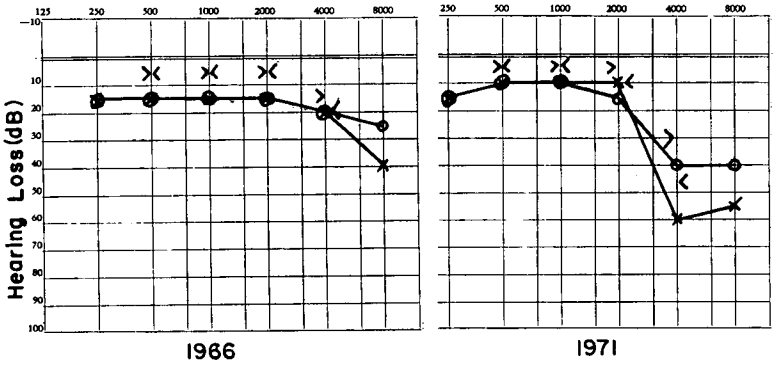


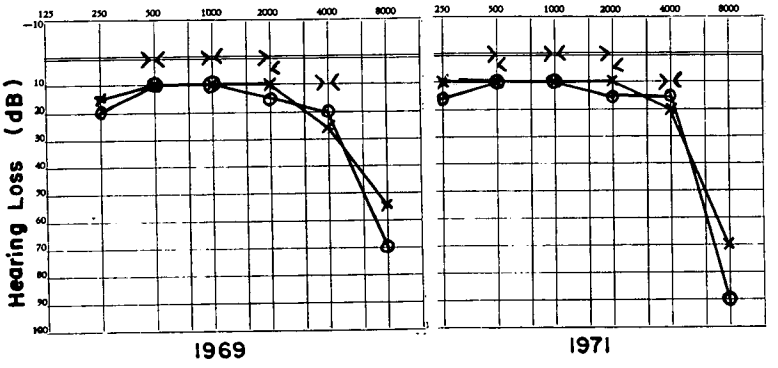
FIG. 3. x represents left ear air conduction; O represents right ear air conduction; >, < indicate right and left bone conduction, respectively. **A,** Case no. 14, 28-year-old diver, 6 years of diving. The first audiogram, carried out after 6 months of training, shows a perceptive loss, more pronounced at left. The last audiogram shows a severe U-shaped perceptive loss. Tympanic membranes are retracted and sclerotic. Mild ear squeeze during a descent in 1971. **B,** Case no. 17, 24-year-old diver, 5 years of diving. In 1970 ear pain during an ascent. Bilateral tympanic sclerosis. The 1969 audiogram shows a conductive loss. In 1971 evolution toward a perceptive loss. **C,** Case no. 18, 24-year-old diver, 8 years of diving. Audiogram almost normal until 1970. The last audiogram 1 year later



D



E



F

shows an evident perceptive loss with dip on 4096 Hz. No previous barotrauma. Mild opacity of tympanic membranes. **D,** Case no. 22, 23-year-old diver, 4 years of diving. Mild opacity of tympanic membranes. No previous ear barotrauma. The first audiogram shows a mixed hearing loss with dip on 4096 Hz, improved after 2 years. **E,** Case no. 44, 32-year-old diver, 5 years of diving. No changes of tympanic membranes. No barotrauma. First audiogram, after 6 months of training. The last audiogram 5 years later, shows a perceptive hearing loss on high tones. **F,** Case no. 63, 34-year-old diver, 13 years of diving. Abrupt hearing loss on 8000 Hz, got worse after 2 years. Normal finding of tympanic membranes. No. barotrauma. Some difficulty to equalize in winter.

Discussion

The observations all refer to the divers that we have under periodic control; therefore, it is affirmed that in these professional military or industrial groups, the incidence of hearing loss is significantly higher than in nondiver subjects of the same age and sex.

Changes in audiographic findings during activity and over the period of a few years would confirm the causal role of underwater activity. The subject history was investigated with reference to exposure to noise, but in no subject were there reasons to think that this factor was of any importance. None of the military divers used firearms for training. Very few industrial divers used hunting guns, except occasionally, and among these hearing loss was not observed.

Among the etiopathogenic factors related to underwater activity, ear "squeeze" with the eventual clinical results such as otitis, hemato-tympanum or rupture of the eardrum, is obviously admitted by all investigators. The apparent disagreement is due to the opinion that without barotrauma there are no changes in hearing. One must admit that professional divers are subject very rarely to this accident. In the divers in this study this accident has been verified only some 30 times in thousands of dives, although it probably occurred many times more during training.

When these cases are examined, it is found that in at least 15 of them, barotrauma could have been the cause of a temporary or permanent hearing change. In all the other cases it must be admitted that either there was mild or evident barotrauma, one or several times, which the diver has not revealed or remembered; or there are other causes in play.

As to the first possibility, one must remember that the professional diver can undergo dives with imperfect ear conditions, even without being so disturbed as to refuse to dive. Furthermore many divers have little pain sensitivity in the ears and may suffer rupture of the tympanum without having forced the dive knowingly and without having previously noticed pain. Others are accustomed to equalizing with the Valsalva maneuver (about 50% of the subjects), thus exposing themselves to great pressure changes, positive or negative. However, even for those who equalize well with deglutition during rapid descents, differences in pressure can occur in the middle ear, such as 50-100 mm Hg, between one deglutition and another.

Without calling upon factors other than barotrauma, one can admit that repeated minor unnoticed barotrauma could have the greatest importance in the genesis of this hearing loss. Therefore, from the clinical point of view, the audiometric changes of conductive, mixed or perceptive type and their evolution toward sensorineural forms, located predominantly on the high tones, are in agreement both with tympanic damage (15) and with cochlear damage.

In addition, 65% of these divers show objective evidence of eardrum alterations of a retractive or sclerotic type. These changes can easily be related to either slight repeated barotrauma which can produce pullings and lacerations of the connective fibers of the eardrum, or to the most evident barotrauma with phlogistic and congestive alterations of the internal layer of the tympanic membrane. Sclerosis of the round window may explain forms of hearing loss not classifiable as purely perceptive, such as the abrupt loss on 8000 Hz, and can lead to ear squeeze or to slight repeated barotrauma. Such changes, which can improve in some cases, as has been seen, would be caused by damage of the basal round of the cochlea.

Nevertheless it is hard to believe that all the divers with hearing loss had such frequent (even if slight) ear squeeze to produce such damage.

Another factor of some importance is cold water, which can act locally on the eardrum for long periods of time during diving. Cold can produce circulatory changes in the ear drum, contributing to cause tympanic sclerosis.

Both factors—pressure and temperature changes—can act directly or by reflex upon vessels of the inner ear, inducing vasoconstriction and passive vasodilatation with the resulting change of capillary permeability, transudation, edema or hydrops of the labyrinth, already amply demonstrated by clinical and experimental observations, and followed (after a long or short period of time) by degeneration of the stria vascularis and subsequently of the ciliated cells of the basal turn of the cochlea. Cold also produces whole body heat loss, thermal stress, increase of arterial pressure, hypovolemia, and blood sludging, which can be coadjuvant factors of circulatory troubles of the inner ear.

An initial evolution toward changes of sensorineural type, observed in many cases, could point toward a direct action of these causal factors on the labyrinth, according to a mechanism of "vasomotor neurosis" maintained by some (4), which would make these slowly progressive forms comparable to those of sudden deafness, already well-known in divers. Another cause, common to sudden deafness, could be the functional problems of the vertebral basilar circle. These can occur during dives made by subjects with static or dynamic changes of the spinal column, as shown by forced movements of the head and which are recognized as being capable of provoking inner ear hearing problems (8, 12).

An intermediate mechanism between such factors as barotrauma, cold, vasomotor neurosis, blood changes, alterations of the vertebral basal circle, and inner ear damage, could be that of hypoxia, the effects of which on the hearing apparatus have been studied in recent years (13). Hypoxia could occur following hemodynamic changes and the phenomena of labyrinth hydrops and edema. In fact, it has been demonstrated that hypoxia causes more evident and significant histomorphological changes at the level of the stria vascularis, and less evident and more delayed changes in the organ of Corti.

The results of these observations demonstrate that an audiographic examination should be made as early as possible, at least during the first preventive visit, and carried out annually, so that therapeutic or preventive measures may be taken. In particular, divers should be carefully instructed with respect to maneuvers of middle ear equalization (the Valsalva should be avoided) and the wisdom of diving when one has difficulties of ear pressure equalization.

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THE ROLE OF THE VESTIBULAR APPARATUS UNDER WATER AND AT HIGH PRESSURE

R. S. Kennedy

This literature review is selective, relating to the increasing importance of vestibular effects in diving. A more complete referencing is provided elsewhere (60).

Snell in 1896 attempted to document "...cases of auditory vertigo...because they have not previously been met with and recognized by writers on the subject" (p. 74, ref. 105). Yet earlier reporters of compressed air work had also recognized such a malady. Curnow, for example (28), published an article in *Lancet* two years earlier than Snell with the term "auditory vertigo" in the title; and Smith (104) 20 years earlier claimed that "Affections of the ears are mentioned by every writer on the subject of compressed air and are extremely common" (p. 25); further "vomiting" (p. 26), "vertigo" (p. 27), and "dizziness" (p. 29) were also described by Smith (104) before Snell as symptoms of "Caisson disease" (p. 25), a term both authors felt they coined; and many of these same symptoms were also mentioned by Bert in 1878 in his famous work (19), and by van Rensselaer in 1891 (116). A lack of awareness of previous workers' reports regarding vestibular symptomatology as part of a constellation of symptoms which occur with some frequency in hyperbaria is not limited to Snell, but occurs often in the history of compressed air work.*

There are several reasons why the full importance of vestibular problems may not have been realized: 1) many articles of merit have been written in German, Italian and French although a major portion of the decompression sickness literature is in English; 2) of the foreign articles cited in the diving medicine "Sourcebooks" (45, 46, 55), English titles were provided only for those studies which were written in Russian; 3) specific pertinent categories (e.g., "ear lesions"), which occured in Volume I of the sourcebook by Hoff (55) were subsequently dropped; 4) pain-type symptoms (type I) (47) are more clear-cut, more common, and usually a cause for self-referral, whereas the various vestibular-type symptoms (e.g., vertigo) can be due to several causes in compressed air work (e.g., syncope, migraine, oxygen toxicity) and are less tangible than pain; and 5) physicians connected with underwater medicine are more likely to be specialists in internal medicine or neurology than in otolaryngology.

*It should be noted that Shilling in 1938 and again in 1941 (99,100) suggested that after joint problems, otological problems were next in importance for future study, but this suggestion does not appear to have been taken up by workers in the field.

This review was undertaken because the Navy Diver's Manual (114) and other guides mention the role of the vestibular apparatus very little. An annotated bibliography (60) of studies concerning the roles of the vestibular apparatus in compressed air work has been published. This bibliography is organized into six categories and the studies are sorted and listed within appropriate categories. Generally, the studies are annotated with some cross-referencing among 1000 references included. The categories are: 1) Vestibular symptomatology reported in connection with compressed air work. 2) Clinical diagnosis of vestibular symptoms with relevance to hyperbaria. 3) Provocative tests of the positive function of the vestibular apparatus to be used as pre-post measurements of normal functions. 4) Illusory phenomena occasioned by water and similar environments which may involve or interact with vestibular functions. 5) The potential use of the vestibular system as a navigation aid under water. 6) Relevant auditory studies.

The purpose of the present report is to summarize and evaluate the main findings of the studies listed under item 1, "Vestibular symptomatology reported in connection with compressed air work." One is directed to the main bibliography for citations in the other categories.

Method

The chief criterion for placing an article in this category was reference to vestibular symptomatology in connection with the exposure of humans (usually) under water or pressure; however, other considerations were also used. For example, not all articles which dealt with aerotitis media were included, since so many exist and since the mechanism appears to be reasonably well understood. However, an attempt was made to include all the literature concerning alternobaric vertigo (72), the high pressure nervous system syndrome (17), etc. Some references which it is felt contain useful methodologies for future study of this problem were also included (38).

Generally, original sources were referenced, although occasionally articles which reviewed the work of others were included. Other criteria were employed and are described in more detail elsewhere (60). For the most part, the literature was assembled by searching the reference lists of modern authors and then proceeding backwards. Particular attention was paid to articles which were not written in English. The three "Sourcebooks" (45, 46, 55) and Shilling and Werts (102) were then consulted for articles which may have been missed by this approach. In addition, the general otolaryngologic literature was surveyed.

Results and Discussion

The number of studies which report vestibular symptoms in hyperbaria is presently increasing although about as many appeared before 1900 as between 1900 and 1940. From the literature review (60) it seems that the number of studies approximately doubles each decade since 1900, and presently the number of articles is probably growing at an equivalent or faster rate than the scientific literature in general. The three "Sourcebooks" (45, 46, 55) and Shilling and Werts (102) show a slower growth rate than this for compressed air work studies in general. For example, studies which mention vestibular-type symptoms are infrequent between 1940 and 1955, a period when diving medicine investigation grew at its

most rapid rate according to the "Sourcebooks" (about 4000 citations in the 6-year period—January, 1946 to December, 1951).

Approximately 300 studies appear in Section I of the larger bibliography (60) which reports vestibular symptoms in compressed air work. The reported incidence of vestibular symptoms in these studies ranges from zero (i.e. none reported) (21, 82), to 28% (52, 53), to 40% where a very liberal criterion was employed (61), and to more than 50% with "ear problems" (87 out of 161) in tunnel workers (10). In the latter study a further breakdown showed "33 complained of dizziness, . . . 6 of vomiting, . . . 6 had blind staggers, that is, labyrinthine vertigo, with nystagmus." Therefore, 28% probably involved the vestibular system itself (c.f. Hill [53]). In modern saturation diving studies, as many as 50% or more of the subjects have been reported to have experienced vestibular-type symptoms (e.g. "dizziness" and "nausea" both were complaints made by subjects [17]). In addition, related CNS (i.e., type II as opposed to type I, pain only, bends) symptoms (47) also appear to be occurring with greater frequency in saturation diving unless special precautions are taken. In these saturation diving studies the group sizes are typically small—generally two to four persons (23, 48, 109). However, in a report of 83 saturation diving accidents (at 11-23 ata), 13% involved the labyrinth (22), and nearly all of these (9 of 11) required treatment; whereas, of the remaining (nonlabyrinthine) accidents, a smaller proportion (49 of 71) required treatment.

Vestibular symptoms in divers have been mentioned in pressure chambers and open-sea dives for various working conditions, gas mixtures, and depths (94). They have also been reported in submarines (113), caissons (53), and following breath-hold dives (83). They have occurred during *compression* (97), and *just* after (89) or *long* after (57) *decompression*, and also under *isobaric* conditions (109). They appear to occur also in guinea pigs and squirrel monkeys under high pressure (74). Vestibular symptomatology ("nausea and vertigo" as reported by Chouteau et al. [24]) is among the symptoms mentioned in connection with the high pressure nervous syndrome (HPNS) (17, 78).

Residual vestibular defects have been reported in both divers (62, 68, 85) and caisson workers (69, 71). Furthermore, Bertoin (20) feels these labyrinthine symptoms specifically worsen with time. Residual central nervous system deficits (EEG abnormality) were high (50%) in a group which experienced labyrinthine symptoms of decompression sickness (DCS) (92), although true control groups were not shown in that study.

In terms of the number of instances of vestibular-type involvement in DCS, probably the best data are found in Rivera (89) where of 93* cases, "dizziness or vertigo" was reported 80 times; "nausea or vomiting" 74 times; "visual disturbances" 64 times; "incoordination" 9 times; "equilibrium disturbances" 7 times; and "auditory disturbance" 3 times. Although these symptoms often occurred along with others—notably localized pain—still either dizziness or nausea, or visual disturbance was a premonitory sign in 5% of *all* the cases.

Studies of a history of vertigo only (as opposed to vestibular symptoms in general) in divers have shown that a high proportion of divers (between 12% and 40%) (72, 111, 122) have experienced vertigo at least once in their careers. Decompression sickness (mainly, type II) was responsible for 10% of civilian diving deaths, yet almost half the deaths (30)

*For an earlier, larger, but less delineated, review (1,361,461 decompressions) Behnke (13) should be consulted.

were of *unknown cause* (12). Possibly disorientation complicated by DCS could have been a factor in these 30.

In tests at pressure, although the vestibular-ocular reflex did not change with increasing depth (4), postural disequilibrium (body sway) did increase as pressure increased (5). It is known that noise is an adequate stimulus for the vestibular system (1, 80) and the "Tullio effect" (112) suggests that noise can produce body sway. It has been shown that pressure chambers are noisy (108), and this factor should be considered in future studies.

Reuter (88) feels that "90% of the medical problems of the sport diver are centered around the middle ear."

From these results it appears that vestibular-type symptoms are prominent in compressed air work, yet the actual incidence may be much larger than is reported here. Several reasons are offered for this belief:

1) In compressed gas work dizziness, nausea, vertigo, vomiting and occasionally ataxia are considered as vestibular symptoms although only vertigo is discussed. However, other symptoms also occur from a stimulation to the vestibular system. These include drowsiness, pallor, sweating, salivation as well as various sorts of visual phenomena (e.g., nystagmus, apparent movement). If a liberal criterion is employed when analyzing U.S. Navy diving accident records, as many as 40% of all DCS accidents (oxygen and compression accidents omitted) contain central nervous system symptoms which may indicate vestibular involvement (61), although 15% is a more realistic figure (cf. Rivera [89]). As shown also by Rubinstein and Summit (94) in their study of vestibular derangement, vestibular involvement appears to be on the increase. This increase may be connected with saturation diving; because of the long periods spent under pressure by caisson workers there may be a connection between them. Perhaps much is to be learned by reviewing the older literature of caisson work.

2) It is generally agreed that CNS-type symptoms occur earlier following decompression than do pain-type symptoms. If so, it is possible that vertigo could precede experiences of localized pain and be ignored or self-limited, either by keeping the head still (c.f. Bennett et al. [16] where this worked while at pressure) or by sleep.* Pain could develop later. Therefore a physician could see a patient who presents with pain symptoms but who may have previously had vestibular symptoms. These vestibular symptoms may be missed in the course of treating the former. In addition, vertigo or dizziness may not be considered manly by respondents (e.g., Navy divers) and may not be reported as frequently for this reason.

3) Diver accident records used by the U.S. Navy contain a broad space in which to record pain-type symptoms and the physician is encouraged by the layout of the form to add descriptive comments for pain beside the time it occurred. However, he is enjoined from doing this for vestibular-type symptoms since the space beside dizziness, vertigo, etc. is blackened out.

4) There may be a tendency to consider type I and type II symptoms of DCS as mutually exclusive categories (see McCallum [73] for a review of DCS studies reported between 1914 and 1966). Thus if a person reported severe pain and mild dizziness there might be a tendency to classify this as type I. Furthermore, the tangibility of the pain-type symptoms, with probable higher cure rate, may also cause this type to be favored as a diagnostic category.

*Alcoholic intoxication could potentiate the vestibular symptoms (18) and/or enhance sleep.

5) Provocative tests of eustachian tube clearing at 50 p.s.i. are conducted prior to Navy diver training (114) but not necessarily prior to civilian SCUBA training. If eustachian tube patency is negatively related to a susceptibility to vertigo, then, other things being equal, data from Navy diver records may underestimate the problem when generalized to include the potential incidence of vestibular problems in all diving.

6) Because a form of apparent movement is experienced less by alcoholics (123) and because a "fullness of habit" ([116] and others) is common in compressed air workers, perhaps experiences of vertigo in career divers may be less in these groups than in sport divers.*

7) Sometimes vestibular-type symptoms are not listed in reports of decompression sickness (c.f. Paton and Walder [82]). One must assume that either too few to mention were obtained, or they were missed on the patient's interview because the response category (e.g., dizziness, vertigo, etc.) was not on the physician's form.

8) The qualitative coding of U.S. Navy DCS symptoms employs higher numerical numbers for vestibular-type symptoms (e.g., "12" and "20" for "dizziness/vertigo" and "equilibrium disturbances") (30) and these symptoms tend not to be grouped together. Computer sorts, performed serially, could underestimate the incidence of these symptoms, if they occur along with other symptoms with lower code numbers, since the computer cards would need to be replaced to check on the incidence of these symptoms after they had appeared in other categories.

9) Nystagmus (spontaneous and otherwise) has been mentioned directly (54, 109) and indirectly (19). In addition, disconjugate eye movements in decompression have also been mentioned (35). These eye movement responses are generally not listed as a sign or symptom of DCS in the reviews which have appeared (c.f. #1 above). Yet characteristics of nystagmus (direction, rate, frequency, etc.) are a useful aid to diagnoses of problems involving vestibular pathways.

10) Reporting the symptomatology of altitude DCS, Gray et al. (43) consider nausea, vomiting, pallor, sweating, faintness as circulatory reactions, although vestibular stimulation causes similar symptoms.

11) In some studies of DCS ([41] and others) only cases "severe enough for the man to bring himself for treatment are included." This approach should overestimate the incidence of type I (pain) and underestimate type II (CNS) and consequently vestibular involvement since the latter are generally considered as type II symptoms.

12) Because the incidence of the individual symptoms reported by investigators usually adds up to 100% (c.f. Erdman [36]), this means that accessory symptoms or benign, accompanying symptoms are often ignored in these reports. Thus there may be a tendency to classify a symptom according to the patient's discomfort rather than other considerations. Pain is tangible and hurts, and type I diagnoses may be made even though mild type II symptoms may also have been present but are not reported or recorded.

13) Although pressure vertigo probably is more common in divers than in flyers, reference to this malady was not seen in the *U.S. Navy Diving Manual* (114) although it is mentioned in the *U.S. Navy Flight Surgeon's Manual* (115).

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*This factor may be a training or natural selection variable since a high fluid exchange rate may be a consequence of "fullness of habit," but a high fluid exchange rate has also been shown to afford some protection from decompression sickness (125,126).

For these reasons and others, it is felt that greater attention should be paid to vestibular involvement in compressed air work.

Conclusions and Recommendations

The results reported above indicate that greater attention should be paid to vestibular involvement in hyperbaria. Future plans and programs should include:

1) *An improved nosology for the differential diagnosis of vestibular involvement should be developed.* Edmonds (33) has suggested a useful classification system for vertigo, but his system should be broadened to include other forms of vestibular symptomatology (e.g., nystagmus, ataxia, nausea, disorientation, etc.), which also occur under water and pressure. Speculation about the potential causes of these vestibular symptoms should be made more freely in order to aid others in differential diagnoses, and so that symptoms of vestibular origin can be separated from the same or similar symptoms due to other causes. In compressed air work the mechanisms which could involve the vestibular system and result in vestibular symptomatology are:

a) Bubble formation and lesions which can be:

i) Cerebellar (36, 116)
ii) Medullary nuclei (91)
iii) Semicircular canal (52)
iv) Other "aural lesions" (e.g., nerve VIII, utricle, etc.) (93)
v) Spinal (96)
vi) Cervical (56)
vii) In areas (floor, ceiling) of the IVth ventricle

b) Caloric irrigation (66, 90, 107) of the external ear by water or *gas*
c) Temporomandibular joint problems (27*, 51*, 58*, 84) from long-term mouthpiece use
d) Noise (1*, 80*) from chambers etc.
e) Wax/cerumen in the ears which could result in different caloric, or pressure, stimuli to the ears (37)
f) Exostoses (11*)
g) Barotraumatic otitis media (14, 63, 75)
h) Differential pressure of external auditory canal due to a seal by the pinna (79)
i) Inner ear barotrauma (33) perhaps due to "pressure...because of blocks in endolymphatic circulation" (3†)
j) Cupulolithiasis (95*,‡)

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*These references do not specifically connect diving with the vestibular problems which are cited although it is felt that such might exist.

†This may be the same as (*a*)—bubble formation/lesions.

‡Aside from compressed air work, whether the otoconia (calcium carbonate concretions) from the utricle can be given off and lodge in the ampullae of the posterior (inferior) canal is not certain but possible (70). However, the symptoms reported by Sundmaker (109) in a deep dive occurred after a long latency following a pressure change. This long latency suggests that a biochemical explanation is more probable than a neural or mechanical one and cupulolithiasis may be an analogous syndrome (70). Further, the fact that Radomski and Bennett (87) showed that increased calcium retention occurred during an exposure to high pressure supports this notation and may also be related.

There are other circumstances or syndromes which have occurred in hyperbaria where vestibular-like symptoms are reported but where it is uncertain whether the vestibular system is or is not directly involved. These include: migraine (7, 34); Valsalva problems (33); syncope (65, 67); oxygen toxicity and related gas mixture problems (c.f. Bennett [15]); the inversion of the stomach contents with respect to gravity when descending or swimming downward (37); cerebral gas embolism (40); circulatory problems (43); perceptual problems occasioned by the environment (viz., visual articulation, submersible motion, neutral buoyancy, etc.) (c.f. Kennedy [59]); sudden deafness which occurs occasionally after deep diving (50) and others.

2) *A questionnaire should be developed to serve as a controlled interview to document the experiences of divers related to vestibular symptomatology and to define the magnitude of the problem.* For leads in such an effort, studies by Clark and Nicholson (25), Flanagan (38), Graybiel and Clark (44), Hardacre and Kennedy (49), Vinacke (117-121), and Pashalian et al. (81) should be consulted.

3) *A study of the following relationships may be interesting:* vestibular DCS (91); Ménière's disease (103); syncope/vagotonia in high pressure and after decompression (24, 26, 32); orthostatic intolerance from water immersion (42); sodium retention in high pressure environments (87); migraine after decompression (7); release of ADH after vestibular stimulation (110); and the advantage of a high fluid exchange rate in protection from DCS (125, 126).

4) *EEG changes have been observed in caisson workers who have had vestibular "hits"* (92). Since the following pertain, these interrelationships should also be studied: EEG changes (microsleep) occur in connection with the high pressure nervous syndrome (39); REM sleep is absent when lesions in the vestibular nuclei have been performed (76, 86); electrooculograms can influence EEG's (71, 77); and high pressure can influence eye movement responses (31).

5) *The incidence of all forms of DCS is probably about 1% of all exposures (29, 57). However, a very low base rate (0.0318%) occurred when three factors were employed to preselect caisson workers:* ear drum inspection; ability to equalize (ear drum) pressure; and no untoward effects on the first work shift (64). In addition, Kelly and Langheinz (58) have shown that dental adjustments made surgically can be used to correct acute otitis media. The relationships between the findings of Kooperstein and Schuman (64) and Kelly and Langheinz (58) should be explored relative to the labyrinth and for whatever aid they may provide in understanding various forms of DCS.

6) *It was shown that while less than 1% of all decompressions result in accidents, more than 15% of all career workers develop aseptic bone necrosis (6), even though they may not have experienced DCS symptoms per se.* Perhaps a similar "iceberg" relationship exists regarding vestibular decompression sickness. The reports by Rozsahegyi (91-93) in caisson workers suggest that a vestibuloneurological examination of divers should definitely be undertaken (c.f. Walder [124]) before and after work in compressed air environments.

7) *If the embryology of sensory systems is considered in connection with DCS, a better understanding of the mechanisms involved may result.* In the case of chemoreceptors (e.g., vision) and osmoreceptors (e.g., taste and smell), development occurs differently from development of mechanoreceptors (vestibular, cutaneous, auditory). The latter develop in embryo from the alar plate which gives rise to what become the vestibular (N VIII) nuclei.

From these nuclei what ultimately become vestibular, auditory and cutaneous-proprioceptive systems develop (103, 106). In addition, the corpora quadrigemina and the cerebellum are also embryologic outgrowths from the same origin. Perhaps auditory, skin, and vestibular symptomatology of DCS should be considered together because they have similar beginnings. Furthermore, tremor may be a cerebellar (or vestibular or spinal) phenomenon related to the dizziness and nausea cited above, and both could also be related to minor pain sensations which are observed in connection with the high pressure nervous syndrome.

Summary

The vestibular apparatus is phylogenetically and ontogenetically a primitive and early developing sensory system. The system is anatomically lodged in the petrous portion of the temporal bone, and vestibular responses are largely automatic and unconscious. Therefore, it is—physically, physiologically and behaviorally—probably the least accessible of all the sensory systems. Yet the evidence is mounting that this system is being implicated more and more in human compressed air work. It is urged strongly that greater attention be paid in the future to the involvement of vestibular functions under water and high pressure.

Other implications not mentioned in this report but cited elsewhere (68) include: 1) the use of the vestibular system as a navigation aid; 2) vestibular problems underwater of a spatial orientation-disorientation nature; and 3) the utility of baseline vestibular pretesting to determine whether vestibular damage occurs following a career in compressed air work.

Acknowledgments

The opinions and statements contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

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PART IX. PERCEPTION, PERFORMANCE, COMMUNICATION*

DISCUSSION

A. Bachrach, Chairman

Dr. Walder: Vestibular symptoms occur frequently in compressed air workers. We occasionally get the man who tells us that he is suffering from nystagmus, but it is usually after a policeman has found him swaying, and there is always this confusion as to whether it is alcohol or some result of the decompression.

Dr. McCormack: To comment on Dr. Hollien's paper, I believe he was convinced that loss of hearing found in his work was most likely a conductive loss at pressure and that there was no evidence in the literature that it might be sensory neural. We have evidence obtained in our laboratory that this may not be the case.

We have studied the cochlear potentials in the cat and the guinea pig at increased pressure to see what kind of hearing loss, if any, they had. Our evidence confirmed the conductive hearing loss in a 300-foot chamber dive on air, but also we found a slight, moderate, sensory neural inner ear loss. Since our measurement is the cochlear potential we can say for sure that this is a deficit of the sensory hair cells in the inner ear. I cannot say what is happening to the brain, but here is a case of sensory neural inner ear loss at pressure. We did calibrate the sound field at increased pressure.

Earlier discussion here concerned Langley's observations of evoked potential losses at pressure, with a question of whether narcosis led to these losses. The cochlear potential we are measuring has been shown not to be sensitive to anesthetics at 1 atmosphere. Therefore, a narcotic mechanism is probably not likely with inert gases at high pressure.

Dr. Brauer and I have done studies in which barotrauma was eliminated, exposing guinea pigs to high pressure neurological syndrome and bringing them back. There may in this case be interaction with high pressure neurological syndrome, but without barotrauma. Measurements are not being made at pressure, but effects could have occurred at pressure. After the exposure we find the same loss that I referred to in the cat. This may be another sensory neural loss at pressure.

Another comment derives from our past work in the porpoise. Dr. Hollien has shown today that when man goes from hearing in air to hearing underwater, he goes from aerial conduction hearing and use of the ear drum and the ear canal to bone conduction hearing. When Weaver, Ridgeway and I carried out our work to determine how the porpoise hears there were many theories about how its ear works, whether by air or bone conduction. It is clear that evolution set up the same experiment that Hollien did, because once the porpoise was a primitive "horse" or "cow" and had an ear which adapted to hear in air just like ours, and then evolution occurred in reverse and the porpoise went into the ocean, just like Hollien's subject goes underwater to have his hearing tested.

Our studies on the porpoise showed that the porpoise hears by bone conduction only, just as Hollien's subjects do underwater. We tested whether there was any function of the ear drum and the ear canal in the porpoise, and this has been lost in evolution.

Dr. Farmer: I agree with Dr. McCormack in his analysis of the last point he made about Hollien's data. It demonstrates that underwater hearing in liquid form is by bone conduction.

In reference to what Dr. Hollien said about previous experiments not showing any neurosensory decrements in hearing at pressure, he was referring to human experiments in which there were apparently no incidences of barotrauma and which were apparently nontraumatic experiments as far as the ear was concerned. Our work at Duke plus his work have not shown any psychoacoustic evidences of inner ear dysfunction, and this is certainly not

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* *Panelists:* J. Adolfson, R. W. Hamilton, Jr., R. S. Kennedy, J. M. Walsh and D. Zannini.

to say that they cannot occur. We have been doing some of the animal experiments at Duke that Dr. McCormack referred to, trying to do some of his work plus some more, and we are having trouble separating the cochlear loss that we see from excessive noise exposure. There appears to be (this is not confirmed yet) a significant noise effect, at least in the guinea pig, with an intact middle ear, and if the middle ear is removed, the cochlear depression that we saw with the intact middle ear is not found.

This brings up another point which Dr. Hollien referred to: the temporary threshold shift leading into permanent threshold shift. The audiograms which our colleagues from Italy showed us look like what we in otolaryngology refer to as classically noise-induced hearing loss. Noise measurements in our chambers have at least confirmed that the noise levels on compression and decompression are very high.

Dr. McCormack: I mentioned that we found sensory neural hearing loss at pressure, and I believe Dr. Farmer is implying that this might have been caused by noise in the chamber rather than pressure itself. We have attempted to eliminate this factor. During compression and decompression, when noise periods occur, we record sensory cell output from the round window at the inner ear. It is well known that to get overstimulation of the ear you will have an output from these hair cells of as much as a thousand microvolts before the ear overloads and you get inner ear damage from noise. At no time during the noise of our compression did we ever have more than 3 microvolts coming out of the ear. Therefore, I do not think noise was a factor in the sensory neural involvement.

Dr. Davis: Dr. Hamilton, there is evidence that time estimation is affected by changes in body temperature and also that it undergoes diurnal variation. Was body temperature constant in subjects when this was tested and was it done at the same time of day each time?

Dr. Hamilton: The subjects were able to control the temperature in the chamber to their desires for reasonable comfort. It was not possible to do all measurements at the same time of day.

On a different topic, we are concerned about the effect of the auditory signal in doing auditory evoked responses. One of the presumed symptoms of nitrogen narcosis is a heightened sensitivity to sound. Divers complain that the noise of the scrubber is a lot louder when the air fills the chamber instead of helium. I wonder what a complicating factor this must be in using the auditory evoked response to study narcosis.

Dr. Farmer: We have thought about this phenomenon of heightened sensitivity to sound. I think this is a central phenomenon, not concerned with getting the signal into the central nervous system per se. I do not think it has anything to do with the ear or organ of hearing per se—conduction across the middle ear and the cochlear threshold per se. It is probably related to a phenomenon going on in the auditory cortex itself.

Dr. McCormack: It is well known that the potential of the sensory hair cells of the inner ear is not affected by anesthesia, nitrous oxide or narcotic agents. I would agree that any effect is probably further up in the CNS, and must be studied with evoked potential equipment.

Dr. Bachrach: With regard to performance in air versus in water, we tend to oversimplify the transference of skills from one medium into the other. Dr. Egstrom has shown with underwater work that actual training in the water is a critical factor.

Dr. Behnke: Captain Bornmann, in the extensive U.S. Navy Experimental Diving Unit test program between 1960 and 1970, is it not true that these individuals had the vestibular disturbances only during the course of subsaturation dives, meaning that decompression was probably rapid in the early stages? In the steady-state equilibrium dives or saturation dives, there appear to have been no vestibular disturbances or injuries.

Dr. Bornmann: Up to my last direct contact in 1969, it was true that no case of ear involvement had occurred as a result of saturation dives conducted in accordance with the U.S. Navy procedures. It is still true according to Commander Sparr, the present medical officer of the Experimental Diving Unit.

Dr. Behnke: With regard to measurement of psychomotor performance and fatigue, would it not be more correct to state that even the excellent tests used, and the refinements which we have today for evaluating performance both in man and in animals, are nevertheless largely relative? By that I mean a series of tests are run on individuals for control value, and then stress is applied. But suppose the basic state of the individual is entirely changed. I speak now of the men who, in World War II, came in from submarine cruises after being out three months and were stale. They just could not respond quickly. Now, suppose you started with this type of individual—the individual in a state of chronic fatigue—and you then established your baseline and applied your stress. You may well get the same type of difference that was reported here.

This very difficult problem of the individuals who are in a state of chronic fatigue is an important one because in many of the diving operations today, that extend over such a long period of time, the state of the individual changes greatly.

Dr. Miller: There was some work done using prisoners to train them to do underwater work in California. These were nondivers; they were taught to dive and subsequently taught to do work. Their first dive was made

blindfolded; all subsequent dives were made blindfolded and they were taught to do work in dirty water where most of the diving work is done. These men not only learned faster but performed better than people who learned in clear water and transferred. To go from the laboratory into the water, the real work situation should be closely simulated.

Dr. Lundgren: There have been young people who have been blind from early age who have learned to dive. They have done some useful and remarkable work under severe conditions (mainly in the Stockholm area) and were considered in some ways to perform a lot better than normal-seeing divers.

Part X. THERMAL BALANCE

THERMAL STRESS IN UNDERSEA ACTIVITY

P. Webb

Thermal problems in diving have gradually become more prominent as improved techniques for respiratory gas exchange have permitted men to live and work for longer times at greater depths. Thermal papers in preceding Symposia on Underwater Physiology reflect the changes in both thermal research and the techniques of diving. In 1963, Beckman (1) was concerned with heat loss from swimmers and from experimental subjects who were immersed to the neck in cold water. He drew attention to the insulating value of subcutaneous fat, defined convective heat loss from the skin to water, and described how foam neoprene wetsuits effectively reduced this loss. He predicted the need for supplemental heating, which he assumed would be in the form of electrically heated underwear. In 1967, Raymond (22) was mainly concerned with the increased convective heat transfer in hyperbaric environments, particularly those in which helium was the inert gas. Craig (5), in 1971, summarized the studies carried out on the Japanese and Korean ama, who have been diving in cold water for upwards of 2000 years. Among the many interesting things learned from these women was that they stopped their day's diving activities when their internal temperatures reached 35 °C—although thermometers are not part of their regular equipment. Craig also reported on the water temperature in which nude subjects could maintain thermal balance; the harder people exercised, the lower was the neutral water temperature. He warned that standard methods for estimating total body heat loss from the theoretical computation of the change in mean body temperature were suspect, and that definite results could come only when experiments were done with direct calorimetry. Rawlins and Tauber (21) focused on the thermal problems of a diver working at depths of 600 feet and beyond. They pointed out that foam neoprene wetsuits are poor insulators at depth, even when partially re-expanded with the breathing gases of an underwater habitat. They emphasized the serious thermal drain involved in breathing gas at the prevailing temperature and pressure of water at 600 feet. They estimated that the respiratory and body surface heat losses could be balanced only if 1500 to 3000 watts of supplemental heating were added to the diver. Their theme was that better insulation could be developed, breathing gas could be warmed, hot water could be flooded through the suits, and personnel transfer capsules and deck decompression chambers could and should be insulated and heated. In other words, there were direct engineering solutions for the serious problem of heat loss at great depth.

Within the past few years, research in thermal stress has grown considerably in two major directions: first, specific measurements of the thermal and energetic balance in hyperbaric environments; and second, a continued and more sophisticated effort aimed at measuring the quantity of heat lost in cold water, both from the body surface and from the respiratory tract. What is the background for thermal research in the present stage of diving technology?

Diving is traditionally a cold occupation, but divers are tough and expect to be uncomfortable, to have numb hands and feet, and to shiver at the end of a dive. In commercial and salvage diving in cold water, relief can be had by hot water delivery to the diver's suit from a surface source, but the thermal problems in excursion dives from a deep habitat or during long transfers in a personnel transfer capsule are not so easily remedied. A diver leaving the habitat for an excursion dive or leaving a personnel transfer capsule in deep cold water badly needs a well-insulated suit and good supplemental heating, neither of which is readily available as yet. In these deep dives there is not only direct heat loss from the body surface to the water, but respiratory heat loss is also a major drain. As heat is lost, a diver may unknowingly begin to lose critical mental faculties and, if he gets in trouble, he is terribly remote from topside help.

Adding to the problem, when the diver returns to the habitat, it now looks as if this is not an ideal place to rewarm, since the highly convective gas environment appears to drain body heat despite temperatures that feel comfortable. Ordinarily a commercial diver is expected to dive only once a day, and his rewarming can continue until the next day. In the present era of saturation diving, it is common to expect a man to dive more than once a day, since there are no long decompression schedules to endure. But how can the diver, or a trained observer for that matter, tell if rewarming is complete? To start a new dive with lowered body temperatures means experiencing the penalties of being severely cold sooner: poor performance, lethargy, confusion. Only the most general guidelines exist to warn when these dangerous signs will appear in terms of loss of heat content; moreover, precise procedures for knowing how much heat a man has lost have not yet been established.

It should be clear that major loss of body heat is being referred to here—and not just cold hands and feet or brief shivering. The physiological responses of vasoconstriction and shivering are too feeble to make much difference, and the diver can be virtually helpless, physiologically, while heat drains away until some tolerance point is reached. It is not definitely known whether these severe losses of body heat are responsible for some of the unexplained losses of life in diving, but Rawlins and Tauber (21) hinted strongly that a repeat dive for an already cold man may have contributed to the fatality which brought the Sealab III project to a premature end.

It is true that many cold diving situations can be handled with protective clothing and supplemental heating, either through hot water flooding in the suit or from electric heating. Jegou (11) has described a successful 560-foot working dive carried out off the coast of Labrador in water temperatures around $-1\,^{\circ}C$, where the thermal problem was successfully handled with a constant-volume dry suit, electrically heated underwear, and electric heating for the breathing gas. And there have been adventuresome dives under the polar ice, as described by Bright (3) and by MacInnes (15), carried out with conventional SCUBA and well-made insulated suits. Notice, however, that they were relatively shallow, and the men operated from shelter at sea level. A constant-volume foam neoprene-insulated dry suit, like

the Swedish Unisuit, works very well for this sort of diving, especially when the depth is no greater than 50 or 60 feet. But, as both Beckman (1) and Rawlins and Tauber (21) have pointed out, foam neoprene is severely compressed and loses much of its insulating effectiveness at greater depths.

There is evidence that better insulation at depth and effective supplemental heating are becoming available, although their use is just beginning. Noncompressible insulation has appeared in prototype form, and the constant-volume dry suit with insulation, worn as underwear, was successfully demonstrated by Jegou (11) and Rawlins and Tauber (21). Hot water delivery, either from the surface or from an umbilical connected to an underwater habitat, circumvents the major problems of body heat loss, and there are portable heat sources under development which show promise. One of these (the Conox heater developed for the Navy by General Electric Co.) makes use of the thermal energy involved in oxidizing metallic magnesium; it delivers 6 kilowatt hours (20,000 Btu) at a maximum rate of 2.4 KW, and circulates water at the rate of 3.8 L/min (one gallon per minute) in a package weighing about 35 pounds.

Excellent new literature in the area of survival in cold water exists. Although these reports do not bear directly on thermal stress in undersea activity, they contribute materially to understanding man's response to the severe cold stress of cold water immersion, and to the principles involved in protective equipment. *Survival in Cold Water*, by Keatinge (13) deals with recent knowledge in this general area. Human temperature response to simulated survival in Arctic waters, using various life rafts and clothing assemblies, has been reported by Veghte (27). Hall (8) has developed a model that allows prediction of tolerance time for subjects with various types of clothing, either in water or in life rafts. The model includes the variables of compression by hydrostatic forces and decreased insulation caused by wetting of the clothing.

The present study is concerned with the following topics: convective heat transfer in water; the effects of exercise; quantifying body heat loss by calorimetry; respiratory heat loss at depths beyond 600 feet; the problem of rewarming; comfort and heat loss in hyperbaric helium; and energy balance and weight loss under hyperbaric conditions.

Heat Loss in Water

Direct loss of heat to the water is the dominant thermal problem of the diver. He limits his dive time probably as much because of body heat loss as from any other factor. This study summarizes the current knowledge of heat loss from the body surface to the water and of how the body limits heat loss from the core as a response to cooling; it also tries to answer the question of how much heat loss is critical.

Direct measurement of the loss of heat from the skin surface to water has been reported by Boutelier et al. (2), with the technique of partitional calorimetry. Using nude subjects in bath temperatures between 32.5° and 33.5°C, they found that in a well-stirred bath the combined convective and conductive heat loss coefficient was 63 watts/meter^2-°C (53 Kcal/m^2-hr-°C). This value closely matched several other values in the literature. They pointed out that in still water the coefficient is 44 watts/m^2-°C (38 Kcal/m^2-hr-°C), but even the small agitation of water from shivering is enough to raise the value to that for stirred water.

Analytical approaches have been taken with rather different results. Rapp (20) showed that the coefficient of heat transfer by conduction is small and constant, despite various swimming velocities; his value is 11 watts/m²-°C. The convective transfer coefficient (h_c) is given as 94 watts/m²-°C in still water, increasing steadily with increasing water velocity up to 400 watts/m²-°C at a swimming speed of 0.5 m/sec. Using a slightly different analytical approach, Witherspoon et al. (34) show even higher values for the combined transfer coefficient as a function of water velocity, but in the same report their values derived from measured heat loss from a copper mannikin in moving water are fairly close to those of Rapp. This work is summarized in Fig. 1, which shows heat transfer coefficient plotted as a function of water velocity, or swimming speed, from all three authors. Boutelier et al. did not give a figure for velocity for their stirred bath, so a velocity of 0.05 m/sec has been assumed. The figure emphasizes the considerable discrepancy between calculations, measurements taken on a physical model, and measurements on humans. There may be several explanations for this: the surface temperature measurements on humans may be in error or the assumed values for the skin to water gradient may be in error; the assumption of cylin-

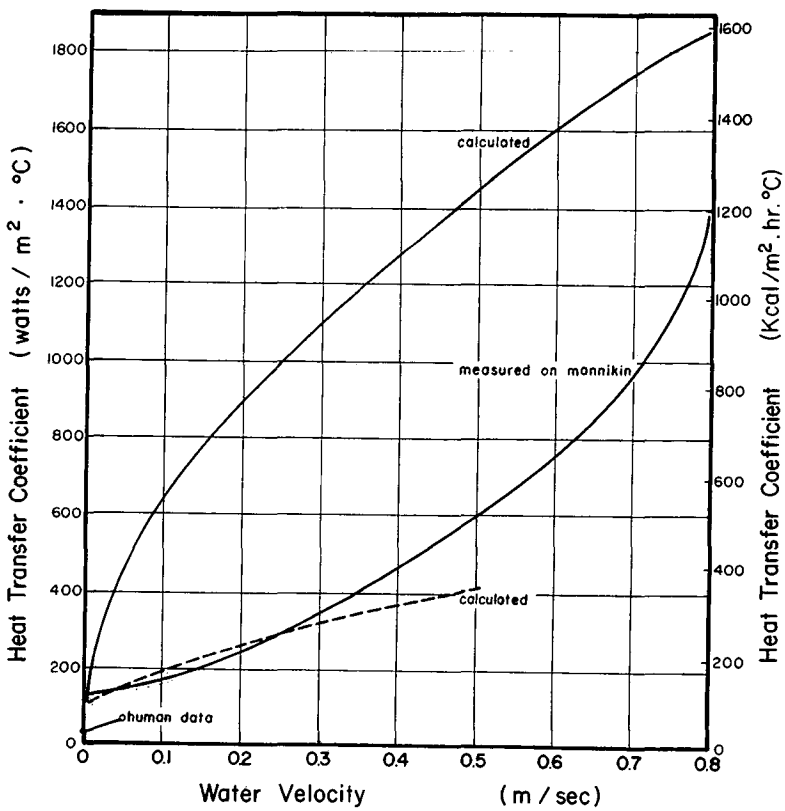

FIG. 1. Values for the combined convective and conductive heat transfer coefficient from body surface to water as a function of water velocity, according to several authors. The solid lines labeled "calculated" and "measured on mannikin" are from Witherspoon et al. (34); the dashed line from an analysis of Rapp (20); and the "human data" from Boutelier et al. (2). Possible reasons for the great discrepancies between these curves are given in the text.

drical shapes for the analytical models may be misleading; countercurent heat exchange in blood vessels to the limbs and the matter of shell cooling while the core temperature is maintained may throw off the theoretical analysis; and the whole problem of measuring heat storage or assuming some value for it may account for much of the discrepancy. The way to find the correct value for h_c is by direct calorimetry in a water bath, a technique which Craig and Dvorak describe (6). In my laboratory a different direct calorimetric approach is being combined with exposure to cold water while wearing a lightly insulated diver's suit. The storage term seems to be the one which is the most difficult to handle and the one which is most likely to lead to errors of estimate.

The response of the body to strong cooling is a distinct one which serves to conserve body heat, at least in the core. There is a considerable literature on this topic, the majority of it concerning the nude resting man in cold air. However, some work has been done on resting and exercising man in the water, and this topic has been most recently reviewed by Bullard and Rapp (4). It is useful to restate the major points concerning the body's attempt to conserve heat during cold-water exposure.

The strong cooling sensation one experiences on entering cold water is matched by a rapid drop in surface temperature, the result of both the cooling by the water and the constrictive response of the cutaneous blood vessels. During the first 10 or 15 minutes of a given cold exposure, while a new level of skin temperature is being established, the conductance of heat from the center of the body to the surface is being reduced. The ratio of conductance with full cutaneous vasodilation compared to the conductance in cold with full vasoconstriction is on the order of 15 to 1. The change in conductance is not uniform over the body; it is most marked in the hands and feet, next in the arms and legs, less in the trunk, and least of all in the head. In his review, Beckman (1) noted how important the loss from the head is. Since laboratory studies of immersion are usually done with subjects whose heads are out of the water, this area, as a site for heat drain in divers, has probably been underestimated. The importance of the head as a major site for heat loss has been emphasized in the studies of Nunneley et al. (18) and Schvartz (23), who showed that direct cooling of the head can remove considerable heat with no evidence of local cutaneous vasoconstriction. Loss of heat from the head and neck is considerably greater than its small area (7% of the body) would suggest. Incidentally, the ama of Japan and Korea who are daily exposed to cold water make a ritual of wrapping the head and neck in protective clothing (5). Realistic values for body heat loss should be measured in men totally immersed and swimming.

The concept of greater heat loss from the shell of the body, as compared to the body core, is closely related to the preceding statement concerning how conductances in various parts of the body change differently in response to cold. The shell is sacrificed, thermally speaking, as blood flow is restricted in the skin, and there is significant heat conservation in deep arteries and veins of the limbs by the countercurrent heat exchange process. Thus the hands, feet, arms, and legs become colder and colder while the deeper portions of the trunk and head are maintained at or near the predive core temperature. This concept has considerable experimental data to support it. The difficulty for the experimenter is that standard methods for computing change in body heat content or reduction in heat stores, which are normally estimated from the surface and core temperature measurements alone, do not estimate the large losses possible from limb tissues as compared to those of the trunk and head.

Another caution—experimental data should come from working men, yet most of the literature deals with resting subjects. Note some of the differences. Convective transfer increases sharply with water velocity, as shown in Fig. 1. The effect of exercise on the core-shell concept, and hence on values for conductance in different parts of the body, is not known, but is surely important. Underwater swimming involves major muscle masses of the legs and back, as the total heat production becomes three to six times the resting heat production. There is not only more heat on the positive side of the heat-balance equation, but also blood flows and tissue temperatures are very different in active limbs compared to resting values. A general difference between exercising and resting men was described by Craig (5). He noted that the neutral temperature, that is the water temperature for nude men whose ear canal temperature did not change in an hour of exposure, was 35°C for resting men, just under 32°C for men working at two times resting metabolism, and 26° for men working at three times resting metabolism. In addition, Moore et al. (16) have shown that heart rates are significantly lower for men working in cold water compared to their heart rates for the same work loadings and oxygen consumptions in air.

Some preliminary results from current work in my laboratory illustrate several of these points. Two subjects of about the same weight, 70 and 72 kg, one of them with a thin layer of subcutaneous fat, the other with a heavier layer on his torso (trunk skinfolds 8 mm vs. 18 mm), swam underwater at water temperatures of 5°, 10°, and 15°C. They wore cotton underwear and a diver's rubber dry suit. Dives lasted 1 hour unless terminated sooner by the subjects; some were as short as 35 or 40 minutes. Temperature data before, during and after the dive were taken in the core, thigh muscle, subcutaneous fat layer, and on the skin. Calorimetric heat balance was established before the dive, and a calorimetric restoration of initial thermal state was accomplished by rewarming in a suit calorimeter (33) after the dive. Table I shows the change in several temperatures from before the dive to the end of the dive, and also the quantity of heat lost for each water temperature. Core temperatures are not given, since the major drop in rectal temperature occurred during the rewarming period, and changes in ear canal and esophageal temperatures had different time courses. Note the large quantities of heat lost, especially by subject B, the fatter man. This was measured

TABLE I
PREDIVE AND POSTDIVE TEMPERATURES AND BODY HEAT LOSS

Water Temp. (°C)	Subject	Change in Mean Weighted Skin Temp. (°C)	Change in Subcutaneous Temp.		Change in Thigh Muscle Temp. (°C)	Heat Loss (Kcal)
			Chest (°C)	Calf (°C)		
5	A	−11.0	−2.9	−12.3	−6	172
	B	−14.7	−15.7	−17.3	+1.4	292
10	A	−10.6	...	...	...	184
	B	−13.8	−10*	−12.6	...	243
15	A	−11.1	−6.7	−9.0	...	194
	B	−9.7	−5.7	−9.0	+1.2	194

*Estimated.

both calorimetrically, as shown in the table, and also estimated from the various ways available for computing change in mean body temperature (not shown). Incidentally, subject A's higher losses in the warmer water are explainable by such factors as varying lengths of exposure and uncontrolled variation in the quantity of insulating air trapped in the dry suit.

The skin and subcutaneous temperature changes for subject B with the thicker fat layer on the torso are greater than those for subject A with the thin fat layer. Thus B's heavier shell gave up more heat. Notice also that B's muscle temperature increased across the dive in both the 5° and 15°C water, and the same increase has been observed in a third subject, also thin, in a 10°C dive.

Thermally speaking, the body mass must be not just core and shell, but at least three compartments: the skin and subcutaneous tissue including hands and feet; the active muscle mass; and the central organs. Certainly these three compartments have different temperature behaviors. In earlier work, when only resting man was considered, the muscle compartment was thought to participate in the shell-temperature changes—in other words, it was thermally disposable. This is clearly not true in exercise.

The subjects occasionally had to stop swimming in order to adjust equipment, and it was during these moments that the cold became especially noticeable. There was never any shivering during work for these lightly clad men in the 5°, 10°, or 15°C water, but whenever they stopped swimming, shivering came on quickly. In all cases the skin temperatures were low, but low skin temperature alone is not an adequate signal for shivering. In these experiments, rectal temperature was as high or higher than at the predive level because of the exercise involved, and only in the coldest water did rectal temperature begin to drop during the latter part of the exposure. But although rectal temperature may have been high, the temperature in the ear canal and usually the esophageal temperature were lower than the predive value, suggesting that both the central and peripheral signals for shivering were present. Apparently muscular activity itself suppresses shivering.

How much heat loss is critical and how is heat loss quantified? The answers to both questions are presently unsatisfactory. Without direct calorimetry the usual method of quantifying heat loss is from body temperature measurements; normally one has the rectal temperature and the mean skin temperature to work with, and the changes in these temperatures are supposed to be additive using weighting coefficients, traditionally Burton's 0.65 times T_{re} and 0.35 times $\overline{T}_s$. For the standard 70 kg man, a loss of 150 Kcal is generally thought to be critical, yet Kang et al. (12) estimated that the Korean ama regularly lose 200 to 300 Kcal. Preliminary data, and that of Craig and Dvorak (6), using direct calorimetry, show that heat losses of 200–300 Kcal are what laboratory subjects are willing to endure voluntarily.

Another approach to determine critical cooling is from the rectal temperature measurement alone, despite the fact that this is a notoriously slow body temperature to change. In brief immersions of an hour or two, rectal temperature usually rises, especially with exercise, then slowly drops; it is common to see the major change in rectal temperature occur after an acute cold exposure is finished. But in the long slow cooling procedures used in clinical medicine and in the occasional cases of protracted body cooling from exposure, the body appears to cool more uniformly, and various symptoms have been related to lower-than-normal levels of rectal temperature.

An attempt to define how much heat loss is critical is shown in Fig. 2. The list of symptoms and how they relate to the two scales is speculative. The hope is that others, who have

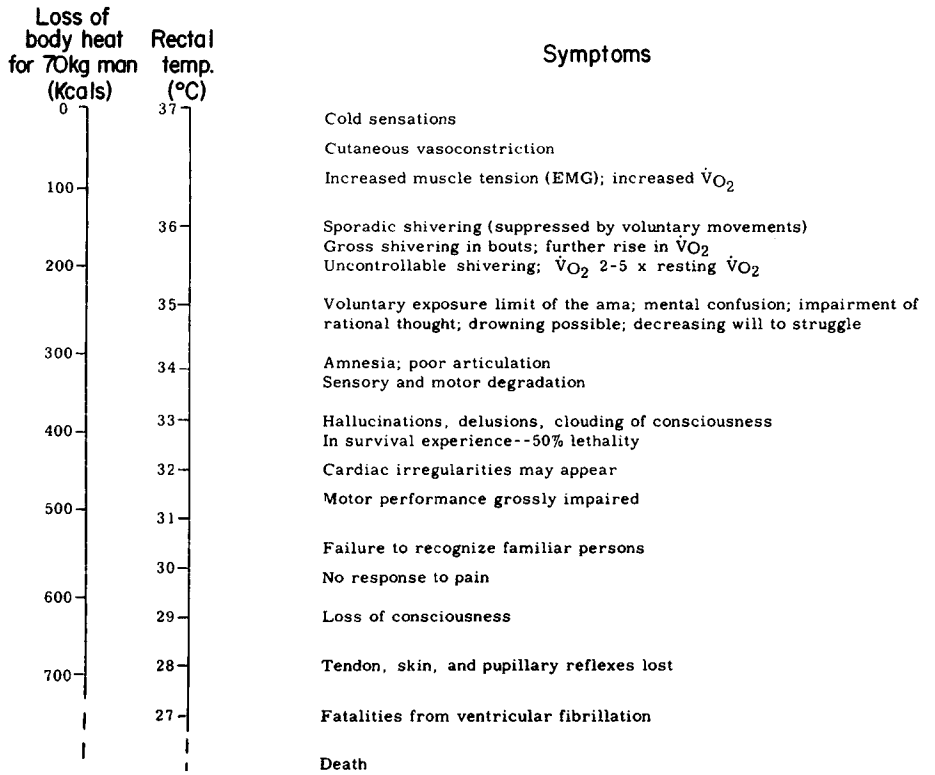

FIG. 2. A tentative ordering of symptoms of severe and prolonged cold exposure related to scales of body-heat loss and rectal temperature. The heat loss scale is linear, the rectal temperature scale is nonlinear at the upper end since the rectal temperature does not reflect the large heat losses from the body shell.

better data, will modify the figure. Perhaps future investigators will give substance and precision to such a chart. However, no one should be encouraged to let volunteer subjects be cooled below a final rectal temperature of 35 °C or permit a heat loss of more than about 300 Kcal, which means we will have to be content in measuring the relatively mild physiological and behavioral changes which occur above this level.

How does one estimate body heat loss outside of the laboratory? It is obviously impractical to measure several core temperatures, many skin temperatures, subcutaneous temperature, and muscle temperature. Nor is it likely that techniques of direct calorimetry can be adapted to field usage. A temperature which should reflect the thermal state of the whole body is that of the central blood, but if arms and legs are shut off during severe cold exposure, their share of the total heat loss and their contribution to the mean temperature of the body would be understated in the central blood. This is an argument against relying on esophageal temperature or the temperature of the auditory canal. And, from present data, estimating caloric loss from deep body temperature and skin temperature, by any of the conventional schemes for deriving changes in mean body temperature, should be discouraged. So there is no good method yet for estimating heat loss, and the matter carries a high priority in current research efforts.

Respiratory Heat Loss

Under normal conditions heat loss from the body via the respiratory tract is something like 10% of the metabolic heat production. The process of warming and moistening inspired air increases with exercise, matching the increased respiratory ventilation, so the percentage stays about the same even in cold dry air. But a man breathing hyperbaric gas is moving in and out of his body a fluid with considerably greater heat capacity than air at 1 ata. As deep diving on helium became more common, respiratory heat loss became a topic of considerable interest. Simple calculation shows that as gas density increases its heat capacity increases accordingly, and if cool inspired gas is warmed to body temperatures and expired at something near body temperature, then a great many calories can be carried away in the exhaled air. Extrapolation of the data from this laboratory (32) indicated that breathing helium–oxygen mixtures at 5 °C, at a pressure equal to that of water at 600 feet would cause the entire metabolic heat production to be drained away independently of whatever additional heat loss there was from the body surface. There was one major question to be answered by direct experiment: was the exhaled gas significantly cooler than 37 °C, hence representing a conservation of body heat? Studies of respiratory heat loss at 1 ata with very cold inspired air had shown that expired air temperatures were progressively lower as the inspired temperature was lower (30), the phenomenon being that of a passive heat exchange in which the tissues of the respiratory tract, having been cooled by inspiring cold air, then absorbed heat from the warm expired air. Definitive studies in hyperbaric gas have now been made, and data from one of these studies (7) will be used. Another is presented in detail by Hoke et al. (9) and by Varène et al. (26).

The magnitude of the heat conservation represented in exhaled gas leaving the body at lower than body temperature should be examined. Goodman et al. (7), in their careful study of respiratory heat loss of divers in cold water in a pressure chamber at depths equivalent to between 450 and 1000 feet, state that the actual loss of heat from the respiratory tract is only 60 to 80% of that which would be calculated if the gas temperature were assumed to be 37 °C. More specifically, they presented data for the exhaled gas temperature as a function of inspired gas temperature, as shown in Fig. 3. Figure 3 also shows curves from Jacquemin et al. (10) and Boutelier et al. (2), but these last two studies were of men breathing air at 1 ata or 5 ata. Notice how much steeper is the curve of Goodman et al., showing, as expected, that the cooling effect of the dense inspired cold gas is pronounced, and the recovery of heat is therefore more than studies at 1 ata would suggest. In fact, at an inspired temperature of near 0 °C breathing helium–oxygen mixtures at 14 to 31 ata, the exhaled air temperature is about 23 °C, which is the same as the exhaled air temperature breathing air at 1 ata at -40 °C (30).

The equation for calculating respiratory heat loss is:

$$H_{resp} = \dot{V}_e \varrho C_p (T_e - T_i) + \dot{V}_e 0.58\,(W_e - W_i)$$

where: H_{resp} is the rate of respiratory heat loss in Kcal/min; $\dot{V}_e$ is the respiratory minute volume in liters/min; ϱ is the density of the gas in gm/L; C_p is its specific heat in Kcal/gm; T_e is the temperature of expired gas and T_i the temperature of inspired gas; 0.58 is the latent heat of vaporization of water in Kcal/gm; W_e is the water content of the expired gas; and

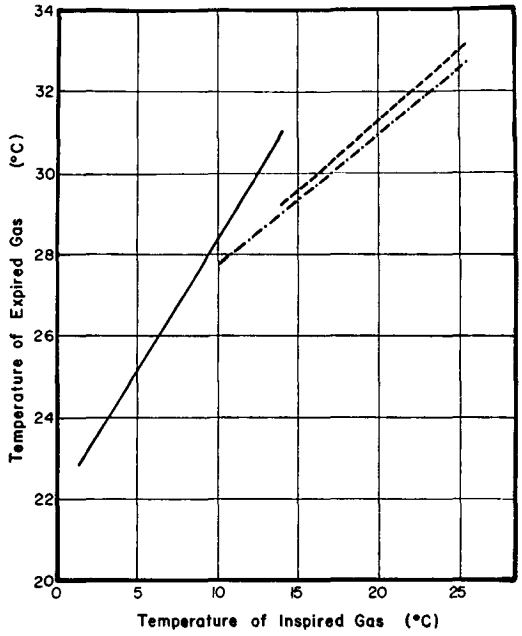

FIG. 3. The temperature of expired gas as a function of inspired gas temperature under hyperbaric conditions according to several authors. The solid line is from Goodman et al. (7) at pressures from 14 to 31 ata, while the two dashed lines are from Boutelier et al. (2) and Jacquemin et al. (10) at pressures of 1 to 5 ata.

W_i the water content of inspired gas in gm/L. The equation makes it clear that once the expired and inspired temperatures have been established, the major variable is respiratory minute volume; the greater the minute volume, the greater the heat loss. Since the density of gases respired at these depths is high, the dominant term is the convective group, which includes the term ϱ. The second term for the water vapor loss, which is dominant in respiratory heat loss at 1 ata, is only about 14% of the respiratory heat loss at 400 feet and about 7% at 1000 feet, according to Goodman et al. (7).

The measured respiratory heat loss in divers swimming in water of 1.67 °C (35 °F) is shown in Fig. 4 for a range of respiratory minute volumes from 0 to 40 L/min and at three depths of water—450, 650 and 850 feet. The same kind of data are shown for men in water of 7.2 °C (45 °F) in Fig. 5. Both of these figures are from Goodman et al. (7). The authors took pains to simulate diving situations realistically and to get correct measurements for the important variables of expired gas temperature and respiratory minute volume.

The values for respiratory heat loss are alarmingly high. It is clear that even at a water temperature of 7.2 °C all of the metabolic heat is being lost at depths of 850 feet or greater. At a respiratory minute volume of 20 L/min, a man's heat production would be expected to run about 120 Kcal/hr, which is the value for respiratory heat loss shown. Similar findings have been presented by others (9, 17, 26).

A further problem arises from breathing cold dense gas: it causes, at least in some men, a copious flow of mucus and watery secretions from the respiratory tract. This makes it difficult to keep the mouthpiece and connecting hoses clear, which has caused the early termination of several experimental dives (7, 9).

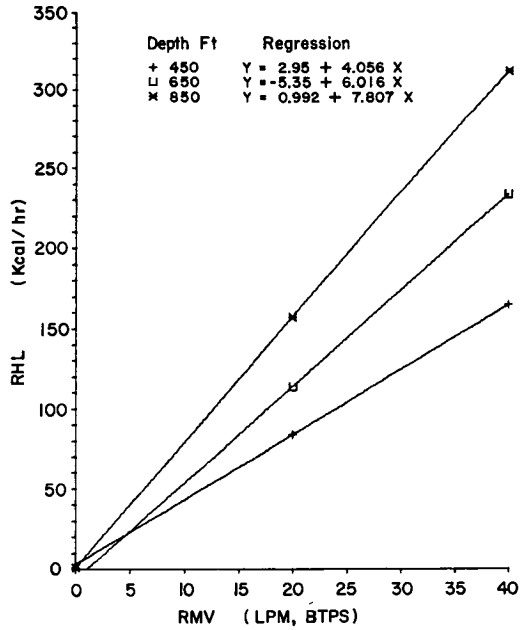

FIG. 4. Respiratory heat loss (RHL) as a function of respiratory minute volume (RMV) during dives with unheated breathing apparatus in water at 1.67 °C (35 °F) at the three depths shown. (From Goodman et al. [7].).

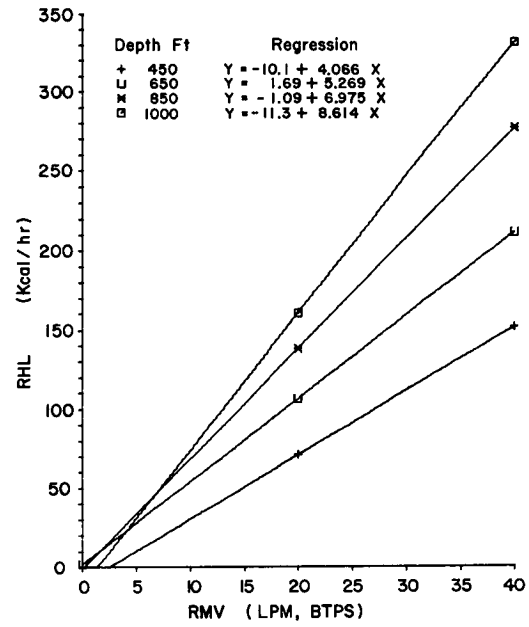

FIG. 5. The same as in Fig. 4, but in water at 7.2 °C (45 °F) and at the four depths shown. (From Goodman et al. [7].)

How important is this information? In the study of Goodman et al. (7), the divers were protected from the cold water with hot-water heated suits. Nevertheless, they had difficulty in completing their swimming tasks at the colder water temperatures and deeper depths because of being severely chilled, which caused continuous severe shivering. Their core temperatures were falling steadily despite minimal heat loss from the body surface. Quoting from the conclusions of this report:

> (1) Dives to 850 feet for exposure durations in excess of 90 minutes in water of 45 F (or colder), i.e. with a 250 kcal/hr (290 watts) respiratory heat loss are, in this context, hazardous without supplemental heating of the inhaled gas; (2) dives to 650 feet, or deeper, for exposure durations in excess of 90 minutes in water of 35 F, i.e. with 150 kcal/hr (about 175 watts) respiratory heat loss, are, in this context, liable to be discomforting to the point of distraction and task performance degradation. (7)

Jegou (11) stated that diving in Arctic water causes all the metabolic heat production to be balanced by the respiratory heat loss at a depth of approximately 280 meters (930 feet). He states: "a greater heat loss cannot be tolerated and adequate compensation by heating the suit is impractical because dissipation of heat from the suit is limited by the comfort of the diver." Jegou reports that heating the respiratory gas was an important adjunct to electrically heated clothing in an operational dive in cold waters at 171 meters (560 feet) off the coast of Labrador. In a report on divers who worked in water in a chamber with pressure equivalent to 1000 feet and water temperatures as low as $-2\,^{\circ}C$, Workman emphasized the importance of heating the respiratory gas with a hot water heat exchanger, in addition to heating the divers with hot-water heated suits (35). There is now general agreement that supplemental heating is required for the diver, not only for his surface heat loss but for the heat loss from his respiratory tract.

How deeply does the cold respired gas penetrate the respiratory passages? This question is as yet unanswered. It is known that the warming of cold inspired air at 1 ata takes place in the first 10 cm or so of the upper respiratory tract (30), but it is suspected that with dense cold gas this penetration is considerably deeper. Suggestive evidence is to be found in the report of Jacquemin et al. (10). They measured esophageal temperatures at depths of 17, 25, and 33 cm, noting that these all showed that the local temperature was considerably less than $37\,^{\circ}C$. The implications for calculations of respiratory gas flow are important. Most respiratory models assume that the gas temperature in the respiratory tract is $37\,^{\circ}C$. If the gas is colder it would be denser and affect flow characteristics in these passages.

Rewarming

Heat losses from the body surface and from the respiratory tract are serious problems in diving. How do divers rewarm? The traditional hot shower and hot drink following a cold water dive are partially effective and certainly make a man feel better. However, people are poor judges of their own thermal state, and it is likely that rewarming by these methods is not complete in the sense that all the lost calories have been restored. Shivering stops in the early stages of rewarming, and a person feels comfortable long before his rectal and skin temperatures have returned to normal. There is no great danger in this situation if the man

does not have to make a second dive the same day, but in saturation diving one expects a man to make several excursion dives each day. It is vital to be able to say that rewarming is complete prior to the start of a second or third or fourth dive.

Observations made during current studies in my laboratory provide a description of rewarming. A thoroughly chilled subject comes out of the cold water into a thermally neutral chamber, where his body heat is restored by means of a suit calorimeter, in which a network of fine plastic tubing lying against his skin carries warm water all over his body. Heat is pumped in at the rate of 2 to 4 Kcal/min for the first 10 or 15 minutes, then in decreasing quantity over the next 30 to 40 minutes, until rewarming is judged to be complete. The man's own metabolic heat production is an additional source of heat, at the rate of 1.5–2.0 Kcal/min.

When is rewarming complete? An obvious and gross sign is when sweating begins. This is the sort of endpoint a physiologist would choose since, according to theory, thermal sweating should not begin until central and peripheral signals are both above the comfort level and vasodilation has not prevented a continued rise. Or to put it in another way, sweating is a means of increasing heat loss when there is a need for increased heat dissipation in order to keep the body heat content from becoming higher than normal. In the present study experiments were begun using early sweating as an endpoint for determining the completion of rewarming, but when cool water was subsequently sent through the water-cooling garment, it was noticed that the man delivered up to 5 Kcal/min initially—far more than his metabolic heat production—indicating that he had been overwarmed. When the data was examined, it became evident that—something like 20 minutes before sweating first appeared—the subject, who was usually sound asleep with a low resting heart rate, suddenly showed an increase in heart rate of 15 to 20 bpm. It was inferred that this signalled the point where cutaneous vasodilation occurred as the body attempted to regulate its surface heat loss by vasomotor adjustment. The fact that the vasodilation was inappropriate in the face of warm cooling tubes lying on the skin is not the issue. The body makes the same futile response in air temperatures of 35 °C and higher but then invokes sweating to cause, in normal circumstances, a high surface heat loss.

It was learned from the calorimeter data (if the heat were not pumped too aggressively into the man) that, in the course of a rewarming period, the subjects accepted less and less heat from the suit after the first 10–15 minutes; finally they accepted none and then began to release heat to the suit. The jump in heart rate occurred 2–4 minutes later. The predive heat balance of heat removal equal to heat production could then be re-established and sweating could be avoided entirely. Thus the calorimeter could indicate when rewarming was complete.

Granted that these heart rate and calorimetric signals are perhaps too subtle for field use, nevertheless sweating is easily detected. Some overwarming prior to a second dive is not a bad idea; this will be discussed subsequently.

Incidentally, the body temperatures monitored did not show that heat restoration was signalled by a return of predive temperatures. Skin temperatures were higher and internal temperatures lower than predive levels, when rewarming was evidently complete.

Once a diver becomes cold, complete rewarming is vital before he dives again. As to the method, a hot bath or a water-heated suit is preferable to a shower. Furthermore, if the man can exercise and generate heat internally, the process would be considerably speeded up.

Another mode of rewarming in the habitat would be to take advantage of the high rate of heat exchange in the respiratory tract. Using heated breathing gas at a temperature of, for example, between 40° and 45 °C, a considerable quantity of heat could be added directly to the body core. The process could be improved by having the man exercise, which increases his respiratory minute volume as well as causing an increase in metabolic heat production. If maximum speed in rewarming were desired, exercise in a water-heated suit could be combined with the subject's breathing warm hyperbaric gas. It should be possible to reduce the present time of about 40 minutes for replacing a caloric deficit of 200–300 Kcal down to less than 20 minutes.

There is one further trick which might be useful in preparing for diving in cold water. Some years ago it was learned that men could tolerate severe heat exposures approximately twice as long if they were prepared for the heat exposure by precooling to lower their core temperatures by about 1 °C (28). The same thing should work in reverse. In the face of a known cold exposure, a man might be better prepared if he were preheated, with a rise in core temperature and a significant storage of body heat. When several excursion dives are required each day from an undersea habitat, rewarming from each dive could be carried beyond the point of restoring normal heat content, i.e. well past the sweating threshold, so that for each subsequent dive a man could start with a surplus of heat.

The Thermal Drain of Hyperbaric Environments

Evidence indicates that divers living in hyperbaric shelters—whether in dry chambers in the laboratory or in saturation dives at sea—are under thermal stress like that of cold exposure, even though the temperature feels comfortable to them. The analysis made on this subject several years ago (31) was somewhat speculative, but later studies have supported the idea that living in hyperbaric environments presents a considerable thermal drain. One of these was a 5-day saturation dive in a habitat at 516 feet (16.6 ata) in warm Hawaiian waters (19), which turned out to be distressingly cold for the divers. Another was a 1200-foot (37 ata) saturation dive in the hyperbaric chamber at the University of Pennsylvania Institute for Environmental Medicine (14), where the thermal aspects of the apparently comfortable environment were studied. And Moore et al. (17) gave a thorough description of a second dive off Hawaii where the habitat pressure was 16.1 ata, but the gas temperature was kept up to the recommended level.

The ocean saturation dive at 16.6 ata at the Makai Undersea Test Range, Hawaii, was characterized by Pegg (19) as producing divers who were almost continually cold and whose behavior was that of individuals under stress. The habitat temperatures were between 22° and 25 °C, and the water temperature outside between 19° and 21 °C. The 22 KW of heat available could not raise the habitat temperature higher. Contributing to the cold and discomfort was the failure of the hot water shower used for rewarming following a dive. At night sleeping was interrupted by shivering despite heavy covers. The divers estimated that they could have spent three times as much time diving if they had been able to rewarm adequately. Forty-five excursion dives were made, with an average length of about one-half hour. Because it was impossible to rewarm, the divers could not make a second comfortable dive in the same day. One expression of the cold stress, despite food as desired, was the divers' loss of an average of 2.9 kg (over 6 lbs) in weight, which Pegg felt should be attributed to

increased metabolism due to excessive heat loss. This account of a saturation dive in the relatively warm waters off Hawaii emphasized two major points about living in hyperbaric environments: first, that thermal comfort requires a higher-than-normal gas temperature; and second, that men can apparently adjust to a steady thermal drain by increasing metabolic level, which causes weight loss despite the availability of adequate food.

A closer examination of the comfort question reveals the following. When lightly clothed, mildly active men are confined in a hyperbaric chamber or undersea habitat and allowed to choose the temperature in which they feel comfortable for days of exposure, the literature shows that the higher the pressure the higher the comfort temperature and the narrower the temperature band. Figure 6 shows this effect graphically (31). A scale of "convective character" was invented as a device to arrange hyperbaric gas mixtures and other immersion fluids in a consistent manner. The density (in gm/L) times the specific heat (in cal/gm-°C) times the thermal conductivity (in cal/min-cm-°C) was divided by viscosity (in centipoises). Then this number was related to the number for air at 1 ata, which was 0.61. In this scale of convective character, air became 1 and water was 167. In the 1200-foot saturation dive at the University of Pennsylvania, the four subjects who spent 6 days at this pressure chose a temperature between 32.5° and 33.5°C and the convective character of that environment was 144. This new point would fall within the temperature range shown on the figure. Moore et al. (17) would agree, since their comfort temperature was 29°C for $He-O_2$ at 16.1 ata, and Varène et al. (26) have similar data for several pressures. Table II summarizes these observations.

There is strong evidence that the convective transfer coefficient increases remarkably with pressure (22, 26), but it is difficult to estimate one important component, the velocity of the gas. It is known that the skin temperature is much closer to the gas temperature than one observes for men in air. In the 1200-foot dive, for example, the gradient between skin temperature and gas temperature was only about 2°C. Nevertheless, in these environments

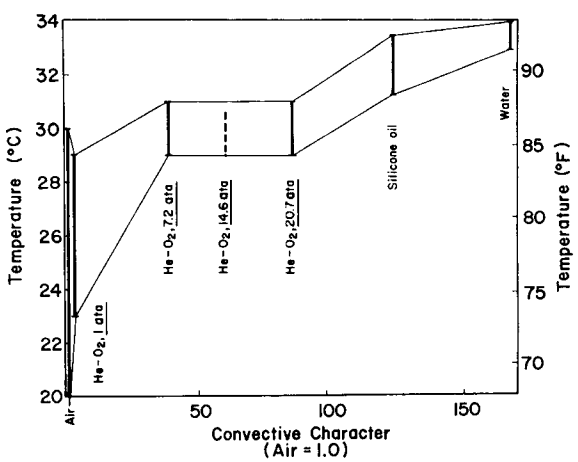

FIG. 6. Comfortable temperatures for prolonged stay in various fluids by men lightly dressed and mildly active. From Webb (31), by permission.

TABLE II
COMFORT TEMPERATURES FOR NORMOXIC HELIUM-OXYGEN MIXTURES AS A FUNCTION OF PRESSURE

Gas Pressure (ata)	Gas Temperature (°C)	Reference
1.5	28.7	(26)
2.3	29.6	(26)
4.4	30.0	(26)
8.4	30.7	(26)
13.1	28.5-31.5	(14)
16.1	29.0	(17)
16.1	31.8	(26)
22.2	29.0-31.5	(14)
28.3	30.0-32.0	(14)
30.8	32.4	(26)
37.4	32.5-33.5	(14)

heat transfer from the skin to the environment is predominantly convective. In a previous analysis (31), it looked as though a fluid with a convective character of about 120 would cause a heat loss of 100 Kcal/hr, approximately the normal resting metabolism. If that data can be extrapolated, the suggestion is that with fluids which are more convective, for example, the helium-oxygen mixtures at 1200 feet, convective drain would be higher than normal resting metabolism despite the presence of a thermally comfortable gas temperature.

The following is a description of the environment observed in the 1200-foot saturation dive. At this pressure of 37 ata, the temperature of the walls and various structures inside the chamber was virtually equal to gas temperature. The velocity of gas motion at several locations in the chamber was so low as to be undetectable using a wind vane anemometer. Significant gas motion was measurable only at the intake and discharge of the blower which circulated gas to the CO_2 scrubber. One could see even with the naked eye the leisurely gas motion and lethargic gaseous diffusion: when a different gas from that in the chamber, for example, a breathing mixture containing neon or nitrogen, spilled from a mask being held by a subject, the new gas with a refractive index different from that of helium could be seen falling slowly toward the floor, rather than immediately mixing and diffusing as one expects at lower pressures. An attempt was made to describe the evaporative character of the gas by measurements with a resistance hygrometer, with a wet bulb-dry bulb thermistor pair, and with a dewpoint hygrometer. It was agreed that the humidity was high, probably above 90% saturation, despite the fact that dry gases were being added throughout the day and night and the fact that there were no large water surfaces or wet equipment in the chamber. The rate of weight loss of an open Petri dish was also observed and it was noted that it evaporated some 5 to 10 times more slowly than from the same Petri dish in air at 1 ata. Low gas velocity and low diffusivity in a hyperbaric gas would explain the poor evaporative rate and the sense of high humidity which these subjects and those in similar dives report.

The most tangible evidence of thermal drain is the body weight loss observed in men who live in hyperbaric environments. Table III shows body weight losses from four saturation dives in laboratory chambers and four saturation dives in the sea. All dives were conducted

TABLE III
BODY WEIGHT LOSS IN SEVERAL HYPERBARIC SATURATION DIVES

Source	Gaseous Environments	Mean Weight Loss (kg)	Food Intake (Kcal/day)	Number of Subjects	Duration	
					at depth (days)	total dive (days)
Tektite I (31)	Air—2.15 ata	1.6	"adequate"	4	60	
Helgoland (25)	Air—3.3 ata	3-5	6000	9	10	
Genesis E (31)	HeO_2—7 ata	0.6	4200	3	12	
U. Hawaii (17)	HeO_2—16.1 ata	0.6	$\geq$4000	6	2	9
Sealab II (31)	HeN_2O_2—7.2 ata	2.0	>4000*	28	15	
Aegir (19)	HeO_2—16.6 ata	2.9	ad lib	6	6	14
USN-EDU (29)	HeO_2—19.2 ata	1.2	ad lib	7	8	16
U. Penna. (14)	HeO_2—37 ata	4.0	3510	4	6	21

*Estimated.

in comfortable temperatures except for Aegir (19) and Helgoland (25) which both involved exposure to cold. From the table it is evident that the higher the pressure and the longer the dive the greater the weight loss. The Aegir and Helgoland experiments are somewhat out of order in this sequence, but their higher weight loss for the pressure and duration shown can be ascribed to the increased heat drain of the cold environment. The table also shows what was known about the food intake. Where estimates were made, the dietary intake was generous. In the University of Pennsylvania 1200-foot He–O_2 dive, a serious effort was made to count calories and the average level of 3510 Kcal per man per day should have prevented weight loss in young men who were cooped up in the small space of a hyperbaric chamber.

There is a bit of biochemical evidence which suggests increased metabolism in hyperbaric helium. Uddin et al. (24) and Vorosmarti et al. (29) reported a reduction of the level of blood glucose during saturation dives, and Uddin et al. reported, in addition, an increase in serum lactic acid and a decrease in serum free fatty acids. Such changes in blood chemistry are consistent with an increased carbohydrate metabolism.

There is one additional curious observation which might be part of this picture of increased metabolic activity. The body temperature, measured either in the rectum or by mouth, was reportedly about 1 °C higher than normal during the Tektite and Sealab II experiments (31). In the 1971 University of Pennsylvania dive to 1200 feet, early morning rectal temperatures were found to be consistently elevated in all four subjects. This is illustrated in Fig. 7. However, it should be noted that midday rectal temperatures were not higher than expected. In fact, when two of the subjects exercised rather vigorously for 30 minutes, their rectal temperatures did not rise, suggesting that the increased heat production

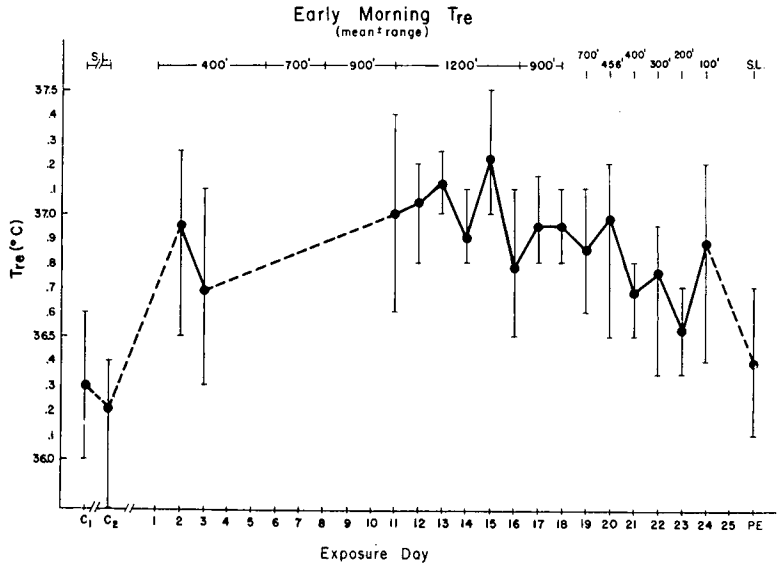

FIG. 7. The mean early morning rectal temperature (with range) for four subjects during a saturation dive at the University of Pennsylvania Institute for Environmental Medicine (14) to the depths shown at the top of the figure.

of exercise was rapidly drained in the highly convective environment. If the early morning rectal temperatures are high, then perhaps this indicates a higher than normal level of metabolism during the night.

Such are the lines of evidence to indicate that subjectively comfortable hyperbaric environments represent a thermal drain. That drain is met by an increased metabolic heat production in order to maintain body temperature. Despite high levels of food intake, men are not in caloric balance and lose weight.

The implications of the persistent loss of body weight observed in men who are apparently unwilling to eat enough food to meet their caloric requirements should be considered. Assume that the men in the 1200-foot saturation dive at the University of Pennsylvania needed approximately 3000 Kcal per day to maintain their weight in the confined space, if the chamber held air at 1 ata. Judging from the weight they lost, in the 37 ata $He-O_2$ environment, they had a caloric deficit of about 1500 Kcal per day; in other words, they should have been eating about 4500 Kcal per day instead of the 3500 observed. If this had been an ocean dive, one would have to allow for the caloric demands of the exercise of excursion diving and the possible extra heat loss in cold water. The caloric need might well rise to 6000 Kcal per day. Such intakes are not unheard of for lumberjacks who work hard all day and regularly consume such quantities. However, with the exception of the divers in the Helgoland project (26), men in undersea shelters have not shown much desire to eat this much. A way must be found to supply such quantities and to encourage men to eat them or the weight loss will continue. This is not necessarily bad for men who carry excess body fat, but it could be a problem for thin men, and it could become an increasing problem as the depth and duration of saturation dives continue to increase.

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RESPIRATORY HEAT LOSS AND PULMONARY FUNCTION DURING COLD-GAS BREATHING AT HIGH PRESSURES

B. Hoke, D. L. Jackson, J. M. Alexander and E. T. Flynn

Diving in cold water presents a severe thermal stress to the diver and necessitates insulation and supplemental heat to prevent excessive heat loss through the skin. In addition in deep diving, significant heat loss through the lungs—both in warming and in humidifying the inspired cold gas—occurs due to the increased thermal capacity of the breathing-gas mixture. Other factors which increase respiratory heat loss (RHL) are a decrease in inspired gas temperature (T_I) and an increase in respiratory minute volume ($\dot{V}_E$).

The purpose of this study was to measure RHL in two divers at rest and at four graded levels of exercise while breathing cold gas at simulated depths to 1000 feet of sea water (fsw). In earlier studies, Webb and Annis (6) measured RHL during immersion in cold water at a simulated depth of 200 fsw, with the cold water acting as the heat exchanger to cool the inspired gas. Tauber et al. (4), on the basis of the studies by Webb and Annis, constructed a theoretical model of thermal balance in deep diving. Assuming that inspired gas temperature would be at ambient water temperature, they predicted that RHL would exceed metabolic heat production at 850 fsw in 4.2 °C water. Since the optimum condition for safe, efficient working dives is maintenance of homeostasis in the diver, the predictions required empirical testing. The data presented here substantiates those predictions. A secondary purpose was to study cardiopulmonary function and to investigate the possibility of pulmonary damage from dense, cold gas acting directly on the respiratory tract mucosa.

Materials and Methods

Since actual immersion is not required to quantitate RHL at high ambient pressure, this study was performed in a dry chamber with an independent system for delivery of cold breathing gas to the subjects. RHL was measured in two subjects at simulated depths of 0, 200, 400, 600, 800, 850, and 1000 fsw, while breathing warm (23–32 °C) and cold (0–7 °C) He–O_2 mixtures in a dry pressure chamber with an ambient temperature of approximately 30 °C.

Both subjects were dressed in swim trunks and were subjectively in thermal comfort and objectively in thermal balance during the control rest period that preceded each cold-gas

exposure. The subject was seated on a bicycle ergometer* and, with a noseclip in place, breathed gas from a mouthpiece. Exercise was performed by pedalling at a constant speed, while the external workload was increased in calibrated steps from 30, 60, 90, to 120 watts at intervals to produce an increase in $\dot{V}_E$. For both warm-gas control experiments and cold-gas exposures at each depth, each subject rested for 15 minutes, worked at 30 watts for 7 minutes, at 60 watts for 7 minutes, at 90 watts for 6 minutes, at 120 watts for 6 minutes, and then rested for 19 minutes. (The 120-watt work rate was not attempted at depths greater than 600 feet during the cold-gas exposures due to excessive breathing resistance.) This 60-minute period marked the end of each warm-gas control run and the end of one of the two cold-gas exposures at each depth. The second cold-gas experiment at each depth was continued by alternating 10 minutes of work at 60 watts with 10 minutes of rest for a total exposure to cold gas of 4 hours.

The He-O_2 breathing mixture (Table I) was prepared automatically by an Airco Mixmaster. For the cold-gas studies the gas passed through a 200-foot double coil of 0.5″ inside diameter thin-wall copper tubing immersed in an ice and brine slurry. For the warm-gas control studies, the cooling system was bypassed. A short piece of 1.25″ I.D. flex-hose, insulated with a noncompressible, open-cell urethane foam, led from the regulators to the mouthpiece assembly. A shielded thermistor was placed in the lumen to measure T_I immediately before entering the mouthpiece. The mouthpiece assembly† (Fig. 1) was also heavily insulated with foam. The dead space was 80 ml. Three penetrations were made in the mouthpiece: 1) for a thermistor to measure expired gas temperature (T_E); 2) for a pressure transducer to record changes in pressure in the mouthpiece during the respiratory cycle; and 3) for a gas sampler. T_I and T_E were measured with separate, directionally-oriented rapid acting (0.1 sec) thermistors.‡ The thermistor outputs were plotted continuously on separate channels of an eight-channel Gilson recorder. The thermistors were calibrated for ranges of 0°–40°C against a Bureau of Standards certified mercury thermometer at the beginning of each experiment. This permitted T_I and T_E to be read

*Monark Co.

†Collins Co., "Double J" valve, No. P-307.

‡Series 520, Yellow Springs Instrument Co.

TABLE I

INSPIRED GAS DENSITIES

Depth (ft)	He-O_2 Mixture (%)	P_{IO_2} (mmHg)	Density (gm/L)	Density Relative to Air
0	72.0-28.0	200	0.48	0.43
200	95.8- 4.2	228	1.47	1.33
400	97.8- 2.2	228	2.43	2.19
600	98.5- 1.5	230	3.38	3.05
800	98.8- 1.2	232	4.34	3.92
850	98.9- 1.1	230	4.58	4.13
1000	99.0- 1.0	256	5.33	4.80

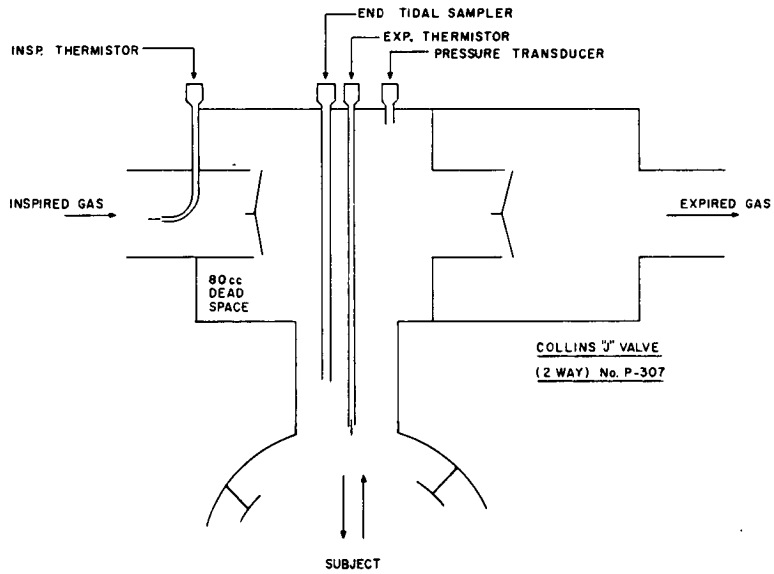

FIG. 1. Mouthpiece assembly—cold gas study.

directly from the graph. The pressure transducer* was calibrated with a water manometer in the chamber before each experiment, and its output was recorded continuously. A two-lead electrocardiogram was also continuously recorded during each experiment. Skin and rectal temperatures were measured and continuously recorded on a 12-channel Leeds-Northrup recorder. Mean skin temperature was calculated by the Teichner approximation (5).

Mixed expired gas was collected in a 150-liter Douglas bag during the final 5 minutes of the rest period, the final 2 minutes of the 30- and 60-watt work periods, and the final minute of the 90- and 120-watt work periods. After thorough mixing, a sample for analysis was removed with a 100 cc glass syringe lubricated with dilute lactic acid, and the remaining gas volume was measured in a 120-liter Tissot gasometer. A sample of inspired gas was also obtained during each collection period from the gas supply system, using a similarly prepared syringe. Empirically derived correction factors were applied to the observed spirometer temperature to correct for pressure effects on the alcohol thermometer.

On the surface, the fractions of O_2 and CO_2 in inspired and expired gas were measured using the micro-Scholander technique. At depth, the gas samples were passed through the chamber wall via needle valves. Oxygen fractions were measured by a Beckman F-3 paramagnetic analyzer and recorded on a Sargent strip recorder; CO_2 fractions were measured by a Beckman LB-1 or IR-215 infrared analyzer and recorded on an Esterline-Angus strip recorder.

VC, FEV_1, MVV, and FIV_{25-75} were measured before and immediately after each cold-gas exposure with a 13.5 liter Collins spirometer filled with the ambient chamber gas. In addition, measurements of FEV_1 were obtained while breathing cold gas after 5 and 55 minutes of exposure by connecting the exhalation hose to the 13.5 liter spirometer.

*Statham Co., PR-23.

RHL was calculated using a formula modified from Webb and Annis (6). To derive a usable value for the water content of expired gas, which was not measured, it was assumed that the inspired gas was dry and that the expired gas was saturated at the measured T_E. The basic equation used was:

$$RHL = \dot{V}_{E_{STPD}}\left(\frac{\Delta T \times (0.2215\ F_{E_{He}} + 0.31124\ F_{E_{O_2}})}{1000}\right) + \dot{V}_{E_{STPD}}\left(\frac{0.8162 \times P_{H_2O} \times [590 - 0.55\ (T_E - 20)]}{(P_B - P_{H_2O}) \times 1000}\right)$$

Where:

RHL = respiratory heat loss in Kcal/min

RHL (watts) = (RHL [Kcal/min]) (69.77 watts/Kcal/min)

$\dot{V}_{E_{STPD}} = \dot{V}_{E_{BTPS}} \left(\frac{P_B - 47}{760}\right)\left(\frac{273\,°K}{310\,°K}\right)$

$\Delta T = T_E - T_I$

0.2215 cal/L/°C = ρCp for He = $\left(\frac{4\text{ gm/mole}}{22.43\text{ L/mole}}\right)$ (1.242 cal/gm/°C)

0.31124 cal/4°C = ρCp for O_2 = $\left(\frac{32\text{ gm/mole}}{22.393\text{ L/mole}}\right)$ (0.2178 cal/gm/°C)

$F_{E_{He}} = 1.00 - F_{E_{O_2}} - F_{E_{CO_2}}$

$\frac{1}{1000}$ = conversion from cal to Kcal

0.8162 gm/L = density of water vapor at STP

P_B = ambient barometric pressure, in mm Hg

P_{H_2O} = vapor pressure of water at T_E, in mm Hg

[590 − 0.55 (T_E − 20)] cal/gm = latent heat of vaporization of H_2O, corrected for temperatures other than 20°C.

The first part of the formula calculates the amount of heat required to heat expired gas from T_I to T_E, while the second part calculates an approximation of heat loss due to humidification of dry inspired gas.

Standard equations were used for all pulmonary function calculations. $P_{A_{CO_2}}$ was computed assuming volume dead space (V_D) equal to the subject's weight in pounds. Inspired gas densities are shown in Table I. Peak inspiratory and expiratory pressures measured in the mouthpiece are shown in Table II. The pressures increased with increasing depth and workload. Peak positive pressures during expiration were, in most cases, substantially less than peak inspiratory pressures.

Results

Tables III and IV show the data for RHL and pulmonary function in both subjects. Figures 2 and 3 show the RHL versus $\dot{V}_E$ for the warm- and cold-gas experiments, respec-

tively. The observed RHL during the warm-gas controls at 1000 fsw (Fig. 2) was less than at 800 fsw because of the difference in inspired gas temperature. On the surface when breathing warm, 30°C, gas with $\dot{V}_E$ of 40 L/min the RHL was 50 watts, and 95 percent of this was due to humidifying the inspired gas. With the same minute volume, at 800 and 1000 fsw on warm gas, the RHL was between 150 and 200 watts. For comparison, Fig. 3 shows that breathing cold gas with $\dot{V}_E$ of 40 L/min at the surface the RHL is about the same as with warm gas, 50 watts. At 800 and 1000 fsw, however, RHL ranges from 400 to 500 watts. The highest observed RHL, 778 watts, occurred during heavy work at 1000 fsw with $\dot{V}_E$ of 64 L/min. Table V shows the percentage of calculated RHL due to water vaporization. Only 10 percent of RHL is due to this factor at depths of 800 to 1000 fsw.

Figure 4 shows the percentage of the metabolic heat production lost through the lungs. The results were derived by converting $\dot{V}_{O_2}$ to watts, subtracting the watts of external workload, and dividing the remainder into RHL. At 800 fsw, 80–100 percent of the available metabolic heat was being lost through the lungs, and the resultant negative thermal balance is shown in Fig. 5.

At 800 fsw, with T_I = 0.5°–1.7°C, two significant problems occurred. The first was excessive body heat loss in spite of exercise and an ambient temperature of 30°C. Subject J. M.'s body temperature decreased 1.5°C in 2 hours, and the experiment was stopped because of the marked discomfort and violent shivering. Rectal temperature decreased more rapidly during periods of work than at rest.

The other significant problem was acute respiratory difficulty. After 31 minutes of breathing gas at T_I = 0.5°–1.0°C, subject T. G. suddenly terminated the experiment because thick and excessive upper respiratory tract secretions made breathing exceptionally difficult. Auscultation of his chest at that time revealed coarse rhonchi. After breathing the chamber atmosphere for a few minutes, the subject's difficulty subsided. To determine if this was a consistent reaction, the same subject was retested under the same conditions 2 hours later. His reaction was the same: thick, copious secretions created acute respiratory difficulty, and he stopped after 35 minutes.

Because of these findings at 800 fsw, the cold-gas exposures planned for 1000 fsw were changed. A T_I of 6°–7°C was used and was calculated to be almost as severe a cold stress as 0.5°–1.0°C had been at 800 fsw. Subject J. M.'s body temperature dropped 1.0°C during the 1-hour exposure. The other subject's (T.G.) planned 4-hour exposure was stopped after 74 minutes because of discomfort and uncontrollable shivering, although his rectal temperature had dropped only 0.5°C. His secretions were moderately thick and copious but not sufficient to induce acute respiratory difficulty.

Cold-gas breathing at high pressure had no consistent effect on pulmonary ventilation when compared with warm-gas controls at the same depth (Fig. 6). However, subject T.G. did demonstrate a small decrease in $\dot{V}_E$ and a small increase in $P_{A_{CO_2}}$ (Table III) during cold-gas breathing at 400 and 600 fsw.

During cold-gas breathing there was a reduction in the FEV_1 at each depth when compared to the warm-gas control FEV_1 (Fig. 7). This reduction was particularly noticeable in the first 5 minutes at depths of 600 fsw and greater. When the FEV_1 was repeated at 55 minutes while the subjects were still breathing cold gas, there was again some reduction but not as great as seen at 5 minutes.

Table VI shows the results of spirometry before and shortly after cold-gas inhalation for

TABLE II

PEAK INSPIRATORY AND EXPIRATORY PRESSURES (cm H_2O)

Depth (ft)	Workload (watts)	Subject: J.M.				Subject: T.G.			
		Warm Gas		Cold Gas		Warm Gas		Cold Gas	
		Inhalation Resistance (cm H_2O)	Exhalation Resistance (cm H_2O)	Inhalation Resistance (cm H_2O)	Exhalation Resistance (cm H_2O)	Inhalation Resistance (cm H_2O)	Exalation Resistance (cm H_2O)	Inhalation Resistance (cm H_2O)	Exhalation Resistance (cm H_2O)
0	0	4.7	0.5	6.0	1.0	2.5	1.7	4.0	—
	30	4.7	0.5	6.0	1.0	2.7	1.5	—	—
	60	5.5	1.0	7.2	2.0	3.3	1.5	—	—
	90	7.7	1.2	7.5	2.0	3.7	2.0	—	—
	120	8.0	3.0	8.0	2.0	4.7	2.3	—	—
200	0	3.7	1.0	5.5	1.2	3.7	1.0	5.3	1.3
	30	8.5	2.3	7.0	1.5	5.0	1.0	6.0	1.5
	60	10.0	2.3	7.3	2.0	6.5	1.0	6.5	1.7
	90	10.5	2.5	8.7	2.3	8.0	1.2	7.0	2.3
	120	13.5	4.3	12.0	3.0	9.5	2.0	7.7	3.3
400	0	6.2	1.0	8.0	1.3	4.7	1.0	6.5	1.0
	30	8.5	2.0	9.0	1.7	6.7	1.0	8.0	1.5
	60	9.5	2.5	10.0	2.3	7.0	1.3	8.5	2.0
	90	10.5	3.3	12.5	3.5	8.5	2.0	9.5	2.3
	120	13.8	4.3	15.5	4.5	9.5	3.0	10.5	2.7

	0	5.0	1.0	7.0	1.3	4.5	1.7	6.0	1.2
	30	7.5	2.5	9.8	2.0	6.0	1.7	7.5	1.2
600	60	10.0	2.7	10.8	2.7	7.7	2.0	9.5	2.3
	90	14.0	4.0	13.7	5.0	9.5	3.5	10.0	2.5
	120	17.5	4.0	18.5	5.0	12.5	4.2	12.0	4.5
	0	6.5	1.5	8.8	1.3	3.7	1.7	7.2	1.7
	30	9.0	3.0	—	—	5.0	2.2	8.7	2.2
800	60	11.0	3.5	21.0	4.5	6.2	3.7	10.5	3.5
	90	13.5	3.5			7.2	4.2	25.0	6.0
	120	22.5	4.5			10.5	4.2		
	0	4.0	1.2	5.2	1.0	4.5	1.5	3.7	2.0
	30	6.5	2.7	10.5	1.5	7.5	2.5	8.7	2.7
850	60	11.0	4.0	15.0	3.0	10.5	4.0	9.7	3.7
	90	13.5	4.5	16.0	3.5	13.5	4.7	11.0	4.5
	0	7.5	1.2	6.0	1.0	5.5	2.0	4.7	1.0
	30	10.0	2.7	10.0	2.0	9.0	1.7	7.3	2.0
1000	60	12.0	4.3	12.7	2.5	12.0	4.0	10.5	2.0
	90	17.0	5.0	14.5	4.0	16.0	4.5	11.0	2.5
	120	20.5	5.5						

TABLE III

RESPIRATORY HEAT LOSS DATA—SUBJECT T.G.

			Warm Gas											Cold Gas									
Subject	Depth	Work Rate	T_I (°C)	T_E (°C)	$\dot{V}_E$ (L/min. BTPS)	f (Breath /min.)	$\dot{V}_{O_2}$ (L/min. STPD)	$F_E O_2$	$F_E CO_2$	$P_A CO_2$	R	RHL (watts)	T_I (°C)	T_E (°C)	$\dot{V}_E$ (L/min. BTPS)	f (Breath /min.)	$\dot{V}_{O_2}$ (L/min. STPD)	$F_E O_2$	$F_E CO_2$	$P_A CO_2$	R	RHL (watts)
T.G.	Surface	0	31.2	33.4	15.0	14.4	.450	0.234	0.0303	29.7	.83	22	2.3	33.7	18.4	16.5	.483	0.239	0.0269	25.8	.85	34
		30	31.2	32.3	22.3	15.5	1.119	0.213	0.0442	39.3	.73	30	1.6	32.6	25.7	19.0	1.350	0.226	0.0347	31.3	.54	46
		60	29.8	31.6	33.5	15.4	1.677	0.211	0.0491	40.2	.81	42	1.1	33.1	27.2	18.4	1.443	0.222	.0454	40.0	.70	50
		90	30.7	32.0	39.6	15.0	1.911	0.211	0.0541	43.1	.92	53	1.0	32.5	37.8	20.7	1.904	0.226	0.0473	39.9	.77	68
		120	31.4	32.1	49.7	18.0	2.320	0.213	0.0502	39.8	.88	57	0.9	33.6	43.5	21.3	2.105	0.225	0.0502	41.5	.85	82
	200′	0	23.7	34.0	9.6	9.3	.354	0.0364	0.0050	37.3	.85	24	4.3	29.0	9.3	10.4	.366	0.0365	0.0054	42.2	.84	32
		30	26.2	33.4	18.0	10.6	.823	0.0349	0.0062	40.0	.84	38	2.6	27.3	20.7	11.8	.961	0.0350	0.0067	42.7	.89	70
		60	27.3	33.6	24.3	10.8	1.266	0.0341	0.0070	42.9	.83	50	2.6	27.8	31.8	11.7	1.645	0.0344	0.0073	43.7	.88	110
		90	28.5	33.8	31.8	11.0	1.636	0.0340	0.0074	43.9	.89	90	1.9	28.0	35.2	13.7	1.841	0.0343	0.0076	45.5	.90	126
		120	28.9	34.3	41.3	13.1	2.154	0.0343	0.0075	43.9	.93	39	1.8	27.7	49.2	14.7	2.333	0.0348	0.0077	44.5	1.00	174
	400′	0	19.2	32.6	8.1	10.1	.285	0.0199	0.0026	40.6	.86	30	1.27	26.9	10.1	10.4	.490	0.0188	0.0028	39.9	.67	56
		30	22.3	32.4	18.7	11.3	.763	0.0194	0.0033	40.7	.96	59	.87	26.9	19.7	12.4	1.025	0.0186	0.0035	42.7	.78	112
		60	24.4	32.4	20.9	12.2	1.099	0.0184	0.0037	44.9	.82	58	.47	26.6	26.2	11.5	1.347	0.0185	0.0038	44.3	.87	148
		90	25.3	33.6	30.8	12.3	1.512	0.0187	0.0037	42.4	.89	91	.47	26.9	31.6	13.0	1.695	0.0185	0.0040	45.6	.87	182
		120	26.1	34.2	38.0	13.4	1.971	0.0185	0.0041	45.5	.91	113	.47	27.9	39.7	14.0	2.198	0.0182	0.0043	47.9	.90	237
	600′	0	28.9	35.3	12.1	12.0	.460	0.0137	0.0017	35.6	.78	10	.8	28.4	13.2	11.9	.459	0.0139	0.0019	38.7	.96	110
		30	28.9	35.6	17.3	12.2	.804	0.0132	0.0021	39.7	.79	59	.8	28.7	15.9	10.2	.710	0.0133	0.0024	43.1	.91	133
		60	30.2	35.6	23.0	11.4	1.1681	0.0131	0.0023	40.3	.79	70	.8	28.9	22.3	12.0	1.029	0.0132	0.0026	45.2	.96	188
		90	30.7	36.4	36.4	12.9	1.669	0.0132	0.0025	41.9	.95	116	1.2	30.4	32.0	13.6	1.520	0.0131	0.0028	46.6	1.00	283
		120	30.4	36.9	44.4	13.6	2.052	0.0132	0.0026	43.0	.98	156	1.2	30.2	37.3	12.5	1.985	0.0128	0.0030	49.8	.98	328
	800′	0	23.7	35.0	9.8	11.6	.341	0.0106	0.0013	39.9	.88	53	0.9	28.4	13.8	13.4	.365	0.0106	0.0015	36.7	.89	100
		30	26.3	35.6	28.6	11.6	1.269	0.0104	0.0016	34.8	.80	138	.52	28.7	24.6	13.6	1.127	0.0099	0.0018	39.3	.82	235
		60	28.1	36.1	25.7	12.9	1.223	0.0101	0.0019	43.1	.90	114	.52	30.0	25.7	14.8	.878	0.0101	0.0020	42.2	.95	199
		90	28.8	37.1	33.2	12.6	1.704	0.0102	0.0019	42.7	.86	154	—	—	—	—	—	—	—	—	—	—
		120	29.5	37.6	39.4	12.7	2.120	0.0101	0.0021	45.4	.89	182	—	—	—	—	—	—	—	—	—	—
	850′	0	23.0	34.4	10.3	12.8	.295	0.0103	0.0011	36.8	.96	58	5.0	30.5	11.6	11.1	.463	0.0097	0.0012	34.3	.72	119
		30	25.4	35.4	24.7	13.2	1.074	0.0097	0.0016	39.4	.89	130	5.0	29.4	29.0	12.3	1.362	0.0093	0.0015	36.4	.79	308
		60	27.9	37.0	40.5	13.3	1.806	0.0096	0.0017	39.0	.92	206	5.0	30.0	32.9	13.5	1.700	0.0090	0.0017	41.0	.81	304
		90	29.4	37.9	47.8	15.0	2.101	0.0097	0.0019	42.6	1.02	206	5.0	30.5	42.7	15.4	2.083	0.0091	0.0018	41.6	.89	415
		120	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	1000′	0	30.6	35.5	13.6	13.5	.4219	0.0095	0.0009	32.1	.86	50	7.1	29.2	12.4	13.2	.414	0.0095	0.0010	36.0	.87	131
		30	31.3	35.2	22.9	13.0	1.002	0.0091	0.0012	36.7	.81	75	6.1	30.2	25.1	14.9	1.169	0.0090	0.0014	40.4	.83	288
		60	31.7	35.9	32.4	14.4	1.549	0.0089	0.0014	39.1	.83	112	6.9	30.0	30.1	14.6	1.466	0.0090	0.0015	41.6	.85	332
		90	32.1	36.3	45.4	16.1	2.106	0.0090	0.0015	40.5	.91	159	6.9	30.6	58.7	15.6	2.902	0.0089	0.0016	41.1	.89	681
		120	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

TABLE IV

RESPIRATORY HEAT LOSS DATA—SUBJECT J.M.

		Warm Gas											Cold Gas									
Subject	Depth	Work Rate	T_I (°C)	T_E (°C)	$\dot{V}_E$ (L/min. BTPS)	f (Breath /min.)	$\dot{V}_{O_2}$ (L/min. STPD)	F_E O_2	F_E CO_2	P_A CO_2	R	RHL (watts)	T_I (°C)	T_E (°C)	$\dot{V}_E$ (L/min. BTPS)	f (Breath /min.)	$\dot{V}_{O_2}$ (L/min. STPD)	F_E O_2	F_E CO_2	P_A CO_2	R	RHL (watts)
J.M.	Surface	0	25.0	35.0	15.5	13.9	0.5361	0.245	0.0312	29.2	.75	26	4.4	27.6	15.2	15.6	0.453	0.252	0.0232	23.0	.65	20
		30	28.0	32.6	29.4	23.1	1.087	0.241	0.0361	32.6	.81	42	3.4	27.3	26.9	22.4	0.997	0.242	0.0325	30.0	.75	36
		60	29.6	33.0	46.5	25.7	1.755	0.241	0.0352	29.5	.77	68	2.9	26.9	41.2	23.5	1.435	0.243	0.0339	28.6	.81	54
		90	30.2	33.0	62.5	30.5	2.024	0.243	0.0371	30.3	.95	91	1.6	26.3	54.0	28.9	1.708	0.246	0.0330	27.6	.87	69
		120	30.9	34.0	96.7	36.0	2.770	0.247	0.0380	30.1	1.10	149	6.6	26.7	59.2	25.0	1.834	0.246	0.0355	28.6	.97	78
	200′	0	24.9	34.3	14.2	12.5	0.428	0.0379	0.0042	29.6	.87	33	3.1	31.2	11.7	13.8	0.413	0.037	0.0043	33.6	.76	46
		30	28.1	34.5	28.8	18.0	1.051	0.0369	0.0051	32.8	.87	61	1.6	30.8	26.2	19.8	0.940	0.036	0.0050	33.7	.87	106
		60	28.5	34.3	33.7	18.7	1.327	0.0365	0.0054	33.7	.84	67	1.2	30.2	31.8	21.3	1.173	0.036	0.0052	33.8	.87	127
		90	28.9	34.5	50.9	22.4	2.033	0.0365	0.0056	34.0	.87	104	.8	30.4	40.1	17.9	1.663	0.036	0.0059	36.1	.89	163
		120	29.3	35.0	65.5	25.6	2.468	0.0367	0.0057	34.1	.94	104	.8	30.0	63.4	26.6	2.284	0.036	0.0056	34.4	.97	254
	400′	0	20.3	34.0	11.5	14.6	0.392	0.0200	0.0024	36.2	.83	45	1.31	33.65	11.3	14.4	0.391	0.0198	0.0022	34.8	.76	82
		30	24.8	34.0	26.4	16.6	1.052	0.0196	0.0029	34.7	.84	83	1.31	30.2	25.6	16.6	1.096	0.0190	0.0028	32.2	.72	157
		60	25.8	34.0	35.3	17.9	1.486	0.0194	0.0031	36.2	.86	105	1.31	29.7	31.3	18.2	1.339	0.0191	0.0029	34.4	.79	196
		90	27.3	34.3	54.1	22.4	2.024	0.0197	0.0032	35.8	.99	151	1.31	30.45	45.7	20.5	1.979	0.0192	0.0031	35.1	.83	294
		120	27.8	35.1	61.1	24.9	2.502	0.0195	0.0033	36.9	.93	154	1.31	31.6	61.7	24.8	2.599	0.0192	0.0032	35.7	.88	415
	600′	0	27.2	36.8	15.5	11.1	0.4552	0.0143	0.0015	28.3	.90	66	1.6	31.8	15.4	13.1	0.520	0.0138	0.0015	29.9	.79	142
		30	28.7	36.8	25.1	14.9	0.8760	0.0138	0.0018	32.1	.90	97	1.4	30.7	31.8	15.6	1.178	0.0135	0.0018	30.8	.83	283
		60	28.7	37.2	36.9	15.8	1.3423	0.0137	0.0019	32.5	.92	147	1.2	29.7	32.2	16.8	1.219	0.0135	0.0019	32.6	.86	277
		90	29.1	37.6	47.6	19.0	1.68	0.0137	0.0020	32.8	.96	193	1.2	30.7	41.9	16.4	1.614	0.0135	0.0020	335	.90	376
		120	29.1	38.0	88.9	22.5	3.289	0.0136	0.0020	32.6	.95	373	1.0	30.2	50.5	19.5	1.828	0.0136	0.0019	32.3	.93	447
	800′	0	25.6	36.7	11.5	10.5	0.415	0.0106	0.0013	33.9	.82	64	.9	30.2	10.2	12.8	0.326	0.0107	0.0012	36.0	.87	115
		30	27.8	36.4	31.4	12.6	1.409	0.0099	0.0014	31.9	.74	147	.5	30.4	25.8	14.4	1.042	0.0104	0.0015	34.2	.84	296
		60	28.5	37.1	34.6	14.1	1.399	0.0099	0.0015	33.0	.84	164	.5	29.4	23.0	13.2	0.799	0.0105	0.0015	34.1	.87	255
		90	28.9	37.1	39.5	16.1	1.531	0.0106	0.0015	33.8	.90	182	.7	29.0	33.0	—	1.317	0.0104	0.0015	—	—	358
		120	29.6	38.0	52.0	20.2	2.16	0.0105	0.0016	35.7	.89	231	1.7	27.5	32.4	—	1.384	0.0102	0.0016	—	—	321
	850′	0	24.6	35.0	9.1	9.7	0.375	0.0099	0.0013	37.8	.76	14	5.1	29.5	11.6	10.5	0.408	0.0098	0.0013	36.6	.91	99
		30	27.9	35.3	29.9	15.4	1.149	0.0099	0.0014	34.7	.90	49	4.6	29.8	24.8	15.8	1.005	0.0094	0.0015	39.0	.93	218
		60	29.5	36.7	41.3	18.2	1.587	0.0099	0.0015	35.6	.94	129	4.6	29.9	40.2	19.9	1.698	0.0094	0.0016	38.7	.92	285
		90	30.1	37.2	46.9	20.3	1.926	0.0099	0.0016	38.6	.96	180	4.6	30.5	50.1	20.8	2.060	0.0094	0.0016	38.4	.96	416
		120	—	—	—	—	—	—	—	—	—	205	—	—	—	—	—	—	—	—	—	—
	1000′	0	30.3	35.3	11.7	12.2	0.385	0.0096	0.0010	33.7	.85	44	6.2	30.8	13.1	13.5	0.465	0.0095	0.0010	34.1	.81	154
		30	31.2	35.5	32.1	17.9	1.041	0.0095	0.0011	32.6	.99	111	4.9	30.3	32.4	18.1	1.310	0.0094	0.0012	33.6	.82	390
		60	31.9	35.9	45.2	20.9	1.720	0.0094	0.0012	34.2	.91	152	5.5	31.0	40.2	20.9	1.624	0.0094	0.0012	34.7	.86	488
		90	31.9	36.5	53.4	27.7	2.032	0.0094	0.0012	35.5	.93	197	5.5	31.2	63.5	22.7	2.641	0.0093	0.0013	36.4	.92	778
		120	32.3	36.7	81.5	28.2	3.10	0.0094	0.0013	34.9	.96	296	—	—	—	—	—	—	—	—	—	—

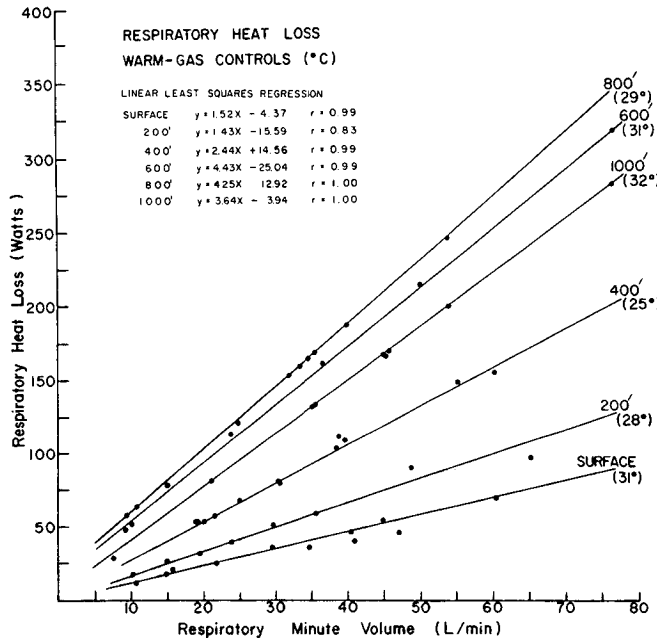

FIG. 2. Respiratory heat loss during *warm*-gas inhalation at various depths, as a function of respiratory minute volume ($\dot{V}_{E_{BTPS}}$). The lines were derived from linear regression equations fitted to the measured RHL and $\dot{V}_E$.

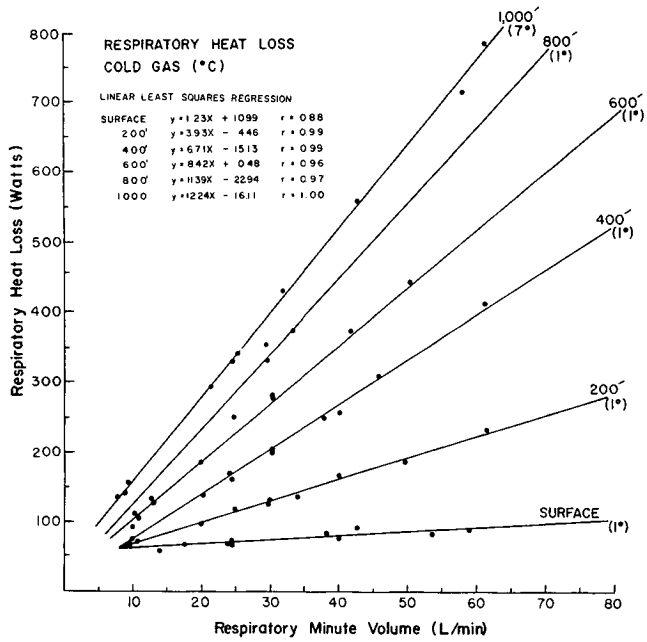

FIG. 3. Respiratory heat loss during *cold*-gas inhalation at various depths, as a function of respiratory minute volume ($\dot{V}_{E_{BTPS}}$). The lines were derived from linear regression equations fitted to the measured RHL and $\dot{V}_E$.

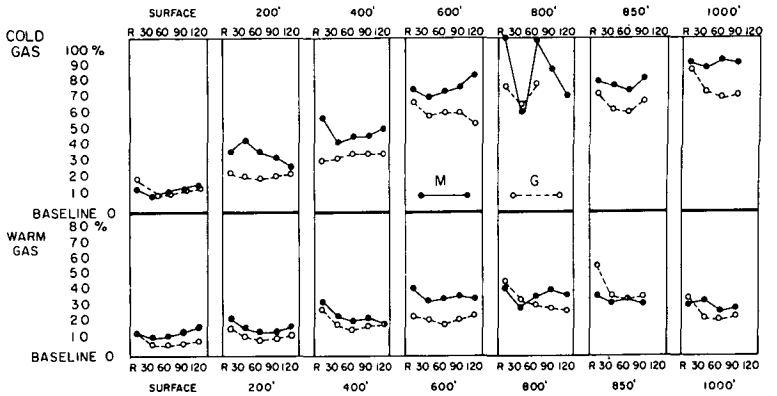

FIG. 4. Percentage of metabolic heat production lost through the lungs at various depths and external workloads. The results were derived by converting $\dot{V}_{O_2}$ to watts, subtracting the external work, and dividing RHL by the remainder.

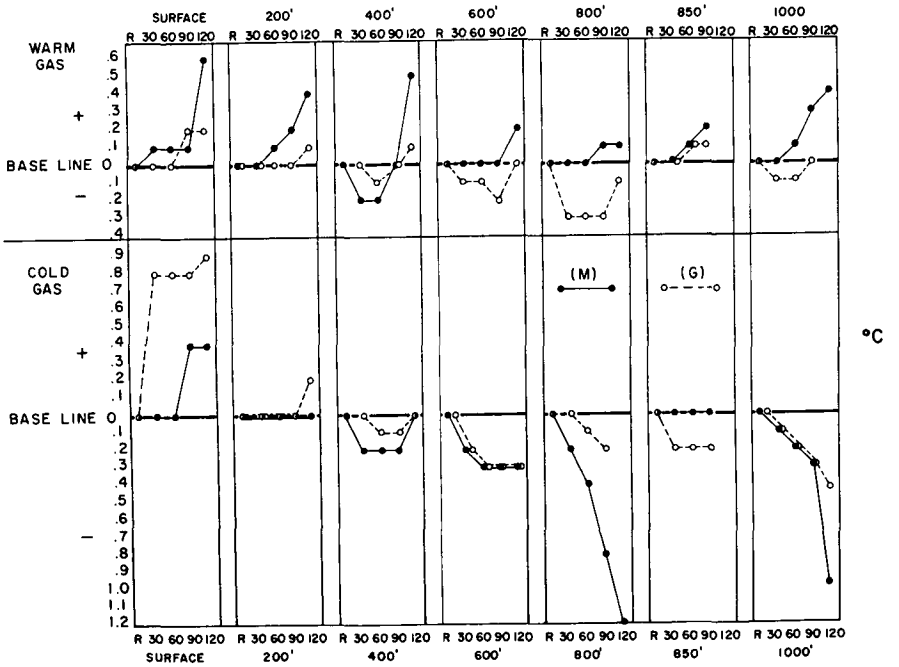

FIG. 5. Change in rectal temperature (°C) (T_R) during warm- and cold-gas inhalation at various depths and workloads. The T_R during the rest period was taken as the baseline for each experiment.

TABLE V

PERCENTAGE OF RHL DUE TO WATER VAPORIZATION

Depth (ft)	Gas Temperature	
	Warm (%)	Cold (%)
0	95	77
200	75	30
400	50	20
600	50	14
800	40	10
850	40	10
1000	40	10

either 1 hour or 4 hours at each depth. FEV_1, MVV, and FIV_{25-75} decrease with increased gas density as expected (2). In general these parameters were unaffected by cold-gas inhalation, with the exception of a reduction in FIV_{25-75} following cold-gas breathing at 850 and 1000 fsw, in subject J. M.

The incremental increase in heart rate during the sequence of graded exercise ($\Delta HR/\Delta \dot{V}$) appeared to be essentially unaffected by cold-gas inhalation at all depths except at 1000 fsw. Both subjects. however, demonstrated a marked reduction in the slope of the cardiac response to exercise during cold-gas breathing at 1000 fsw (Fig. 8).

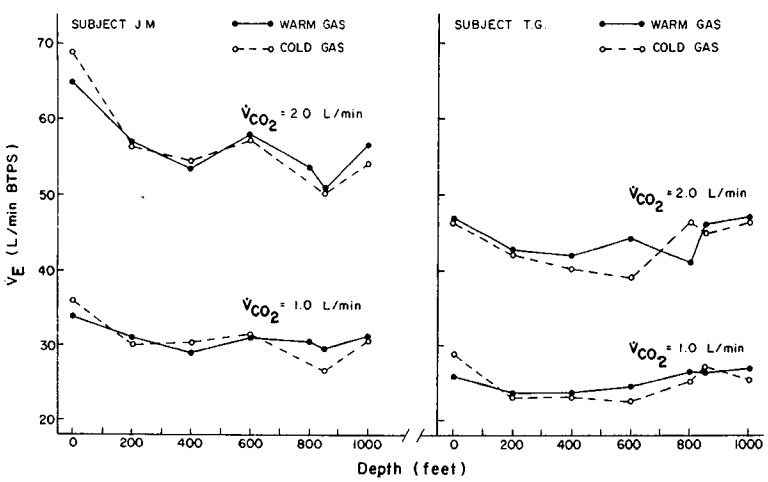

FIG. 6. Pulmonary ventilation during warm- and cold-gas inhalation at various depths. The data was derived by fitting linear regression equations to the measured $\dot{V}_E$ and $\dot{V}_{CO_2}$ during exercise under each condition, and obtaining estimated $\dot{V}_E$ at 1.0 and 2.0 L/min $\dot{V}_{CO_2}$. Correlation coefficients for these regression equations ranged from 0.96 to 0.99.

TABLE VI

PULMONARY FUNCTION BEFORE AND AFTER COLD-GAS BREATHING

Subject	Depth (ft)	Exposure Time (min)	Vital Capacity (ml BTPS)		Maximal Ventilatory Volume (L/min BTPS)		FEV_1 (L/sec BTPS)		FIV_{25-75} (L/sec BTPS)	
			Pre-Exp.	Post-Exp.	Pre-Exp.	Post-Exp.	Pre-Exp.	Post-Exp.	Pre-Exp.	Post-Exp.
J.M.	0	60	4702	4800	162	151	2.9	3.1	9.0	8.5
	200	60	4761	4856	108	117	2.7	2.8	6.7	5.8
	400	240	4614	4593	92	111	2.6	2.5	5.3	5.7
	600	60	4849	4985	100	107	2.6	2.6	4.6	4.7
	800	120	4974	4807	101	85	2.7	2.5	4.6	4.2
	850	60	4702	4702	94	110	2.4	2.4	4.9	3.4
	1000	60	4601	4433	88	87	2.3	2.2	4.0	2.7
T.G.	0	60	7576	7533	183	193	6.1	6.0	10.0	9.7
	200	240	7671	7672	141	132	5.8	5.9	6.8	6.7
	400	60	7483	7546	110	108	5.2	5.1	6.0	5.7
	600	240	7704	7514	101	100	4.9	4.9	5.0	5.2
	800	31	7419	7514	93	95	4.6	4.3	4.4	4.6
	850	60	7357	7525	108	107	4.5	4.8	4.5	4.6
	1000	74	7965	7640	94	112	4.3	3.9	4.3	4.5

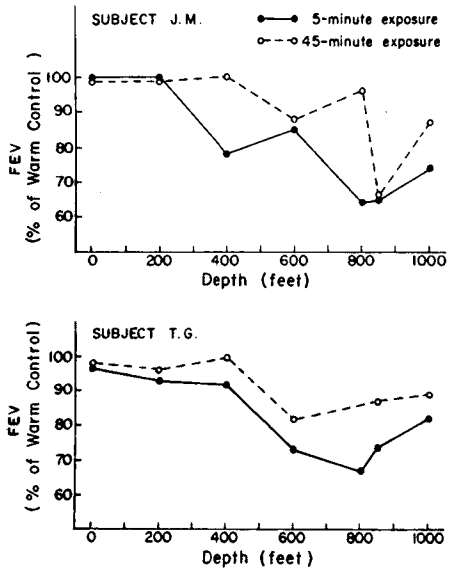

FIG. 7. Change in FEV_2 after cold-gas inhalation for 5 and 45 minutes as percentage of the warm gas control determined at each depth.

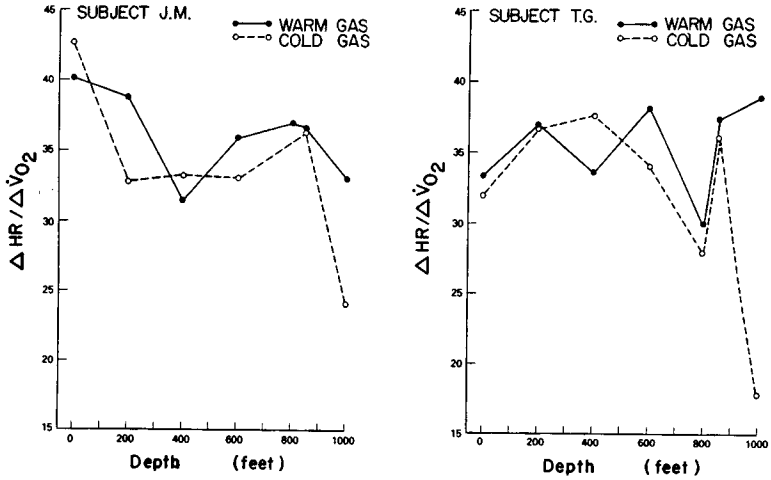

FIG. 8. Incremental increase in heart rate with exercise at each depth and condition ($\Delta HR/\Delta \dot{V}_{O_2}$, beats/L). The data was derived by fitting linear regression equations to the measured HR and $\dot{V}_{O_2}$ and plotting the increase in HR as $\dot{V}_{O_2}$ increased by 1L/m. For subject J.M. the cold gas data at 800 fsw was insufficient due to technical difficulties.

Discussion

This study demonstrates that cold-gas breathing at high pressure has two important aspects: negative thermal balance and acute respiratory difficulty. Rawlins and Tauber (3) calculated that at 600 fsw, breathing cold He–O_2 at rest, the RHL would equal the metabolic heat generation and that, since both of these factors would increase with work, they would tend to cancel each other in the heat balance equation. As indicated in Fig. 5, their prediction was verified because during cold-gas breathing at 600 fsw the rectal temperature did not increase during periods of work but remained depressed. Then, at 800 fsw, severe negative thermal balance did occur during cold-gas breathing in spite of the warm ambient environment.

Acute respiratory distress resulting from direct action of cold gas on the respiratory tract has been shown. The subject who showed acute respiratory distress (T.G.) recovered quickly with no change in pulmonary function. The other subject (J.M.), although experiencing no acute respiratory distress. was noted to have a decrease in FIV after breathing cold gas. This is in agreement with Guleria et al. (1) who noted that direct action of cold air on the upper respiratory tract produced mild local airway obstruction and a reduction in FIV.

Since cold-gas breathing did cause a decrease in both subjects in the FEV_1, especially at depths greater than 600 fsw, in an underwater emergency this might be a factor critical to survival. Despite the decrease in FEV_1 and the copious secretions, the subjects continued to show adequate pulmonary ventilation as evidenced by the $P_{A_{CO_2}}$.

Since the slope of heart rate–$\dot{V}_{O_2}$ curve was depressed during cold-gas breathing at 1000 fsw, this raises the question of what the cause and the consequences might be. Thoracic hypothermia is the probable cause; reduced cardiac output during exercise might be a consequence.

In conclusion, because of the negative thermal balance and the possibility of acute respiratory difficulty from the direct effects of cold gas, heating the breathing gas for working dives in excess of 600 fsw is recommended for safety and efficiency.

Acknowledgments

This work was partially supported by the Bureau of Medicine and Surgery, Navy Department, Work Unit 4306.02-6010B.

The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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THERMAL COST OF SATURATION DIVING: RESPIRATORY AND WHOLE BODY HEAT LOSS AT 16.1 ATA

T. O. Moore, J. F. Morlock, D. A. Lally and S. K. Hong

Since the early 1960s, the demonstrated ability of man to dwell in artificial helium-oxygen mixtures under saturation conditions has brought about many subsequent industrial, military and scientific exposures to this environment. The reasons have appeared to be as many and varied as the exposures. "Feasibility" and "screening" dives have been abundant and pragmatically impressive.

In the 1970s, certain environmental features and their biomedical concomitants have become more evident, if not more serious, in man's dwelling under hyperbaric conditions for extended periods of time. Among these are the need to compromise in choice of inert gas components in order to avoid, variously, inert gas narcosis, high gas densities (and the attendant ventilatory limitations), and of course, decompression sickness—all the while providing adequate but safe levels of oxygen for the divers. A series of ingenious experiments is currently balancing these factors in several ways and continually extending the depth capability of "resident" man (1, 2a, 4, 6).

There are, however, some "on-going" physiologic problems which will continue to accompany men to depth unless overcome by engineering or medical technology. One of these, summarized by Webb (10), is the cumulative body heat drain which occurs particularly in the $He-O_2$ environment at depth and is due primarily to the high thermal conductivity, specific heat and density of that medium. A high convective loss results, with respiratory losses increasing with depth, which could lead to nutritional management problems in order for divers to maintain metabolic balance. Problems involving fluid and electrolyte balance have been less documented but can be likened to situations in Arctic survival where these imbalances may tip the scale away from survival (8, 9).

In order to systematically quantify the physiologic impact on these three—thermal, fluid, and metabolic balances—a "dry" dive was undertaken in January 1972, in which six divers occupied the undersea habitat "Aegir"* for a total of 9 days in a helium-oxygen environment. Maximum depth exposure was 500 feet (16.1 ata). The anthropometric and physiologic characteristics of the divers appear in Table I; the dive "profile" and pertinent characteristics of the environment are shown in Fig. 1. While each diver had particular housekeeping and

*Makai Range, Inc.

TABLE I

PHYSICAL CHARACTERISTICS OF DIVERS

Divers	Age (yrs)	Height (cm)	Weight (kg)	Surface Area (m^2)	Vital Capacity (L)	Pulse Rate (per min)	Blood Pressure (mm Hg)	
							Systolic	Diastolic
BR	29	167.0	64.9	1.74	3.90	56	110	60
JM	25	182.9	69.4	1.92	4.65	48	108	70
EH	32	165.1	75.3	1.82	3.65	80	128	90
WB	41	174.0	69.7	1.84	4.20	78	134	86
CyC	33	185.7	81.2	2.05	4.91	64	146	94
CCh	22	170.5	84.6	1.90	4.90	56	108	70
Mean	30.3	174.2	74.2	1.89	4.368	63.7	122.3	78.3
SE	2.7	3.5	3.1	0.046	0.217	5.3	6.6	5.5

scientific duties, a typical daily regimen appears in Table II. The exercise portions were terminated during the decompression schedule.

"Aegir" consists of three separate compartments, each with autonomous life support and control capability—laboratory/galley, cenral sphere (diving ready-room), and living quarters. All are equipped with P_{O_2} and P_{CO_2} sensors† which continuously monitor ambient air. Relative humidity was monitored by hygrometers. The environmental parameters in Fig. 1 are those reported by the noon and midnight watch each day. A detailed description of "Aegir" has been reported previously by Pegg (7).

†Minos—Beckman Instruments, Inc.

TABLE II

DAILY REGIMEN

Hours	Activity
0600	Reveille
0630	Recording of vital signs
0700	Breakfast
0730–1130	Heat loss monitoring (rest and exercise)
1200	Lunch
1300–1600	Physiological monitoring (cold pressor test, water diuresis test, etc.)
1600–1800	Psychomotor testing[a]
1800	Supper
1900–2200	Psychological and psychomotor testing
2200–0600	Sleep

[a]Psychological and psychomotor data are not included in this report.

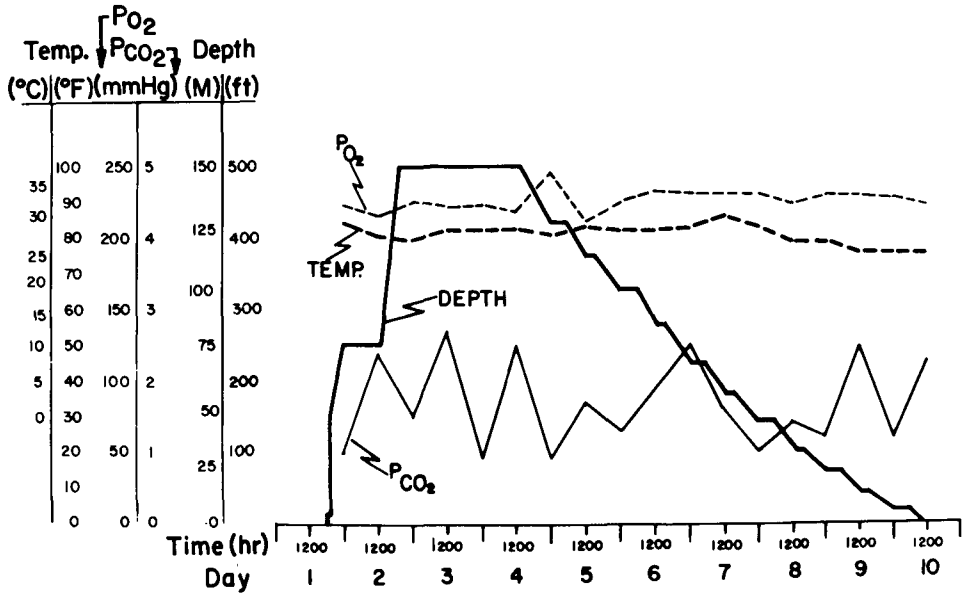

FIG 1. Dive profile and values of environmental parameters.

Methods and Materials

DIVE PROFILE

Pressurization was begun at 1900 hours on day 1. The first overnight stop was made at 250 feet (approximately 8.6 ata). After psychomotor testing during the remaining evening hours, the divers slept for 6 hours. The normal regimen began at 0600. Vital signs—pulse rate, blood pressure, oral temperature and body weight—were recorded; breakfast followed.

After breakfast, the subjects were taken in turn (standardized) for measurement of heat loss. Each subject, with rectal temperature probe in place and clad only in light shorts, sat quietly on a bicycle ergometer‡ for 15 or 20 minutes. He then breathed normally through a standard commercial mouthpiece-double hose assembly* the intake side of which had been shortened to approximately 2 inches upstream of the intake valve. Inserted into this segment was a hygrometer-sensing probe, thermistor and thermocouple probe. The exhaust side (just downstream from the exhaust valve) was similarly equipped, with the exhaust hose terminating at a connection with a Douglas bag valve for expired gas collection. Relative humidities of intake and exhaust gases, rectal temperatures, and intake air temperatures were recorded in the habitat at appropriate intervals during the experimental regimen. The intake and exhaust thermocouples were hard-wired to a polygraph located in the main control room outside the habitat and the EMf difference was recorded; EKG for heart rate count was similarly recorded.

‡Monark, Varberg, Sweden.
*U.S. Divers, Santa Ana, California.

During the last 2–3 minutes of the rest period, expired gas was collected and ΔEMf, rectal and ambient temperatures, and relative humidities were recorded. The Douglas bag was then emptied through a horizontal Plexiglas mixing chamber (approximately 12 inches long, 4 inches diameter) which was fitted and sealed at the bottom over the sensing probe of a P_{O_2} meter. In series downstream from the mixing chamber was an attached gas flowmeter.† Approximately halfway through the bag emptying procedure, the input and output sides of the mixing chamber were clamped off and the P_{O_2} of the contained gas was recorded (approximately 30-40 seconds equilibration time was required). The bag volume was then completely emptied and the expired volume recorded from the flowmeter. The P_{O_2} meter was checked daily against a range of calibration gases carried on board. (These had been analyzed previously by the micro-Scholander technique in the laboratory.) $\dot{V}_E$ and $\dot{V}_{O_2}$ were determined from these procedures.

Simultaneously with the expired gas collections, temperature and heat flux were recorded at several points on the subject's body surface: forehead, chest, abdomen, upper back, upper arm, forearm, hand, thigh, calf and foot. These were monitored by use of an exploring probe consisting of a thermistor and heat-flow disc mounted side-by-side in a Lucite plate. The plate was attached to a handle and spring loaded (with a marker) so that uniform tension was applied in each case. Each subject was indelibly marked at each of various skin points for consistency throughout duration of the dive. Skin temperatures were recorded via telethermometer* in the habitat; heat flux was monitored on the external polygraph.

The subject then proceeded with mild exercise (410 kg·m/min) for 10 minutes, at the end of which (last 2 minutes) the above recording procedures were repeated as well as during recovery for minutes 2–3, 5–6, and 9–10. One ata control values were taken in the unpressurized habitat for the control days just prior to the dive.

At 1300 hours on day 2, after 17 hours at 250 feet depth, pressurization continued to a depth of 500 feet, where the divers remained for 43 hours. The experimental protocol (Table II) was followed, as described above, while at this depth, and fasting venous blood samples were taken on the morning of day 4 for comparison with pre-dive control samples.

Decompression began at 1400 hours on day 4 (Standard U.S. Navy helium schedule), with overnight holds from 0000-0600 hours and a rest stop from 1400-1600 hours daily, until surfacing at 1200 hours on day 10. The experimental procedures (minus the exercise portion) were adhered to until the habitat reached 150 feet (late on day 7) when the habitat went on "power-down" condition to pass through the fire hazard zone. After that point, only the morning vital signs were monitored.

Food and fluid intake were closely monitored throughout. The regular meals were of the frozen variety,‡ and all divers received the identical meal at each time (though the menu varied). The divers began the controlled diet 2 days prior to the dive. Each meal, on an individual basis, was preweighed item by item, tagged as to recipient, and all residue weighed to determine individual consumption. The divers themselves scrupulously logged fluid consumption in its several forms. Sample meals were subsequently analyzed for sodium and potassium content by flame photometry, again on a per item basis, and caloric and water

†A. H. Thomas, Philadelphia.
*Yellow Springs Instruments, Yellow Springs, Ohio.
‡Sky Chef, Inc., Honolulu, Hawaii.

content estimated from nutritional tables (5). Extra food and snacks were allowed for reasons of morale, but again carefully logged and subsequently analyzed.

Feces was not retained, but all urine was collected as voided, the volume noted and triplicate 50 cc aliquots locked out to the surface for subsequent analysis for sodium, potassium, chloride, urea, osmolality and creatinine.

Blood samples drawn on day 4 (Diver WB was an ex-medical corpsman, U.S.N.) were centrifuged in the habitat (after hematocrit determination) and plasma locked to the surface for analysis of osmolality, sodium, potassium, chloride, creatinine, total protein, urea and glucose.

Water diuresis tests (1 liter load) were run on four divers on day 4 at 500 feet, day 6 at 213 feet and at the surface. Cold-pressor tests (6°C water) were done by three divers at 500 feet on day 4, at 180 feet on day 7, and at the surface.

Results

Vital Signs

The daily vital sign data are shown in Fig. 2. Generally speaking, all parameters showed slight decreases early during the compression stage. The systolic blood pressure fell slightly more than the diastolic and both remained somewhat depressed throughout the dive. A significant bradycardia was observed initially with a slow return of heart rate to normal by the end of the dive. A two-day episode of higher rates (compared to control day zero)

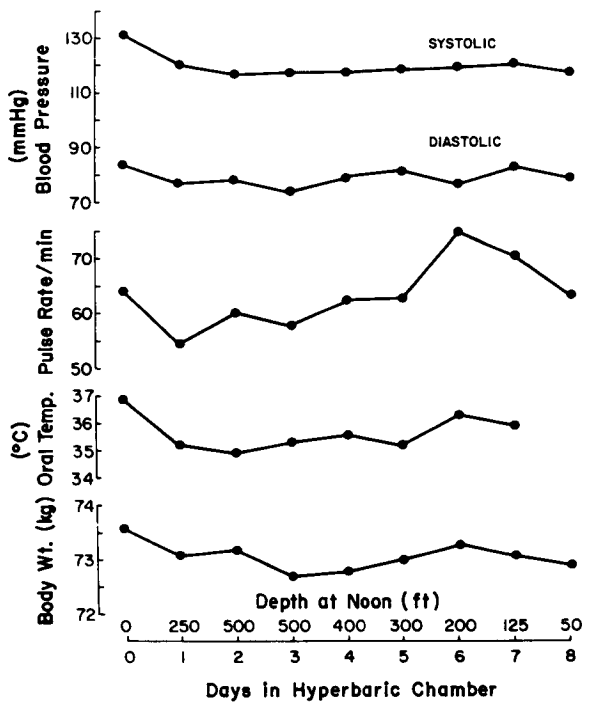

FIG. 2. Daily vital sign data.

occurred on days 6 and 7. Oral temperatures were depressed between 1° and 2°C throughout; rectal temperature was down an average of 1°C. A body weight loss of approximately 1 kg maximum (day 3) was observed; body weights remained lower than control weights throughout the dive. These data will be discussed in detail below. They are generally consistent with peripheral vasoconstriction but only when coupled with reduction of extracellular fluid volume.

Cardiorespiratory Responses

The cardiopulmonary responses recorded at depth are presented in Fig. 3 and show that, even during exercise, heart rates are maintained at lower levels than surface controls. $\dot{V}_{O_2}$, during both rest and exercise, peaks on the first day at 500 feet, returning toward surface values during the second day's exposure at that depth. $\dot{V}_E$ rises at rest, falls during exercise, again inflecting on the second day at 500 feet. This inflection also characterizes the O_2 pulse and ventilation equivalent curves. The common denominator in all these reversals is believed to be the change in ambient temperature which occurred on day 3 (500 feet). As seen in Fig. 1, the temperature of the internal habitat had dropped more than a degree C from day 1 through day 2. On day 3, the second day at 500 feet, the habitat heaters were turned on, bringing the temperature back to approximately 29°C, within the comfortable thermal zone cited by Webb (10). This was subjectively noted by the divers. Mean body temperature was indeed slightly higher on day 3 than on day 2 (see Table III).

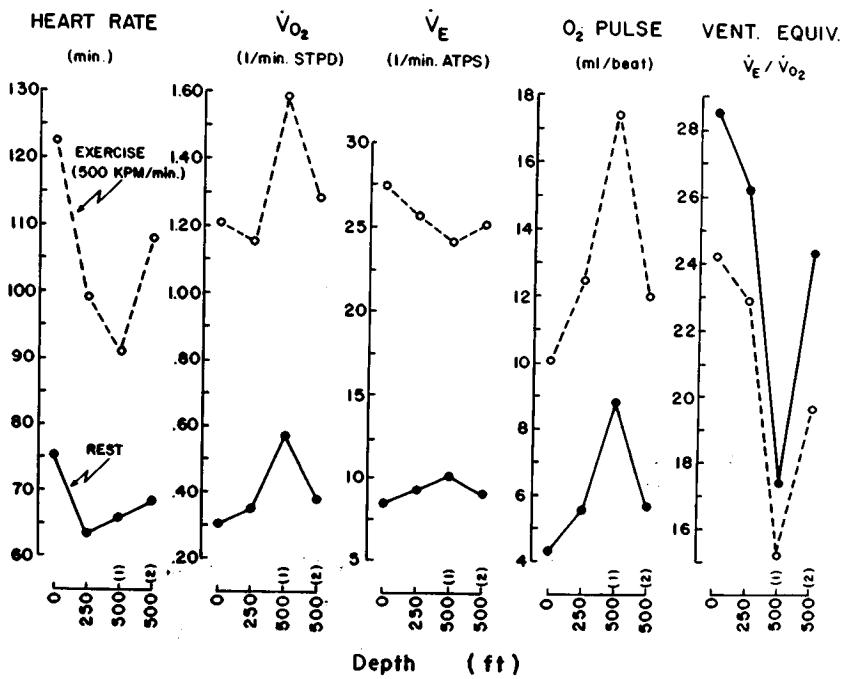

FIG. 3. Cardiopulmonary responses during rest and exercise as a function of depth. [First day at 500 feet—500(1); second day at 500 feet—500(2).]

TABLE III

THERMAL CHARACTERISTICS OF AIR AT SURFACE AND $He-O_2$ AT HYPERBARIC ENVIRONMENT

Physical Characteristics	Air at Surface	250 ft. (He 96.6%) (O_2 3.4%)	500 ft. (He 98.2%) (O_2 1.8%)
Density (ϱ), gm/L	1.11	1.63	2.82
(Relative ϱ)	(1.0)	(1.5)	(2.5)
Specific heat (C_p) cal/gm/°C	0.26	1.11	1.17
(Relative C_p)	(1.0)	(4.3)	(4.5)
Heat capacity (ϱC_p) cal/L/°C	0.29	1.82	3.32
(Relative ϱC_p)	(1.0)	(6.2)	(11.3)

HEAT LOSS RESPONSES

Table III describes the relevant thermal characteristics of the environment, indicating the potential thermal drain facing the divers in this environment, as shown by the relative specific heat *(C_p)* and heat capacity *($\rho\ C_p$)*.

respiratory heat loss becomes an increasing problem as gas density and relative specific heat of the breathing medium increase. Total respiratory heat loss is the sum of the convective loss and the evaporative loss. The following expressions were used for calculation in this experiment:

1) Convective loss: $= \dot{V}_E \cdot \rho \cdot C_p \cdot (T_E - T_I)$

where $\dot{V}_E$ = minute volume in L/min ATPS
ρ = gas density in gm/L
C_p = specific heat of the gas in Kcal/gm · °C
T_E = temperature of expired gas
T_I = temperature of inspired gas

2) Evaporative loss $= \dot{V}_E \cdot \frac{P_{H_2O}}{760} \cdot \frac{18}{22.4} \cdot \frac{273}{273 + T_{amb}} \cdot 580$

where $\dot{V}_E$ = minute volume
P_{H_2O} = vapor pressure of water in mmHg
and = (P_{H_2O}) expired − (P_{H_2O}) inspired
760 = 1 atmosphere of pressure in mmHg
18 = molecular weight of water in gm/mol
22.4 = molar volume of water in L/mol
273 = absolute temperature
T_{amb} = ambient temperature in °C
580 = heat of vaporization in cal/gm

Figure 4 represents the respiratory heat loss components during the experimental conditions of rest, exercise and recovery at indicated points. There was no difference between the

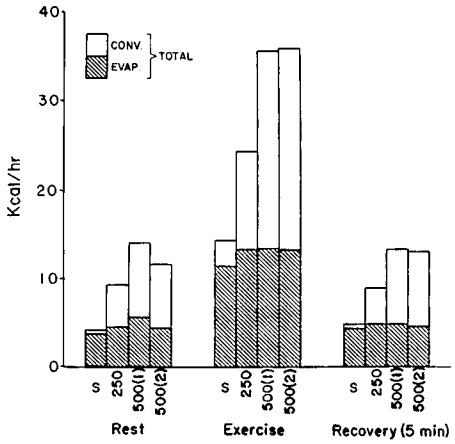

FIG. 4. Convective and evaporative respiratory heat loss during rest, exercise and recovery as a function of depth.

first and second days at 500 feet, so the data can be considered to represent the second day at that depth. The major feature of these data is the fact that with increasing depth and consequent increasing ρC_p of the ambient helium-oxygen mixture the convective expression of respiratory loss increases dramatically while only small increases are seen in the evaporative expression. This is in marked contrast to the case of air at 1 ata, where losses are almost completely evaporative. The respiratory "thermal" recovery was essentially complete after 5 minutes from termination of exercise at this level.

Skin heat fluxes are depicted in Fig. 5 for both rest and exercise at the various simulated depths. For the resting condition, the salient points appear to be an alteration in distribution and degree of flux upon exposure to the He–O_2 environment at depth. For instance, while forehead flux appears high under all conditions, a notable decrease, particularly in the distal extremities, (especially foot, and to a lesser extent, upper arm and hand below 250 feet) was found to occur. On the other hand, the back, lower arm, thigh and lower leg were found to have relatively higher heat flux in the He–O_2 environment at all depths. During exercise, the qualitative pattern was not dissimilar to the resting condition. High fluxes from back, lower arm, thigh and lower leg, with hand and foot (especially the latter) showed low heat flows.

Considering the overall pattern of heat exchange under these conditions then, Table IV provides a summary during rest and exercise. At rest, mean skin temperature falls steadily through the first day at 500 feet; it rises slightly on the second, presumably via the addition of heat to the chamber environment. Mean body temperature (2) follows the same pattern. Heat production, as gauged by metabolic rate, rises to 90.8 Kcal/hr/m^2, then drops on day 2 at depth, while heat loss, both cutaneous and respiratory, follows the same qualitative pattern. The net result is a negative heat balance for all days except the first day at 500 feet where metabolic heat production peaks. During exercise, positive heat balance was maintained throughout, even in the face of higher heat loss, through a higher level of metabolic heat production. Again, the inflection from the first day at 500 feet to the second day at 500 feet was seen.

Figure 6 shows that the relative contribution of respiratory heat loss to total loss increased

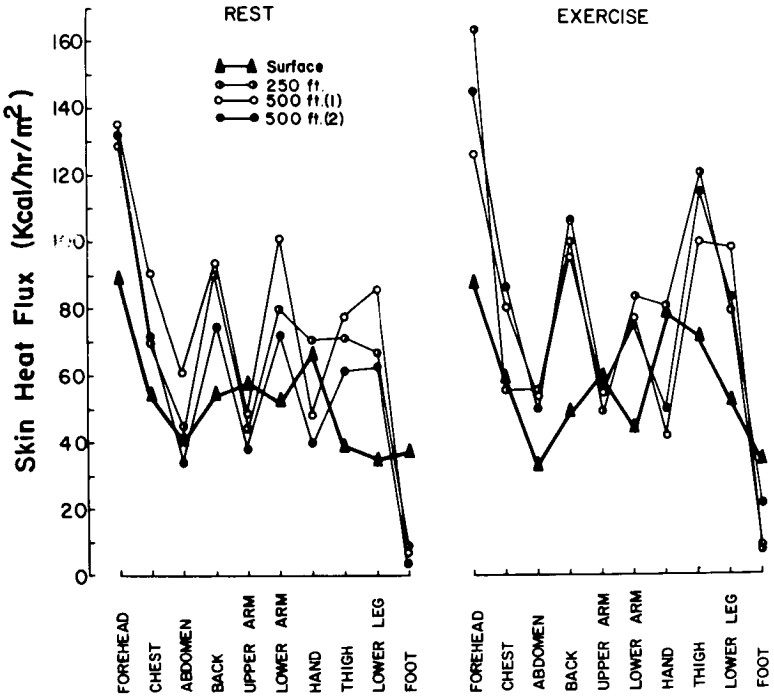

FIG. 5. Cutaneous heat flux during rest and exercise as a function of depth.

predictably with depth and was exacerbated with exercise to the point of comprising approximately 20% of total loss even under the mild workload imposed. Skin flux decreased overall as skin temperature decreased. Figure 7 shows the narrowing of the skin to air gradient under conditions of rest, exercise and at the 5-minute recovery point, when associated with increasing depth. Again, on the second day at 500 feet the gradient narrows as T_{amb} was raised.

The intensity of the peripheral vasoconstrictive reflex is well-illustrated by the results in Fig. 8, depicting the administration of a standard cold-pressor test with the hand immersed in 6°C water for 30 minutes. The blood pressure records are generally typical, but a noticeable lack of "hunting" or vasodilatation and consequent increase of finger temperature was found. The peripheral constriction was maintained even in the face of this added challenge, most apparent at the greatest depths.

FLUID AND ELECTROLYTE BALANCE

As found in other dives (10), the nutritional intake was high in caloric content, averaging over 4000 Kcal/day and ranging as high as 6400 on day 4 of the dive. External water intake was also high, approximately 2.5 L per day; however, negative fluid balance was a characteristic of the entire dive period. Hematocrit changes are given in Table V, in which mean values are misleading, in that two of the six divers were found to have greater than 10% increase. For therapeutic reasons, these divers were instructed to increase both water and salt intake but, unfortunately no further blood studies were made. On the other hand, the two suffered no untoward effects upon decompression, and so presumably the attempts at proper hydration were effective.

TABLE IV

HEAT EXCHANGE AT REST AND DURING EXERCISE

Heat Exchange	Depth (ft)			
	0	250	500[a]	500[b]
Chamber temperature (°C)	29.0	27.8	27.8	29.0
Chamber humidity (%)	78	68	72	76
A. Rest				
Rectal temperature (°C)	37.43	36.91	36.66	37.04
Mean skin temperature (°C)	33.54	31.01	30.13	30.88
Mean body temperature (°C)	36.14	34.96	34.50	35.00
Heat production (Kcal/hr/m²)	44.7	55.7	90.8	60.6
Heat loss (Kcal/hr/m²)	52.3	74.1	87.0	67.5
Heat balance (Kcal/hr/m²)	−7.6	−18.4	+3.8	−6.9
B. Exercise (410 KPM/min)				
Rectal temperature (°C)	37.38	36.83	36.80	36.96
Mean skin temperature (°C)	33.26	30.78	30.06	30.86
Mean body temperature (°C)	36.02	34.83	34.57	34.94
Heat production (Kcal/hr/m²)	191.5	183.5	251.0	203.5
Heat loss (Kcal/hr/m²)	64.5	99.2	103.0	107.5
Heat balance (Kcal/hr/m²)	+127.0	+84.3	+148.0	+96.0

[a]The first day at 500 feet.
[b]The second day at 500 feet.

Figure 9 illustrates the renal excretion patterns found during the dive (pooled 24-hour samples). Urine flow was generally elevated throughout the dive, while creatinine excretion decreased and plasma creatinine levels did not change (Table V). Sodium and potassium excretion remained approximately unchanged, as did plasma levels. Thus, in the face of decreased glomerular filtration rate, relatively constant electrolyte excretion, and increased urinary volume, there is the implication of an impaired electrolyte reabsorption and conse-

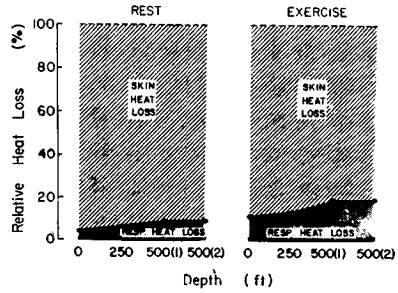

FIG. 6. Respiratory heat loss as proportion of total loss during rest and exercise as a function of depth. [First day at 500 feet—500(1); second day at 500 feet—500(2).]

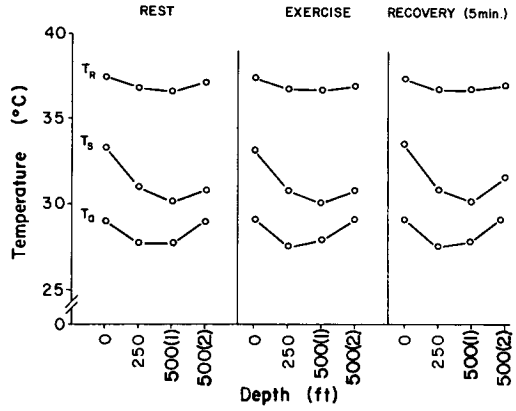

FIG. 7. Rectal, skin and ambient temperature during rest, exercise and recovery as a function of depth. [First day at 500 feet—500(1); second day at 500 feet—500(2).]

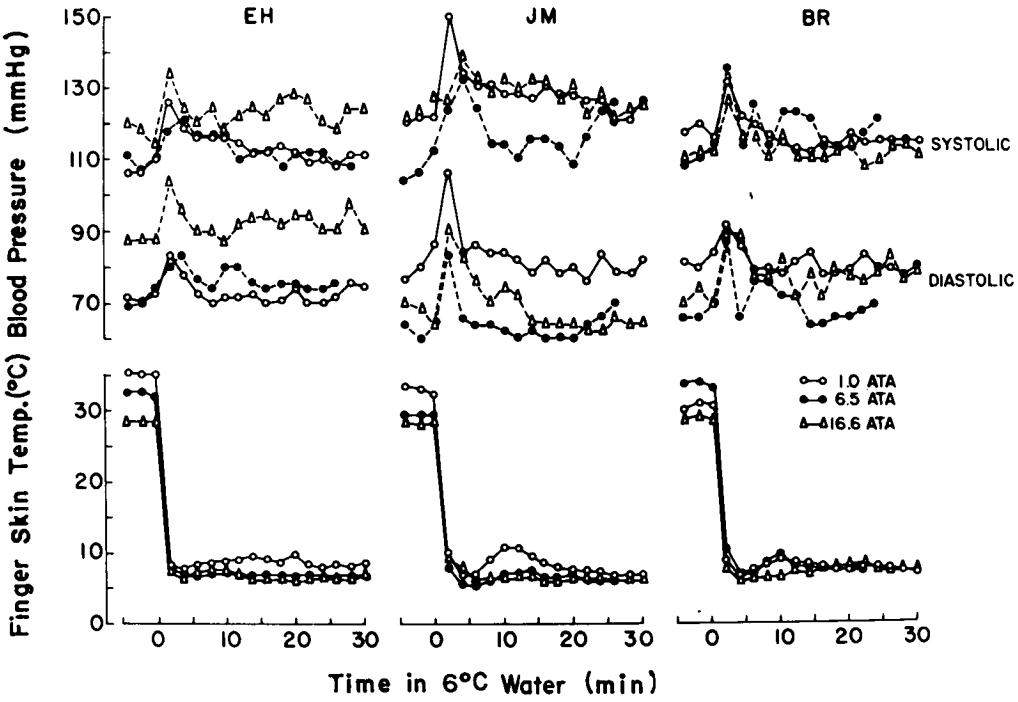

FIG. 8. Blood pressure and finger temperature responses to immersion of the hand in 6°C water as a function of depth.

TABLE V

BLOOD CHEMISTRY BEFORE DIVE AND AT 500 FT. DEPTH IN FOUR DIVERS

Composition	Before Dive	500 Ft.
Hematocrit ratio (%)	40.5 ±1.3	46 ±1.5[a]
Plasma concentration		
Osmolality (mOsm/kg)	285 ±1.3	273 ±0.7[a]
Na (mEq/L)	142 ±0.7	141 ±1.3[a]
K (mEq/L)	4.0 ±0.1	4.2 ±0.1
Cl (mEq/L)	110 ±0.2	106 ±1.5
Creatinine (mg%)	1.03±0.05	1.02±0.05
Proteins (gm%)	7.10±0.17	7.83±0.49[b]
Urea (mg/L)	8.2 ±1.2	7.7 ±0.7
Glucose (mg%)	86.3 ±7.3	97.0 ±8.8

[a]Significantly different from the corresponding value obtained before the dive ($P < 0.05$).

[b]Marginally different from the corresponding value obtained before the dive ($0.10 > P > 0.05$).

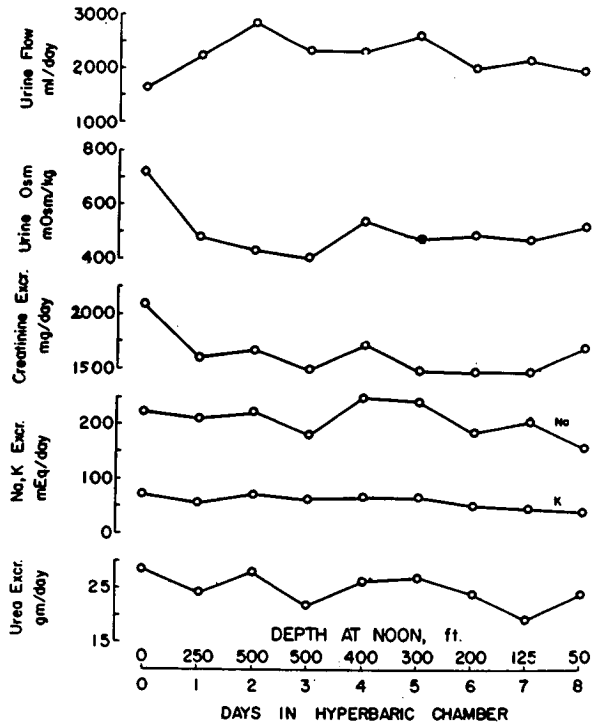

FIG. 9. Urinary excretion data as a function of time and depth of pressurization.

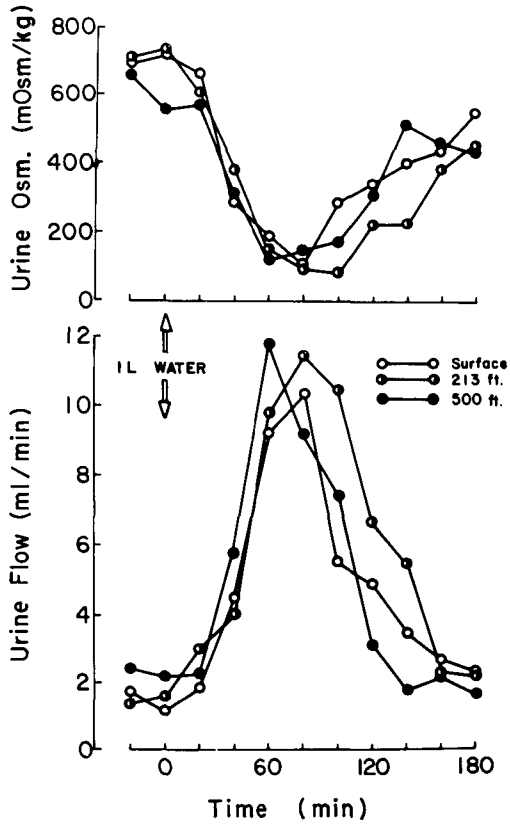

FIG. 10. Time course of urine flow and osmolality in response to a 1 liter water load as a function of depth.

quent solute diuresis. The decreased urinary osmolality is supportive of this. This same pattern of contraction of extracellular fluid volume and diuresis has been observed in men exposed to cold during field experiments in the winter subarctic and ascribed to a decreased aldosterone secretion (3).

It was of interest in the context of fluid shifts and decreased plasma osmolality to determine whether the anti-diuretic hormone (ADH) system was in any way affected. Under the conditions of this experiment, this was most simply checked by following the elimination of a 1-liter water load. These data appear in Fig. 10 as taken at the surface, at 213 feet, and at 500 feet depth. While it appears that peak urine flow occurs somewhat sooner at 500 feet, there was no statistical difference in flow pattern or urine osmolality related to simulated depth. Therefore, it appears on the basis of this test, that there is no change in time course or amplitude of the ADH system response.

In summary, these data suggest that, in the face of the thermal drain associated with a high pressure helium-oxygen environment, attendant deviations occur in fluid and electrolyte balance which are similar to responses seen in other cold-stress situations. This thermal drain, with the increasing importance of respiratory heat loss at depth, would be exacerbated during water excursions and the concomitant cutaneous heat losses. In any event, the relative

dehydration which accompanies cold-induced vasoconstriction could be a predisposing factor in the onset of decompression sickness.

ACKNOWLEDGMENTS

The authors wish to thank Dr. Jon Pegg for medical backup and Mr. Al May, Head of Operations, Makai Range, Inc., for his close cooperation.

This study was supported in part by Sea Grant GB 8393, and a fund provided by the State of Hawaii Marine Affairs Coordinators Office.

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ENERGY BALANCE OF MAN IN SIMULATED DIVE FROM 1.5 TO 31 ATA

P. Varène, J. Timbal, H. Vieillefond, H. Guenard and J. L'Huillier

Experimental determinations of animal or human metabolism in He-O_2 atmospheres are numerous in the literature. After the early work of Cook et al. (6) an increase in oxygen consumption ($\dot{V}_{O_2}$), if any, is usually related to an increase in skin heat losses (2, 7, 8, 12, 18, 19, 25), rather than to a cellular effect, although He might alter metabolic pathways in some biological systems (20).

Theoretical considerations show that skin convective heat losses must increase in the He-O_2 atmosphere at depth (9, 16, 23). However, few studies have been carried on in man to ascertain this point experimentally (17); the same remark holds true for the respiratory convective heat losses for which only predictive assumptions are available (22, 24).

This paper describes an attempt to make an experimental determination of the different parameters in the body heat balance equation at several levels of ambient pressure and He-O_2 atmosphere. For a resting subject this equation may be written as:

$$M \pm R \pm C_S \pm C_{Res} - E = \pm S \qquad \text{(watts/m}^2\text{)}$$

Where:

M = metabolic heat production
R = radiant heat exchange
E = evaporative heat loss
C_S = skin convective heat exchange
C_{Res} = respiratory convective heat exchange
S = heat body storage

In this equation where M, C_S and C_{Res} are the first points of interest, M, R, C_{Res}, E and S are directly estimated and C_S computed.

Experimental Procedure

Measurements were made in four subjects lying nude at rest in a He-O_2 atmosphere ($P_{I_{O_2}} \simeq 300$ mb), at six levels of pressure between sea level down to 300 meters depth (1.49;

2.28; 4.38; 8.36; 16.09; 30.79 ata). Control values were obtained at 1.5 ata in air. Ambient temperatures were always set for the thermal comfort of the subjects.

Metabolism was calculated from oxygen consumption measured by an open-circuit method, during three periods of 10 minutes at each pressure level for each subject. Gas analyses were duplicated using a fuel cell for O_2, infrared absorption for CO_2, and gas chromatography for both.

Heat exchanges by radiation *(R)* were computed from the mean skin temperature $(\overline{T}_S)$ and the mean wall temperature $(\overline{T}_w)$ according to the Stefan-Boltzmann law. Body heat storage *(S)* was computed from variations of the mean body temperature $(\overline{T}_b)$ with $\overline{T}_b = 0.66\ \overline{T}_{re} + 0.34\ \overline{T}_S$. Rectal $(\overline{T}_{re})$, skin and ambient temperatures were recorded using thermocouples, and averaged according to Colin and Houdas (5). Skin and respiratory evaporative heat losses were calculated from a weight loss recording. Respiratory convective heat losses (C_{Res}) were computed from respiratory flow $(\dot{V}_E)$, inspired (T_I) and expired (T_E) gas temperatures picked up with microthermocouples (13).

From these data, skin convective heat losses (C_s) were deduced according to the general equation of the body heat exchange balance:

$$C_S = M - (R + C_{Res} + E + S) \text{ watt} \cdot \text{m}^{-2}$$

A coefficient for skin convective heat exchanges *hc* was established from:

$$hc = \frac{C_S}{\overline{T}_S - T_a}$$

At each pressure level, a supplementary run was made during which each subject was investigated while breathing a precooled $He\text{-}O_2$ mixture for 20 minutes. The first measurements began after a resting period of 1 hour.

Results

The values of metabolism do not show any systematic variation with pressure: $\dot{V}_{O_2}$ does not change in three cases but decreases in one (FS, $P < 0.05$), $\dot{V}_{CO_2}$ increases significantly in two individual cases (BA, BB) and for the subjects, altogether ($P < 0.06$). Consequently R increases significantly with pressure for subjects altogether ($P < 0.01$) (see Fig. 1). Inhalation of cooled gases for 20 minutes does not modify the $\dot{V}_{O_2}$ values of the subjects, in spite of an important increase in respiratory heat loss at the deeper levels (see below). A decrease of 0.2–0.25 °C in rectal temperature (T_{re}) is observed at 30 ata at the end of the cooled gas inhalation period; this appears too small to induce a measurable increase in thermogenesis.

The ventilation ($\dot{V}_E$) does not vary significantly with pressure in three subjects but increases in one (BA, $P < 0.05$) (Fig. 2). No systematic variation of breathing frequency (f) or tidal volume (V_T) is observed.

$\dot{V}_A$ and $P_{A_{CO_2}}$ have been computed with $\dot{V}_E$ and $\dot{V}_{CO_2}$ assuming that dead space does not vary with pressure (21) but is only a function of V_T and f according to the formula given by Bargeton et al. (1). $\dot{V}_A$ does not show any variation with pressure, while $P_{A_{CO_2}}$ increases slightly. Cooled-gases inhalation does not modify systematically $\dot{V}_E$, V_T or f.

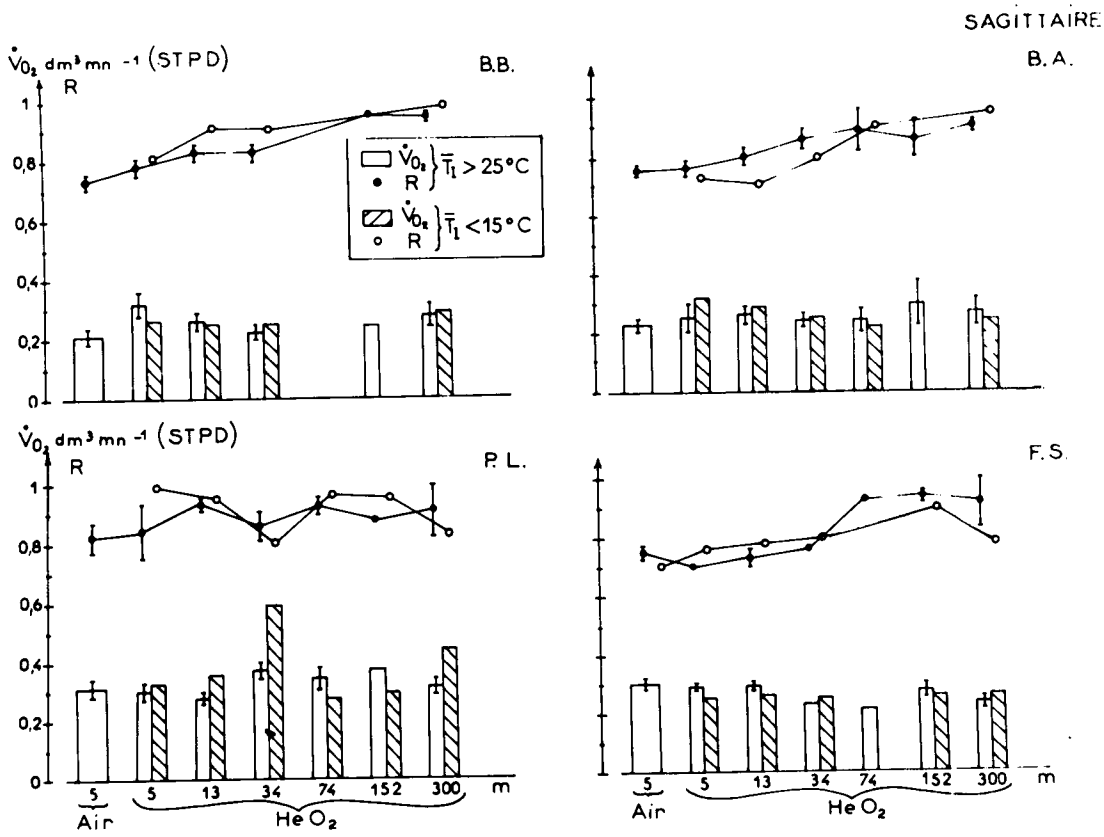

FIG. 1. Oxygen consumption ($\dot{V}_{O_2}$) and respiratory exchange ratio (R) measured for each subject as function of depth (meters of sea water) for two temperatures of inspired gases.

Heat loss by respiratory convection increases as inspired gas temperature *(T_I)* decreases and as depth increases according to predictions. The values obtained on each subject are shown in Fig. 3. Expressed as percentage of metabolism, the mean values computed for four subjects vary from 1 (T_I = 30°C and P_B = 1.5 ata) to 60 (T_I = 10°C and P_B = 31 ata) (Table II).

The values of ambient temperatures set in order to maintain the subjects in thermal comfort increase with depth. They are listed in Table I. Mean skin temperature *($\bar{T}_S$)* increases too as depth increases but less than dry bulb ambient temperature *(T_a)* or wall temperature *(T_w)*. Consequently the difference between T_S and T_w decreases and radiant heat loss decreases.

As a function of pressure, rectal temperature *(T_{re})* slightly increases from day to day. However, heat body storage computed on the 2 hours of each daily experiment from T_{re} and $\bar{T}_S$ shows no systematic variation with depth. Its value averaging 7 or 8 watts/m² is always found negative showing a slight body cooling during each experimental period.

In agreement with predictions, weight loss, from which evaporative heat loss is computed, does not change with pressure. It corresponds only to insensible perspiration.

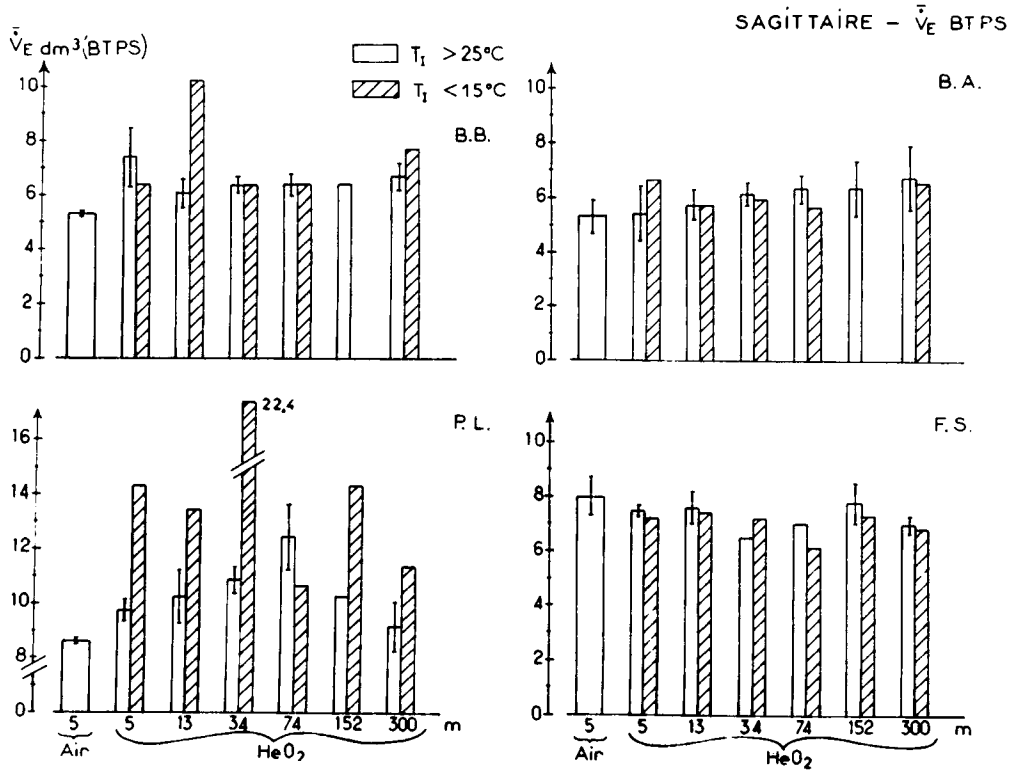

FIG. 2. Ventilation ($\dot{V}_E$) measured for each subject as function of depth (meters of sea water) for two temperatures of inspired gases.

Heat losses by skin convection, C_S, computed from data of M, R, C_{Res}, E and S are widely increasing for each subject with pressure. The mean values found for C_S rise from 14 watts/m² at 1.5 ata to 33 watts/m² at 31 ata in He-O_2 atmosphere. The control experiment at 1.5 ata in air gives a mean value of 8 watts/m². The association of an increase of C_S with a decrease of skin to ambient temperature difference leads to the computation of an exchange coefficient hc:

$$hc = \frac{C_S}{T_s - T_a}$$

which is ten times larger at 31 ata than at 1.5 ata (Fig. 4). The variation of hc with pressure (or other parameters like "convective constant" of Webb [23]) may be represented either by a first degree algebraic equation or by a power function.

Discussion

From a general point of view these results show that energy sources do not vary with pressure or He-O_2 mixture but that the avenues of the energy expenditure are widely modified.

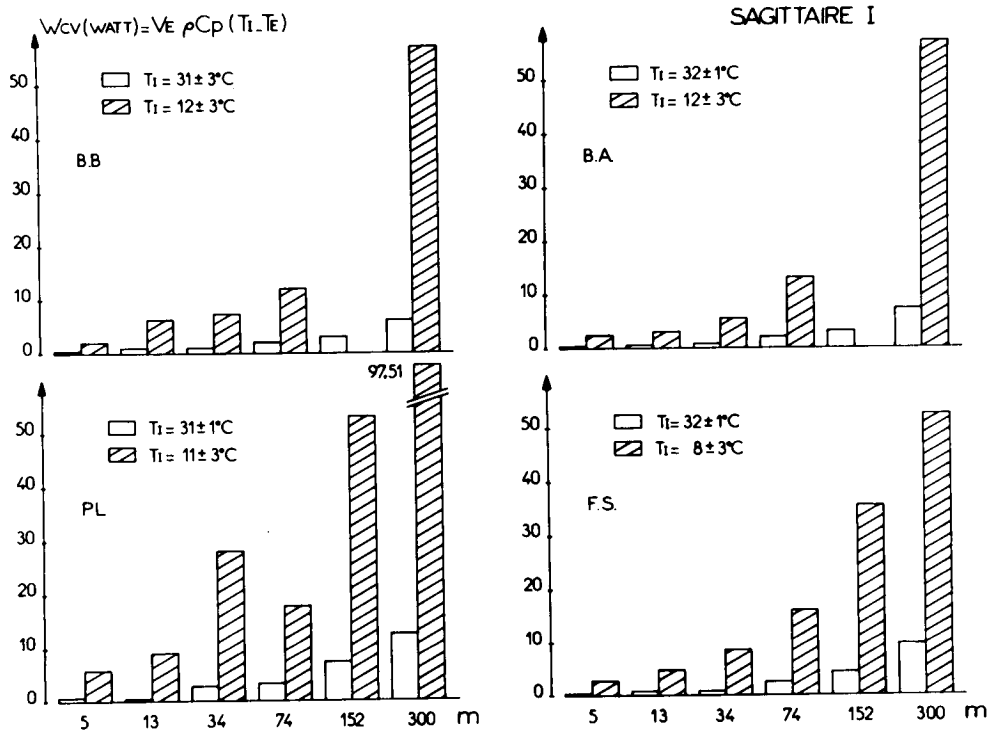

FIG. 3. Total respiratory convective heat losses ($\dot{W}_{CV} = C_{Res} \times A_D$, where A_D is the body area) measured on each subject as function of depth for two ranges in temperature of inspired gases.

The sharp increase in *hc* with the pressure rise agrees with the physical laws for convective heat exchange and implies that, as the pressure increases: 1) for the same temperature difference $(\overline{T}_s - T_a)$, absolute convective heat exchange increases; 2) the diver thermal balance becomes more and more unstable since for the same $\overline{T}_s$, a small variation in T_a induces a larger variation of convective heat exchanges.

These results are in agreement with those previously published up to 14 ata by Raymond et al. (17). Higher values for *hc* found by these authors may probably be related to the con-

TABLE I

MEAN VALUES OF DRY BULB TEMPERATURES (T_a) AND WALL TEMPERATURES (T_w) MAINTAINED IN THE PRESSURE CHAMBER, IN ORDER TO OBTAIN THE THERMAL COMFORT OF THE SUBJECTS

Temperature	Air	$He-O_2$					
	1.5 ata	1.5 ata	2.3 ata	4.4 ata	8.4 ata	16.1 ata	30.8 ata
T_a	26.5	28.7	29.6	30.0	30.7	31.8	32.4
T_w	24.8	26.4	27.0	28.2	28.3	30.1	31.2

TABLE II

RESPIRATORY CONVECTIVE HEAT LOSS (C_{Res}) EXPRESSED AS FRACTION OF HEAT PRODUCTION (M) IN FOUR SUBJECTS AT 30.8 ATA, FOR TWO TEMPERATURES OF INSPIRED GASES

	High T_I		Low T_I	
Subjects	T_I (°C)	C_{Res}/M	T_I (°C)	C_{Res}/M
BB	33.9	0.06	13.6	0.55
BA	33.4	0.08	9.4	0.69
PL	30.6	0.12	8.6	0.66
FS	31.0	0.12	7.5	0.63

vective respiratory heat exchanges which were not taken into account. The value for *hc* that was found at 1.5 ata in air (1.52 watts/m²/°C) agrees with the result of Hardy et al. (0.9 to 1.6 watts/m²/°C) in quiet atmosphere (10). These results are also in agreement with the opinion of authors who think that in He-O_2 atmosphere, no $\dot{V}_{O_2}$ variations occur as long as thermal comfort is maintained (12). The instability of the thermal balance at depth probably accounts for modifications of $\dot{V}_{O_2}$ or even $\dot{V}_E$ often found.

Another noticeable result of this study is the increase of the measured convective respiratory heat losses. The present values are in agreement with previous data obtained in air (11, 22) from which predictions had been made for deeper dives in He-O_2 (Fig. 5). The computation was based on:

1) the experimental fact that T_E may be represented by a simple function of T_I ($T_E = a + b\,T_I$) (see Fig. 5);
2) the assumption that the ratio $\dot{V}_{O_2}$ (STPD)/$\dot{V}_E$(BTPS) is constant with pressure and subjects.

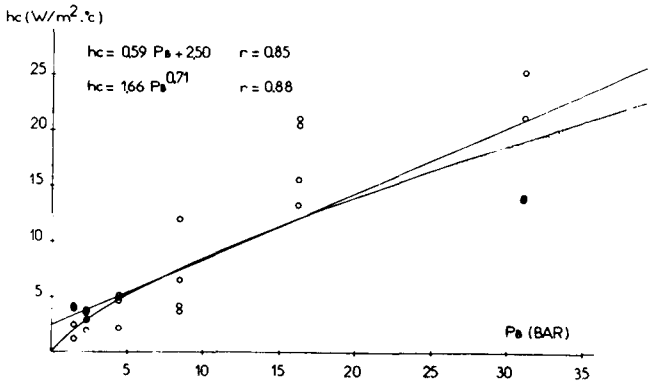

FIG. 4. Evolution of the exchange coefficient of skin convection *(hc)* as function of pressure. The equations give the linear and power functions which can fit the experimental data computed on four subjects.

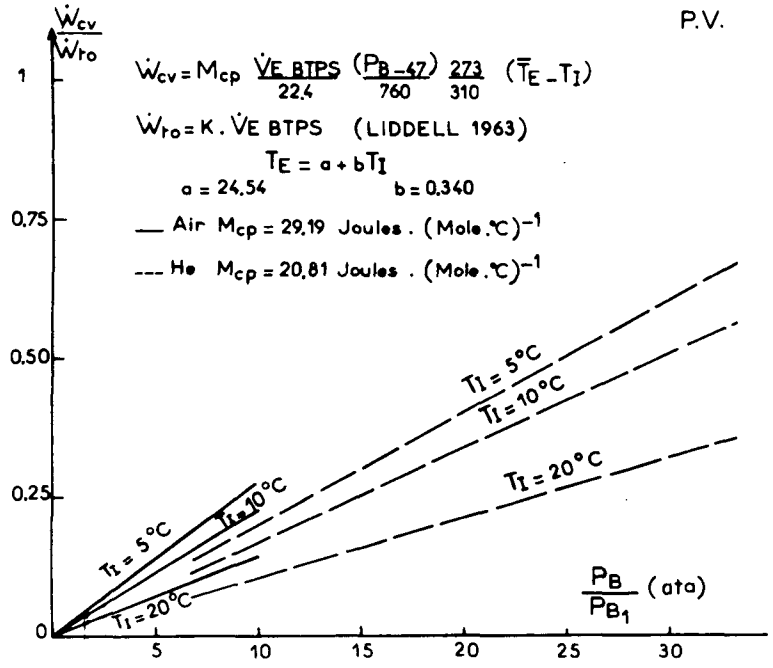

FIG. 5. Fraction of total heat production ($\dot{W}_{to}$) dissipated through respiratory convective heat loss ($\dot{W}_{cv}$) as function of pressure in ata (11).

In this representation

$$\frac{\dot{W}_{cv}}{\dot{W}_{to}} = \frac{C_{Res} \times A_D}{M \times A_D} = \frac{C_{Res}}{M}$$

The computation is made from molar ventilation $\dot{V}_{STPD/22.4}$, molar specific heat M_{cp} and values of a and b previously found in air (see text).

From 1), convective respiratory heat exchanges may be expressed as a function of T_I, $\dot{V}_E$ BTPS, specific heat (Cp.), and either volumic mass (P) or pressure (P_B).

From 2), the ratio of convective respiratory heat exchanges over the total energy sources C_{Res}/M may be expressed as a function of the same parameters without $\dot{V}_E$.

In the present experiment we have found values for coefficients a and b slightly different from those found in air. Coefficient a is higher (26.5 to 28.5 °C against 24.0 to 24.5 °C in air); coefficient b is lower (0.20 to 0.25 °C against 0.32 to 0.34 °C in air). This difference may probably be related to the fact that previous results were obtained in inspired dry air as opposed to wet air in the present work (15). Nevertheless, the values obtained at the deepest level of the present experiment and given in Table II show that they agree with predictions in Fig. 5.

The last main point brought out by this experiment is the increase of R. It may be noticed that such an increase was sometimes found in other work (3, 4, 21) without being discussed. If one admits that alveolar ventilation $\dot{V}_A$ is constant and that $P_{A_{CO_2}}$ is increased, $\dot{V}_{CO_2}$ must increase. Consequently R will increase if $\dot{V}_{O_2}$ remains constant. Such a remark however does

not give an answer to the question: why does R increase? An analysis error consequent to the large decrease of respiratory gas fractions with pressure may probably be excluded: firstly, because the gas analyses were made with two very different technics, and secondly because R always remained in normal range. Actually no physiological explanation may be given on this point with certainty and the true reason of the increase in R remains obscure. Nevertheless, whatever the explanation is, these conclusions on $\dot{V}_{O_2}$ will not be modified since the increase of R is consecutive to an increase of $\dot{V}_{CO_2}$.

ACKNOWLEDGMENTS

This work was supported by grants from the Centre National d'Exploitation des Oceans (Contract No. 70-244).

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HEAT EXCHANGES BETWEEN MAN AND THE WATER ENVIRONMENT

A. B. Craig, Jr. and M. Dvorak

The most obvious fact about the water environment is that when man enters this unfamiliar medium he almost always becomes cold. Numerous attempts have been made to quantitate this observation (5, 6, 8–10, 14, 16, 18, 20–22). Bullard and Rapp (2) summarized many of the reports and remarked that "it is disheartening to see the many small bits and pieces in which the information exists."

It is also worth noting that in most studies observations have been limited to changes in core temperature and measurements of oxygen consumption. Interpretation of such data in terms of heat losses and balances involves many assumptions. To calculate heat loss from the subject to the water, it is necessary to assume a steady state in which the heat stores are not changing. Under these conditions the heat production, as estimated from oxygen consumption, equals heat loss. During head-out immersion it is necessary to subtract an amount for heat loss from the head to the air and for heat loss through the respiratory tract. A proportion of the heat production is usually assumed to be through these two routes, and the remainder of the heat production is that which is lost to the water.

Unfortunately, it is unlikely that a steady state has been reached in any studies of man during immersion. As indicated by Rennie et al. (22), even in water of 30–33 °C rectal temperature decreased continuously throughout the 3 hours during which their subjects were in the bath.

In unsteady states it is necessary to assume that there is an additional loss (or gain) from heat stores to the water. In order to interpret changes in body temperature in terms of heat stores, one must decide what proportion of the subject's total mass is represented by the measured changes in core temperature. It is usually assumed that the periphery of the body mass is at water temperature. The factors which enable the investigator to use these methods of indirect calorimetry were developed for man in the air environment (4); however, to date there is no evidence to indicate they are valid for man in water.

There are only two studies in which the investigators attempted to circumvent these problems by direct measurements of heat loss to water. Lefèvre (16) generally estimated heat loss to the water from observations of the change in temperature in an uninsulated bath of known volume. In some experiments he attempted to maintain the water temperature constant by the addition of measured quantities of ice. Although he used water

temperatures between 5° and 35°C, the periods of immersion were limited to 10 minutes in cold and 30 minutes in the warmer water.

Burton and Bazett (5) constructed a calorimeter by insulating a "household bathtub" and installing circulating pumps. Heat loss was estimated by observing the decrease in the electrical heat which was required to maintain a constant temperature when the subject was in the tank as compared to control observations without the subject. They reported 15 experiments, 10 of which were conducted on one subject. The range of water temperature was between 30° and 35°C. In these experiments they often changed the water temperature after the heat loss appeared to be steady. In view of more recent investigations (9, 21), it is doubtful that their periods of observation were long enough.

In the current studies, heat balance experiments were conducted in a constant temperature water calorimeter at eight different temperatures between 24° and 36°C. Heat production was estimated from continuous measurement of the oxygen consumption. Heat loss from the respiratory tract and from the head, which was not immersed, was also measured. From this information changes in heat stores could be calculated directly and compared to changes in body temperatures. Because a major interest is heat loss in cold water, the results in 24°C water are presented.

Methods

The 10 subjects who participated in these studies were neither obese nor lean. Average height was 181 cm, weight 78 kg. Most of the studies were conducted in the morning no sooner than 1 hour after a light breakfast. All of the subjects wore the same light nylon bathing trunks.

Before the immersion the subjects sat in a semi-reclining position in an open-mesh aluminum rack which was attached to the underside of the cover for the calorimeter bath. The thermistor used to measure the temperature in the insulated external auditory canal (T_e) was inserted, and thermistors were attached, by the methods used previously (9), to measure skin temperature at six different sites. The collar around the subject's neck was made of $\frac{3}{16}$" closed-cell neoprene and was adjustable to insure a close fit.

Heat loss from the head was measured by a Hatfield heat-flow meter disc placed on the left side of the forehead (15). The observed rate of heat flow through the disc was multiplied by half the area of the head as calculated by the formula of DuBois and DuBois (13). Respiratory heat loss was calculated by the methods of Burch (3). Inspiratory and expiratory temperatures were measured on each side of the one-way breathing valve. It was assumed that the expiratory air was completely saturated and that the relative humidity of the inspired air was the same as that in the room.

Heat production was estimated from the oxygen consumption, continuously measured by the open circuit method. A dry gas meter was placed on the inspiratory side of the circuit. Expired gas was sampled continuously from a 2-L mixing can placed on the expiratory side of the valve. The 5-minute collection periods corresponded to the periods of measurement of heat removal from the calorimeter. The methods of gas analysis were the same as described previously (11).

After the subject had been prepared for the experiment three 5-minute control periods were conducted. At the end of this time the cover and attached rack containing the subject

were raised by a motor and positioned over the calorimeter. The calorimeter was uncovered and the subject immersed. These latter procedures took between 10 and 20 seconds.

Schematic diagrams of the water calorimeter are shown in Figs. 1 and 2. The principle of operation is very simple. Without the subject in the calorimeter, a small amount of heat is added at a constant rate by two immersion heaters at each side of the tank. Heat is removed by permitting water to flow through the heat exchanger from a constant temperature bath which is maintained at 14 °C less than the bath temperature. Since the heat exchanger has such a large surface, the temperature of the outflow is equal to the temperature of the bath. The heat removed is therefore the product of the temperature difference and the volume of water which has flowed through the exchanger.

The off-on solenoid valve is controlled by the mean temperature of the bath as sensed by 20 thermistors connected in series and distributed equidistantly at the outer edge of the heat exchanger. The collection selector solenoid valve system permits the measurement of the outflow from one side and then the other. The time of collection is controlled by a clock and relay which also provides a signal to mark all the records simultaneously.

One of the most important aspects of the design is the method of stirring. Two horizontal paddles extend the length of the calorimeter, and the shafts pass through one end. The belts driving each shaft are connected to a common gear reduction system and are driven by a single motor. As indicated in Fig. 2 the water circulates from the top to the sides, across the thermistors, and around the heat exchanger. In addition to this pattern of circulation, baffles placed in the bottom on either side (not shown) cause a slower but significant head-to-foot circulation. Although the water is stirred vigorously, there is no cavitation or bubbles.

When the subject is lowered into the water, the heat which must be removed increases considerably. The rate of outflow from the heat exchanger and thus the cooling capacity can be changed by opening a variable flow control valve located between the flow control

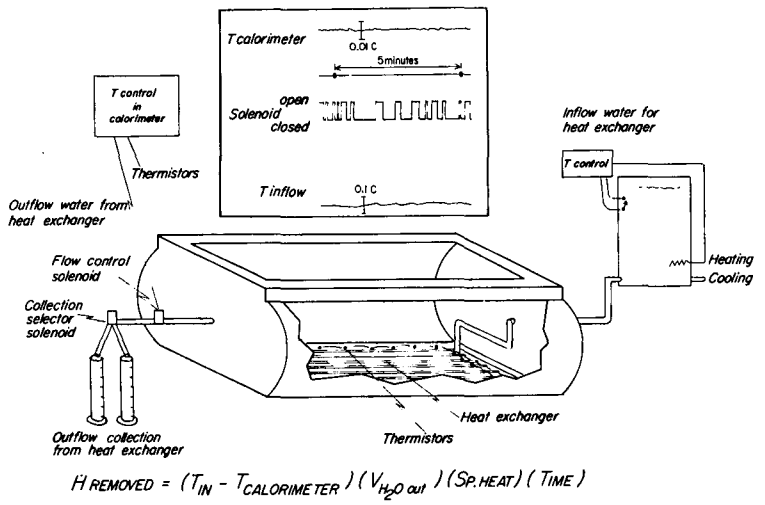

FIG. 1. Schematic representation of the direct water calorimeter.

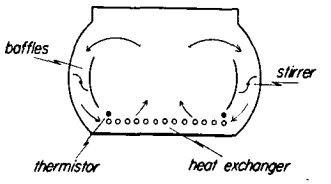

FIG. 2. Schematic cross section of the water calorimeter; arrows indicate the pathway of water flow in relationship to thermistors and heat exchanger.

solenoid and the collection selector solenoid. The maximum cooling capacity is considerably greater than that required in most experiments.

When the calorimeter is operated at warmer temperatures, it is often necessary to add extra heat during the first 5 minutes of immersion. This is accomplished by increasing the electrical power to the heaters at a measured rate for a known length of time.

In addition to running the calorimeter before and after each experiment, it was necessary to perform a number of studies using the rack alone. From the data it was possible to define the magnitude and time course of the changes in heat stores of the rack and to subtract these from the total net heat flow from the calorimeter. The remainder provided measurement of the heat coming from the immersed subject.

Also shown in the box in Fig. 1 is a short strip of record from the continuous recording of temperatures in the calorimeter (top trace) and in the inflow water bath (bottom). The middle record shows the opening and closing of the flow control solenoid. This record enabled one operator to monitor the operation of the calorimeter continuously while also making all of the other observations.

During the control period the bath temperature fluctuated ± 0.002 °C and during maximal rates of heat removal ± 0.005 °C. Actually the fluctuations of water temperature which indicate temporary storage of heat in the calorimeter are not important so long as the temperatures at the beginning and end of the period of measurement are the same. However, rapid and small fluctuations increase the probability of equal temperatures at these two points in time.

Recovery of heat added electrically was studied periodically to check the operation of the calorimeter. Approximately one experiment without a subject was carried out for every four times the subjects were studied. Recovery of heat added at rates between 30 and 270 Kcal/hr varied $\pm 3.3\%$ for the 5-minute periods of measurement. The response of the system was such that, when 500 cc of 40 °C water was quickly poured into the tank at any point, the temperature control system responded in 3–5 seconds. Larger boluses of warm water were completely distributed within 15–18 seconds.

Results and Discussion

The average cumulative heat production for a 1-hour period of immersion of the 10 subjects in 24 °C water is indicated by the top line in Fig. 3. On this graph heat losses from the head and respiratory tract and the heat loss from the subject to the water are subtracted from the heat production. Therefore, the area below the zero line represents heat lost from the subjects' stores.

Since the subjects shivered for a major part of the period of immersion, the rate of heat production by the end of the hour was 98 Kcal/hr as compared to control observations of 64 Kcal/hr. The greatest heat loss to the water occurred in the first 10 minutes of immersion and amounted to 84 Kcal. The total net loss from heat stores during the hour amounted to 183 Kcal, 60% of which was lost in the first 20 minutes.

As indicated at the bottom of Fig. 3, the T_e decreased 0.37 °C during the hour. The major change in the skin temperature occurred in the first 5 minutes, and a slight further decrease occurred from 30 to 60 minutes.

Using the factor of 0.83 Kcal/kg/°C as an average value for the specific heat of the human body, it was possible to calculate that the mean body temperature decreased 2.9 °C. If one assumes that the decrease in skin temperature was representative of the peripheral parts of the body and that the change in T_e reflected the core, weighting factors of 0.31 and 0.69 for the respective regions could be calculated. It should be noted that the large change in the skin temperature compared to that of the T_e is a major determinant in such calculations. Although these factors appear to be reasonable for these particular conditions, it is currently very uncertain that they will apply at other times and in different water temperatures.

From these data it is also possible to calculate thermal conductivity, or its reciprocal—insulation index. These terms are analogous to resistance as calculated from Ohm's Law.

$$C = \dot{H}/(T_e - T_{H_2O})$$

expressed in Kcal/m²/hr/°C where C is thermal conductivity.

At the end of the hour the thermal conductivity was 6.6 Kcal/m²/hr/°C. The insulation index therefore, equalled 0.15 °C/Kcal/m²/hr. Preliminary calculations of results in water of

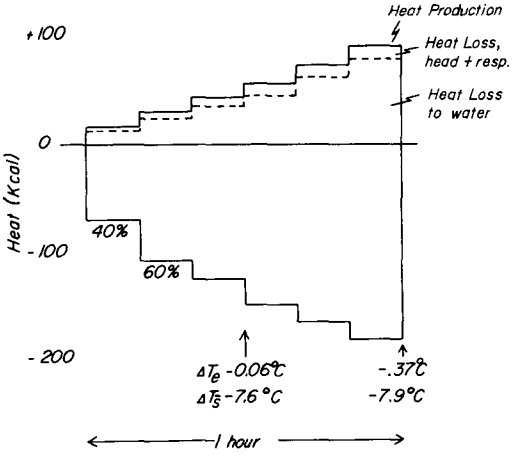

FIG. 3 Cumulative caloric balance during immersion in water (24°C) for 10 subjects. The heat production is plotted *above* the zero line. Heat losses are graphed as values *below* the lines for heat production. Therefore, the area below the zero line indicates the net cumulative heat loss from body stores. Changes in temperature in the insulated external auditory canal are referred to as ΔT_e, and in the weighted mean skin temperature are indicated by $\Delta T_{\bar{s}}$. The proportion of the hour's heat loss from body stores was 40% and 60% and occurred in the first 10 and 20 minutes respectively.

26°, 28°, and 30°C yielded values which do not appear to be different from those in 24°C. Thermal conductivity was greater at 32°C than at 30°C and increased more in 34°, 35°, and 36°C. Such a pattern of the change in thermal conductivity is usually interpreted as indicating the decreased blood flow to the periphery. Measured changes in forearm blood flow reported by Rennie (21) decreased linearly as the water temperature decreased from 34° to 30°C and were minimal at 30°C.

An increase in thermal conductivity was not observed with the onset of shivering as mentioned by Burton and Bazett (5). They interpreted an increase in thermal conductivity as signifying an increase in blood flow to the muscles involved in shivering. In the subjects of the present study, heat production continuously increased after the first 20 minutes, from a rate of 77 to 98 Kcal/hr at the end of the hour. Heat loss to the water decreased from a rate of 234 to 171 Kcal/hr during this time. Since the temperature gradient did not change very much, the calculated C decreased if anything.

Another type of experiment which has been done in the calorimeter was designed to investigate the protective value of the "wet suit" worn by divers. The experimental results, shown in Figs. 4 and 5, were obtained on one subject who wore commercially available 3/16" closed-cell neoprene regular pants and jacket.

The left side of Fig. 4 shows the heat balance data for this subject in 24°C water without the suit. In this experiment the subject lost 226 Kcal from his heat stores. During the control period heat production averaged 66 Kcal/hr, and at the end of the period of immersion it had increased to 108 Kcal/hr because of shivering.

With the full wet suit the rate of heat production did not increase until the end of the second hour when it was 85 Kcal/hr. On the other hand, the heat balance was negative throughout; by the end of 1½ hours the loss from stores equalled that observed without the suit. At the end of 2 hours the subject had lost 276 Kcal of heat from stores.

As reported before (1), the most obvious differences with and without the protection of the suit were noted in the skin temperatures which are shown in Fig. 5. Without protection the weighted mean skin temperature of the immersed parts decreased rapidly, and at the end of the hour was only 1°C greater than the water temperature. With the jacket the cal-

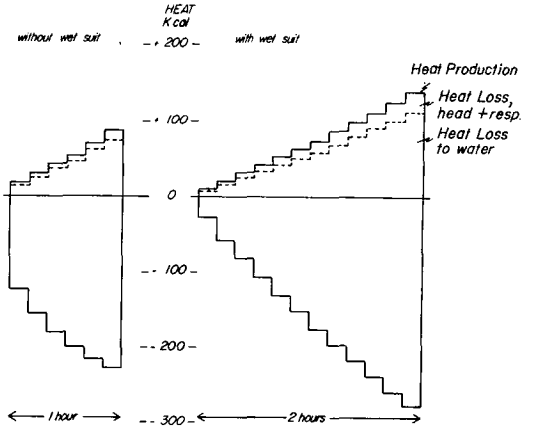

FIG. 4. Cumulative caloric balance during immersion in water (24°C). Heat production and losses are plotted as in Fig. 3. The results of one subject during immersion with and without a "wet suit" are shown.

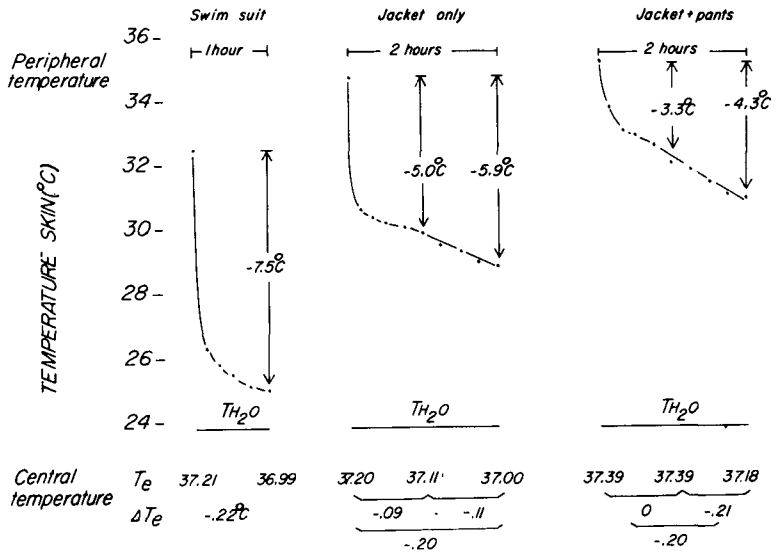

FIG. 5. Central and peripheral temperatures during immersion in water (24°C). Changes in weighted mean skin temperature and T_e are indicated during immersion without a suit, with the jacket alone, and with the full suit for the times indicated.

culated mean temperature remained considerably above the water temperature. Such a result might be expected since four of the six sites at which the temperatures were measured were covered by the jacket. However, even the exposed upper and lower legs were 5 °C warmer than without the suit. With the full protection the skin temperature remained even warmer. However, after the initial increment it decreased at a constant rate.

In all three experiments the T_e decreased by the same amount, 0.2 °C. However, with the full suit the decrease did not occur until the second hour. With jacket alone the rate of fall of the T_e was about half that observed without this added protection. In both experiments with the suit the subject was comfortable and felt cool only at the end of the 2 hours.

In these experiments it was possible to calculate two thermal conductivities or insulation indices. One part of the pathway for the heat loss to the water was from the core to the skin. The other part was from the skin through the suit to the water. Without the suit the usual calculation, using the gradient $T_e - T_{H_2O}$, was also made. In terms of insulation index this latter value was 0.15 at 1 hour, and with the full suit was 0.13 °C/Kcal/m²/hr at this same time. With the suit the insulation index, using the gradient $T_e - T\bar{s}$, was 0.05. The second pathway through which the heat flowed, indicated by $T\bar{s} - T_{H_2O}$, had an insulation index of 0.08 °C/Kcal/m²/hr.

These results indicate the insulative value of the suit. In effect, wearing the suit keeps the skin warm and allows the subject to maintain the blood flow to the periphery at a much greater rate than without the suit. Although the suit decreases the rate of heat loss from the subject's stores, its major value seems to be for the subject's comfort (1).

It was also interesting to note that the subject did not shiver when wearing the suit even though his central temperature had decreased in 2 hours by the same degree as when he was without protection. These observations are in keeping with those of other experiments. It

has been reported (8) that subjects stopped shivering when they were removed from the water and the skin was dried, despite a continued decrease of the T_e. Shivering seems to depend on both stimuli: a decrease in core temperature and a peripheral stimulus from the skin.

In other experiments (12), the subjects were preconditioned by immersion in cool water which caused a decrease in T_e and shivering. They were then allowed to recover in air for ½ hour during which time the T_e decreased but the shivering stopped. They were then immersed a second time. This test immersion was repeated in water of different temperatures on separate occasions. It was necessary to use water less than 32°C in the second test immersion to "turn on" the shivering in these preconditioned subjects.

This water temperature, which can be considered to be a threshold value for the peripheral stimulus, is very similar to the "critical water temperature" described by Rennie et al. (21). Their criterion was the coldest water which the subjects could tolerate for 3 hours without shivering; for American males it was 33°C. These same concepts seem also to apply to man in air (19).

The current studies of heat loss in 24°C water seem to have important implications for the recovery period following this type of cold exposure. If a diver loses 200–250 Kcal of heat, it will take him a long time to return to his predive thermal status. A second dive before significant recovery is always difficult.

Even if a diver were to increase his insulation greatly by putting on many layers of dry clothes after a dive, his major source of heat is still his own metabolism. This might be in the range of 70–100 Kcal/hr, only part of which can be used to replete the heat stores. The most obvious aid would be a bath which was warmer than the core temperature. Not only would most of the heat which the subject produced be trapped, but heat would also be absorbed rapidly from the water.

ACKNOWLEDGMENTS

This work was supported in part by grants from the National Heart Institute HL09676 and the University of Rochester's NIH General Research Supply Grant.

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