# UNDERWATER PHYSIOLOGY VIII

# THE EIGHTH SYMPOSIUM ON UNDERWATER PHYSIOLOGY
# GOVERNING BOARD

# UNDERWATER PHYSIOLOGY VIII

PROCEEDINGS OF THE EIGHTH SYMPOSIUM
ON UNDERWATER PHYSIOLOGY

*Sponsored by*

The University of Pennsylvania

The Undersea Medical Society, Inc.

The U.S. Office of Naval Research

The U.S. National Oceanic and Atmospheric Administration

***Edited by Arthur J. Bachrach and Mary Margaret Matzen***

***Assistant Editor Sally T. McAllister***

Undersea Medical Society, Inc., Bethesda, Maryland, 1984

UNDERSEA MEDICAL SOCIETY, INC.
9650 Rockville Pike, Bethesda, Maryland 20814

ISSN Number 0082-0997

PRINTED IN THE UNITED STATES OF AMERICA BY MCGREGOR & WERNER, INC.
COMPOSED IN TIMES ROMAN BY PHOTO DATA, INC.

# CONTENTS

PART VI

## INTERACTIONS OF VENTILATORY CONTROL, HYPEROXIA, AND INCREASED GAS DENSITY AT HIGH AMBIENT PRESSURES

PART VII

## MOLECULAR AND CELLULAR EFFECTS OF PRESSURE

## LIST OF AUTHORS AND CONTRIBUTORS

**J. Adolfson,** National Defense Research Institute, Swedish Naval Diving Center, Hårsfjärden, Sweden

**T. K. Akers,** University of North Dakota, Grand Forks, North Dakota

**A. Angel,** University of Sheffield, Sheffield, England

**A. Ariel,** Israeli Naval Hyperbaric Institute, Haifa, Israel

**J. D. Balentine,** Medical University of South Carolina, Charleston, South Carolina

**A. Battesti,** E.A.S.S.M.-C.E.R.B., Toulon-Naval, France

**B. Becker,** Naval Medical Command, Bethesda, Maryland

**P. Y. Bell,** Admiralty Marine Technology Establishment (Physiological Laboratory), Gosport, Hants, England

**P. B. Bennett,** F. G. Hall Laboratory, Duke University Medical Center, Durham, North Carolina

**J. C. Betts,** National Medical Committee of the British Sub Aqua Club, London, England

**A. R. Bichard,** University of Oxford, Oxford, England

**G. Bolstad,** Norwegian Underwater Technology Center, Ytre Laksevåg, Norway, and SINTEF, Trondheim, Norway

**L. Booth,** Fort Bovisand Underwater Centre, Devon, England

**R. C. Bornmann,** Defense Medical Materiel Board, Fort Detrick, Maryland

**F. Bowser-Riley,** University of Oxford, Oxford, England

**M. E. Bradley,** Naval Medical Research Institute, Bethesda, Maryland

**R. W. Brauer,** Institute of Marine Biomedical Research, University of North Carolina at Wilmington, Wilmington, North Carolina

**B. Broussolle,** E.A.S.S.M.-C.E.R.B., Toulon-Naval, France

**E. Brown,** Fort Bovisand Underwater Centre, Devon, England

**A. O. Brubakk,** Norwegian Underwater Technology Center, Ytre Laksevåg, Norway, and SINTEF, Trondheim, Norway

**D. W. Burgess,** Admiralty Marine Technology Establishment (Physiological Laboratory), Gosport, Hants, England

**H. Burnet,** G.I.S. Physiologie Hyperbare-C.N.R.S., Marseille, France

**F. K. Butler, Jr.** U.S. Navy Experimental Diving Unit, Panama City, Florida

**E. M. Camporesi,** Duke University Medical Center, Durham, North Carolina

**Ph. Cavenel,** CEPISMER/GISMER, Marine Nationale, Toulon-Naval, France

**J. M. Clark,** Institute for Environmental Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

**J. R. Clarke,** Naval Medical Research Institute, Bethesda, Maryland

**M. R. Cross,** University of Newcastle-upon-Tyne, Newcastle-upon-Tyne, England

**A. Crowther,** St. Thomas' Hospital, London, England

**S. Daniels,** University Laboratory of Pharmacology and Physical Chemistry Laboratory, Oxford, England

**B. G. D'Aoust,** Common Sensing Inc., Bainbridge Island, Washington

**J. M. Davies,** University Laboratory of Pharmacology and Physical Chemistry Laboratory, Oxford, England

**C. J. Detrisac,** Medical University of South Carolina, Charleston, South Carolina

**G. R. Doran,** Charing Cross Hospital Medical School, University of London, London, England

**C. Doré,** Clinical Research Centre, Harrow, Middlesex, England

**T. J. Doubt,** Naval Medical Research Institute, Bethesda, Maryland

**M. E. Duffey,** State University of New York at Buffalo, Buffalo, New York

**K. C. Eastaugh,** University Laboratory of Pharmacology and Physical Chemistry Laboratory, Oxford, England

**R. G. Eckenhoff,** Naval Submarine Medical Research Laboratory, Groton, Connecticut

**O. Eiken,** Defence Research Institute, Hårsfjärden, Sweden

**E. Eilender,** Israeli Naval Hyperbaric Institute, Haifa, Israel

**P. Hayes,** Admiralty Marine Technology Establishment (Physiological Laboratory), Alverstoke, Gosport, Hants, England.

**D. D. Hickey,** State University of New York at Buffalo, Buffalo, New York

**W. M. Hinson,** Institute of Marine Biomedical Research, University of North Carolina at Wilmington, Wilmington, North Carolina

**D. C. Hoffman,** University of Hawaii, Honolulu, Hawaii

**B. Holand,** Norwegian Underwater Technology Center, Ytre Laksevåg, Norway, and SINTEF, Trondheim, Norway

**I. Holmér,** Climatic Physiology Unit, National Board of Occupational Safety and Health, Solna, Sweden

**S. K. Hong,** State University of New York at Buffalo, Buffalo, New York

**C. Hordnes,** University of Bergen, Bergen, Norway

**J. S. Hughes,** Naval Submarine Medical Research Laboratory, Groton, Connecticut

**R. Hyacinthe,** E.A.S.S.M.-C.E.R.B., Toulon-Naval, France

**G. Imbert,** G.I.S. Physiologie Hyperbare-C.N.R.S., Marseille, France

**A. Ishiyama,** Tokyo Medical and Dental University, Tokyo, Japan

**R. Jackson,** Institute for Environmental Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

**W. R. Johnson,** University of Toronto, Toronto, Ontario, Canada

**J. J. Kendig,** Stanford University School of Medicine, Stanford, California

**D. Kerem,** Israeli Naval Hyperbaric Institute, Haifa, Israel

**L. A. Kuehn,** Canadian Department of National Defence, Ottawa, Ontario, Canada

**C. J. Lambertsen,** Institute for Environmental Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

**J. P. Landolt,** Defence and Civil Institute of Environmental Medicine, Downsview, Ontario, Canada

**E. H. Lanphier,** University of Wisconsin, Madison, Wisconsin

**N. M. Lashbrook,** University of Oxford, Oxford, England

**D. C. Legrys,** Naval Medical Research Institute, Bethesda, Maryland

**C. E. Lehner,** University of Wisconsin, Madison, Wisconsin

**C. Lemaire,** G.I.S. Physiologie Hyperbare-OCTARES, Marseille, France

**E. N. Lightfoot,** University of Wisconsin, Madison, Wisconsin

**S. Elnäs,** Climatic Physiology Unit, National Board of Occupational Safety and Health, Solna, Sweden

**Ereth, M.,** University of North Dakota, Grand Forks, North Dakota

**L. Fagni,** G.I.S. Physiologie Hyperbare-C.N.R.S., Marseille, France

**D. G. Fisher,** Institute for Environmental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

**E. T. Flynn,** Naval Medical Research Institute, Bethesda, Maryland

**W. D. Fraser,** Defence and Civil Institute of Environmental Medicine, Downsview, Ontario, Canada

**M. Fried,** Institute for Environmental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

**X. Fructus,** COMEX S.A., Marseille, France

**B. Gardette,** COMEX S.A., Marseille, France

**M. C. Gardette-Chauffour,** G.I.S. Physiologie Hyperbare-OCTARES, Marseille, France

**M. P. Garrard,** Admiralty Marine Technology Establishment (Physiological Laboratory), Alverstoke, Gosport, Hants, England

**R. Gelfand,** Institute for Environmental Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

**M. Gennser,** National Defense Research Institute, Swedish Naval Diving Center, Hårsfjärden, Sweden

**E. W. Gillary,** University of Hawaii, Honolulu, Hawaii

**P. B. L. Giry,** E.A.S.S.M-C.E.R.B., Toulon-Naval, France

**J. M. Goldinger,** State University of New York at Buffalo, Buffalo, New York

**C. G. Gray,** Naval Health Research Center, San Diego, California

**W. B. Greene,** Medical University of South Carolina, Charleston, South Carolina

**Y. Grossman,** Ben Gurion University of the Negev, Beer-Sheva, Israel

**Z. Hajduczok,** State University of New York at Buffalo, Buffalo, New York

**M. J. Halsey,** Clinical Research Centre, Harrow, Middlesex, England

**R. W. Hamilton,** Hamilton Research Ltd., Tarrytown, New York

**A. L. Harabin,** Naval Medical Research Institute, Bethesda, Maryland

**D. J. Harris,** Admiralty Marine Technology Establishment (Physiological Laboratory), Alverstoke, Gosport, Hants, England

**Y. C. Lin,** University of Hawaii, Honolulu, Hawaii

**M. Liner,** Defence Research Institute, Hårsfjärden, Sweden

**H. J. Little,** University of Oxford, Oxford, England

**C. E. G. Lundgren,** State University of New York at Buffalo, Buffalo, New York

**G. W. Mack,** University of Hawaii, Honolulu, Hawaii

**H. Maeda,** Tokyo Medical and Dental University, Tokyo, Japan

**J. Magnusson,** Swedish Naval Diving Center, Hårsfjärden, Sweden

**Y. Mano,** Tokyo Medical and Dental University, Tokyo, Japan

**F. Martin-Chave,** COMEX S. A., Marseille, France

**A. Mayevsky,** Institute for Environmental Medicine and The Johnson Research Foundation, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

**I. B. Mekjavic,** Simon Fraser University, Burnaby, B.C., Canada

**Y. Melamed,** Israeli Naval Hyperbaric Institute, Haifa, Israel

**K. E. Money,** Defence and Civil Institute of Environmental Medicine, Downsview, Ontario, Canada

**S. Monk,** Clinical Research Centre, Harrow, Middlesex, England

**R. E. Moon,** Duke University Medical Center, Durham, North Carolina

**J. B. Morrison,** Simon Fraser University, Burnaby, B.C., Canada

**A. Muren,** National Defense Research Institute, Swedish Naval Diving Center, Hårsfjärden, Sweden

**R. Naquet,** G.I.S. Physiologie Hyperbare-OCTARES, Marseille, France

**N. Naraki,** G.I.S. Physiologie Hyperbare-C.N.R.S., Marseille, France

**M. Nuri,** St. Thomas' Hospital, London, England

**M. Nygogosyan,** State University of New York at Buffalo, Buffalo, New York

**P. C. Odell,** Defence and Civil Institute of Environmental Medicine, Downsview, Ontario, Canada

**J. Onarheim,** Norwegian Underwater Technology Center, Ytre Laksevåg, Norway

**H. Örnhagen,** National Defense Research Institute, Swedish Naval Diving Center, Hårsfjärden, Sweden

**J. A. Paciorek,** Norwegian Underwater Technology Center, Ytre Laksevåg, Norway

**H. Padbury,** Admiralty Marine Technology Establishment (Physiological Laboratory), Alverstoke, Gosport, Hants, England

**Y. S. Park,** Kosin Medical College, Busan, Korea

**A. J. Påsche,** Norwegian Underwater Technology Center, Ytre Laksevåg, Norway

**W. D. M. Paton,** University of Oxford, Oxford, England

**R. R. Pearson,** Royal Navy Submarine Flotilla, *HMS Dolphin,* Gosport, Hants, England

**G. L. Peer,** State University of New York at Buffalo, Buffalo, New York

**R. Peterson,** Institute of Physiological Psychology, University of Bergen, Bergen, Norway

**J. Pisarello,** Institute for Environmental Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

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

**S. Rastan,** Clinical Research Centre, Harrow, Middlesex, England

**D. W. Rennie,** State University of New York at Buffalo, Buffalo, New York

**J. Risberg,** University of Bergen, Bergen, Norway

**R. G. Riusech,** Defence and Civil Institute of Environmental Medicine, Downsview, Ontario, Canada

**P. M. Rowles,** The Middlesex Hospital, London, England

**J. C. Rostain,** G.I.S. Physiologie Hyperbare, Marseille, France

**J. V. Salzano,** Duke University Medical Center, Durham, North Carolina

**D. Sambor,** State University of New York at Buffalo, Buffalo, New York

**M. Shibayama,** Tokyo Medical and Dental University, Tokyo, Japan

**K. K. Shida,** Mahidol University, Bangkok, Thailand

**E. B. Smith,** University of Oxford, Oxford, England

**B. W. Stolp,** Duke University Medical Center, Durham, North Carolina

**W. J. Streit,** Medical University of South Carolina, Charleston, South Carolina

**M. Summerfield,** Admiralty Marine Technology Establishment (Physiological Laboratory), Gosport, Hants, England

**S. Takahashi,** Tokyo Medical and Dental University, Tokyo, Japan

**R. F. Taylor,** Arthur D. Little, Inc., Cambridge, Massachusetts

**E. D. Thalmann,** U.S. Navy Experimental Diving Unit, Panama City, Florida

**P. Tikuisis,** Defence and Civil Institute of Environmental Medicine, Downsview, Ontario, Canada

**S. Tønjum,** Norwegian Underwater Technology Center, Ytre Laksevåg, Norway

**D. Torbati,** Institute for Environmental Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

**Z. Török,** Admiralty Marine Technology Establishment (Physiological Laboratory), Alverstoke, Gosport, Hants, England

**E. J. Towse,** Admiralty Marine Technology Establishment (Physiological Laboratory), Gosport, Hants, England

**I. Tyssebotn,** University of Bergen, Bergen, Norway

**R. Vaernes,** Norwegian Underwater Technology Center, Ytre Laksevåg, Norway

**H. D. Van Liew,** State University of New York at Buffalo, Buffalo, New York

**C. A. Ward,** University of Toronto, Toronto, Ontario, Canada

**B. Wardley-Smith,** Clinical Research Centre, Harrow, Middlesex, England

**D. K. Watanabe,** University of Hawaii, Honolulu, Hawaii

**W. J. Watson,** Defence and Civil Institute of Environmental Medicine, Downsview, Ontario, Canada

**P. Webb,** Webb Associates, Yellow Springs, Ohio

**M. M. Webb-Peploe,** St. Thomas' Hospital, London, England

**J. A. Will,** University of Wisconsin, Madison, Wisconsin

**P. T. Wilmshurst,** St. Thomas' Hospital, London, England

**E. H. Wissler,** University of Texas, Austin, Texas

**D. E. Yount,** University of Hawaii, Honolulu, Hawaii

# PREFACE

*Underwater Physiology VIII* represents the eighth in a continuing series of symposia initiated 30 years ago by the University of Pennsylvania and the Office of Naval Research. This volume was sponsored by the University of Pennsylvania, the Undersea Medical Society, Inc., the U.S. Office of Naval Research, and the U.S. National Oceanic and Atmospheric Administration.

---

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

## ACKNOWLEDGMENTS

Over a period of about two and one-half years, the following persons contributed to the organization and planning of the Eighth Symposium on Underwater Physiology: Paul Webb, John M. Hallenbeck, Kenneth N. Ackles, Arthur J. Bachrach, James M. Clark, Elliott A. Finkle, Richard D. Heimbach, Franklin G. Hempel, Claes E. G. Lundgren, Robert Naquet, and Dennis N. Walder. The final program was comprised of eight sessions, a Keynote Address by Christian J. Lambertsen, and the Suzanne Kronheim Memorial Lecture by Irwin Fridovich.

A novel format for the sessions encouraged discussion and scientific exchange. Each session opened with a review that was intended to develop a background context within which the subsequent papers could be interpreted. All papers were presented as posters rather than read, and each session closed with a general discussion that was guided and stimulated by a discussion leader.

The meeting was managed by the staff of the Undersea Medical Society home office, and C. W. Shilling, C. A. Coppola, Y. P. Desautels, and S. T. McAllister are particularly to be cited for their contributions.

John M. Hallenbeck
*Chairman, Eighth Symposium on Underwater Physiology*

# Part I

## OXYGEN TOXICITY

# PATHOLOGIC CORRELATES OF OXYGEN TOXICITY

*J. D. Balentine, C. J. Detrisac, W. J. Streit, and W. B. Greene*

Biochemical studies have indicated that the mechanisms of oxygen toxicity are protean. Pathways involved in oxidative phosphorylation, electron transfer, membrane transport, neurotransmission, replication, transcription, and protein synthesis are vulnerable to hyperoxia. The pathology of oxygen toxicity correlates well with the diversity of potential biochemical changes (1). It is well known that oxygen is toxic for virtually all species of animals as well as for plants (1,2,3). Lipid peroxidation has been proposed as a common biochemical pathway (1,4,5), leading to cell injury through a number of mechanisms (Fig.1).

A review of oxygen toxicity studies in mammalian organs reveals that virtually all systems are either directly or indirectly altered by exposure to excessive oxygen (1). When one focuses on those studies that specifically deal with ultrastructural pathology, it is apparent that mitochondrial abnormalities appear as early changes in the sequential development of cellular pathology in the presence of high oxygen tensions (Table I). This is true in the major organisms that have exhibited oxygen toxicity, including the lung, eye, central nervous system, and the heart. The general interpretation of mitochondria as a specific site of oxygen toxicity, however, has been complicated by numerous facts. First, there are no specific mitochondrial abnormalities reported in oxygen toxicity that have not been observed following other etiologies of cell injury, such as ischemia, anoxia, and other toxins. Additionally, many of the mitochondrial changes have occurred in very close proximity to, or in parallel with, other cellular abnormalities such as alteration of the endoplasmic reticulum or cell swelling, in models of oxygen toxicity inducing lethal cell injury i.e., cell necrosis. However, selective mitochondrial degeneration occurs in the absence of other cellular changes in renal tubular epithelium exposed to hyperbaric oxygen at 3 ATA for 3–5 hours (1,6). The

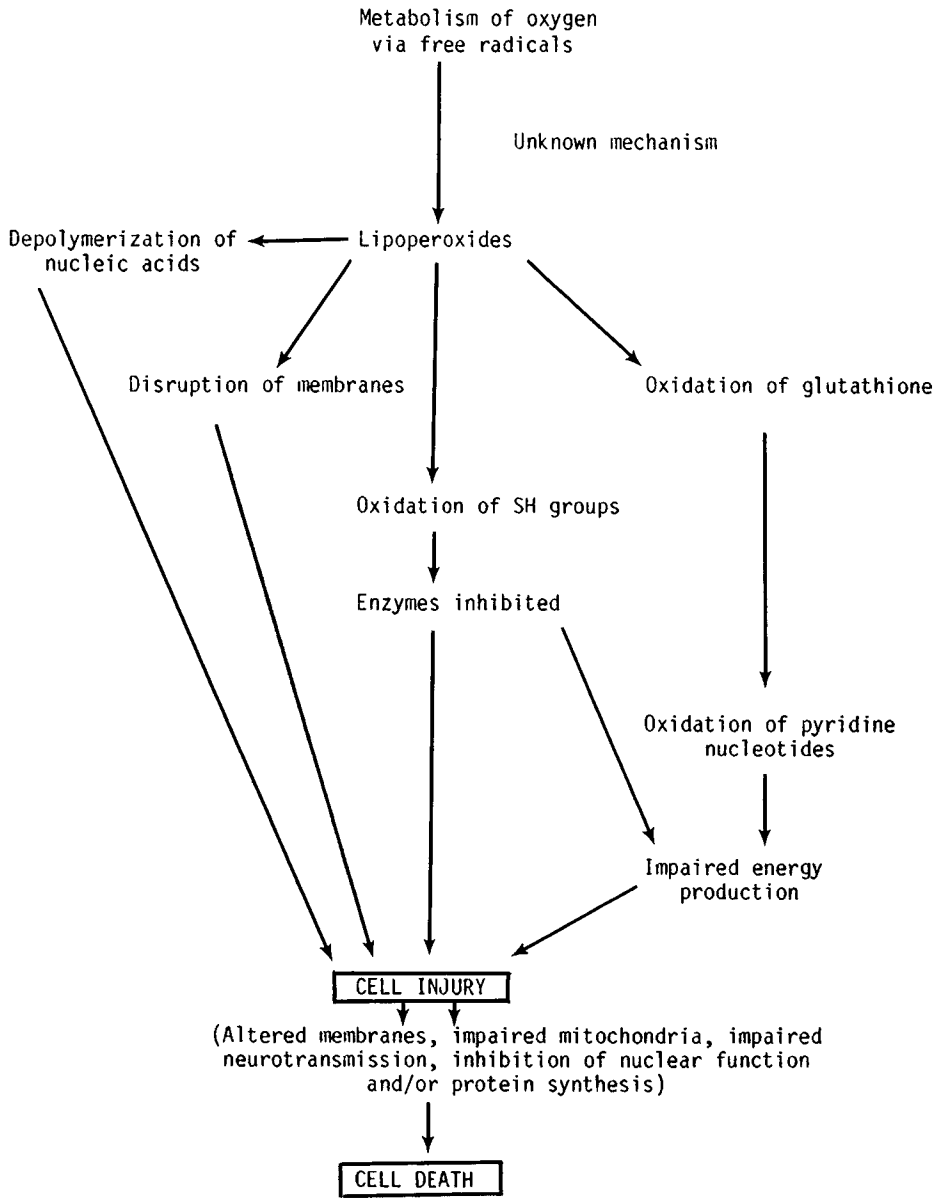



Fig. 1. Summary hypothesis of oxygen toxicity, modified from (1) and based on data presented in (1), (3), and (4).

TABLE I

Mitochondrial Abnormalities Reported in Oxygen Toxicity Studies

1. High amplitude swelling
2. Hypertrophy
3. Membrane changes
   myelin figures
   vesiculation of cristae
4. Flocculent matrical densities
5. Increased autophagocytosis
6. Heterophagocytosis

For references and detailed discussion see Balentine (1).

mitochondria, but not the cells, become lethally altered, acquiring matrical inclusions and being autophagocytized after exposure. This study clearly established the mitochondrion as a specific site of oxygen toxicity.

Oxygen via its radiomimetic effects has been implicated as a nuclear poison as demonstrated in Table II. In addition to the changes described in the table and discussed in more detail by Balentine (1), Wittner and Rosenbaum (7) have demonstrated a significant inhibitory effect of hyperoxia on ribosomal RNA in Hela cells exposed to 2 ATA of oxygen for 15 min. The inhibition of RNA occurred at the level of the cleavage of the 45S precursor molecule into its 32 and 28S preribosomal components, and it was demonstrated in the presence of normal DNA and protein synthesis. Many other studies have illustrated concerted inhibitions of DNA, RNA, and protein syntheses (1). The observation of ribosomal detachment from rough endoplasmic reticulum and their eventual depletion in pulmonary oxygen toxicity correlates with altered protein synthesis (8). Biochemical data suggest that this may be direct (9).

The toxicity of oxygen (10) and its free radical metabolic intermediates (11) for cell membranes has been well documented in the erythrocyte. Other changes consistent with membrane oxygen toxicity are listed in Table III. The

TABLE II

Radiomimetic Changes Produced by Hyperoxia

1. Structural abnormalities in chromatin
2. Mutations
3. Inhibition of mitoses
4. Abnormal mitoses
5. Cataracts
6. Necrosis and giant cell transformation of germinal epithelium of testis

Modified from Balentine (1).

TABLE III

Hyperoxic Changes Consistent with Membrane Injury

1. Hemolysis of erythrocytes
2. Inhibition of sodium transport in epithelia
3. Impaired endocytosis in pulmonary macrophages
4. Myelin figures on organelle membranes
5. Blebbing and loss of cilia in respiratory epithelium
6. Increased autophagocytosis
7. Increased lipofuscin
8. Cell and/or organelle swelling

Modified from Balentine (1).

turnover of organelle membranes stimulates autophagocytosis and the formation of lipofuscin, the product of peroxidation and polymerization of unsaturated lipids. Therefore, increased autophagocytosis and lipofuscin formation is generally considered to be consistent with generalized, i.e., nonspecific, injury to membranes (1). Experimental studies (1) of the morphological effects of hyperoxia have demonstrated both increased autophagocytosis and lipofuscin would support the concept of oxygen as a general membrane toxin. These changes have been observed in the central nervous system (CNS) as well as visceral organs, common sites of observation of the increasing accumulation of lipofuscin associated with aging.

Detrisac and co-workers (12) observed enhanced autophagocytosis and lipofuscin formation in organotypic cultures of newborn mice cerebellum and fetal mouse spinal cord exposed to hyperbaric oxygen. The explants were allowed to mature for 3–4 weeks after which experimental cultures were exposed to 5 ATA of oxygen for 5 h and fixed for electron microscopy 3–5 days after exposure. This dose of oxygen consistently induced severe necrosis of neurons and neuroglia in addition to the enhanced autophagocytosis and lipofuscin formation in surviving cells. The present study was designed to observe possible oxygen-induced changes, using similar CNS organotypic explants, at earlier time intervals as well as after a less severe dose of hyperoxia.

## METHODS

With spinal cord explants, the entire spinal cord was dissected from 13- to 15-day-old ICR mouse embryos and placed in nutrient media containing nerve growth factor (NGF). Each cord was then dissected along the midline axis and transected into 1.5–3.0 mm pieces and placed on collagen-coated coverslips. The explants were allowed to lay flat for 30 min to insure neuritic attachment to the collagen. They were then fed one drop of the nutrient media and gassed for 30 s with 5% $CO_2$ to acidify the media, after which they were sealed in

Maximow slide assemblies. They were processed in batches of 24. The batches were assigned an assession number and maintained at 34–35°C in $CO_2$ in a Napco 5100 incubator. Following an initial 4-day resting period, the explants were fed media without nerve growth factor on a twice weekly schedule until they were used as controls or experiments. The nutrient media was formulated according to Petersen (13) and contained 33% human placental cord serum, 50% Eagle's minimum essential media (MEM) with L-glutamine, $Na(OH_3)_2$ and Hepes buffer, 600 mgm% dextrose, 10% chick embryonic extract, and 1.6 μg/mL tetracycline buffered with ascorbic acid.

For cerebellum, 1- to 3-mm pieces were dissected from 1- to 3-day-old ICR neonatal mice and explanted onto collagen-coated coverslips as described previously. They were placed in nutrient media transferred to Maximow slide assemblies in a manner similar to that described for spinal cord. The cerebellar media contained 40% human placental cord serum, 25% MEM with L-glutamine, $Na(OH_3)_2$ and Hepes buffer, 28.4% balanced salt solution (BSS), 600 mgm% dextrose, 1.6 μg/mL tetracycline buffer with ascorbic acid, and 3 units/mL of low zinc, glucagon-free insulin. These explants were likewise run in batches.

In addition to the periodic feeding with nutrient media during the maturation phase, each explant was examined by light microscopy periodically to determine its viability. Nonviable cultures were discarded, and surviving normal-appearing explants were used for experiments or controls at 21 days of maturity. Approximately 40% of the original explants were found to be suitable. The individual cultures were randomly selected from the surviving 21-day-old mature cultures and placed in one of four groups: a) morphologic controls, b) incubation controls, c) pressure controls, and d) experimental cultures exposed to hyperoxia. The morphologic controls consisted of explants that were fixed at the same time that the experimental ones exposed to oxygen were fixed. Incubation controls consisted of explants that were removed from Maximow chambers and incubated in room air in an incubator for a time comparable to that required to expose the experimental cultures to hyperbaric oxygen. The pressure controls consisted of explants exposed to 60 psig of 4% $O_2$ in 96% $N_2$ for 5 h. Individual explants were taken from their Maximow chambers and placed one each in a small petri dish with 1 mL of nutrient media to prevent dessication. The petri dishes were placed in a small 8/835 cc Bethlehem hyperbaric chamber especially designed with recirculated water to maintain constant temperatures. After the chamber was closed, it was flushed with 100% $0_2$ for 3–5 min and pressurized to 60 psig for either 3 or 5 h. The chamber temperature was maintained at 34–35°C during exposure. Compression and decompression were staged over 3–5 min each. Similar operations were used for pressure controls except that 4% $O_2$ was used. Chamber oxygen tensions for experiments and controls were monitored in outflow samples as previously described (6). After decompression, the experimental explants werer fixed at varying time intervals in 2% cacodylate buffered glutaraldehyde for 2 h followed by 1-h postfixation in 2% osmium acid. Cultures not fixed immediately were put back into Maximow chambers in fresh nutrient media

and incubated as before until fixation. Pressurized control cultures were comparably processed. The other controls were fixed at ages matching the experimental explants.

After fixation, the explants were embedded on edge in Epon and the blocks were trimmed to the maximal diameter, i.e., center, of the tissue. Three to five serial thick sections were taken in the center and stained with Toluidine blue and reviewed in a single-blinded manner for histopathological assessment. Selected thin sections were taken from the blocks, double-stained with lead citrate and uranyl acetate, and viewed by electron microscopy with either a Hitachi HU-12 or Phillips 300 transmission electron microscope. Experimental paradigms indicating the total number of explants studied are shown in Table IV.

The light microscopic assessment included an evaluation of the degree of necrosis present in each explant. The subjective scale of 0 to 4 is shown:

0 = no necrosis or rare single cell nonconfluent necrosis
+1 = focal and confluent necrosis of approximately 2–10 cells
+2 = more extensive necrosis but less than half of the culture
+3 = necrosis of more than half but not the entire culture
+4 = complete necrosis of entire culture

## RESULTS

As anticipated from previous experience (14), necrosis (+1 to +4) of neurons and neuroglia was found in all control groups (Table V). The overall rate for all categories of controls in spinal cord and cerebellum together was

TABLE IV

Total Number of Explants Studied by Experimental Paradigms

| Conditions | No. of Explants | |
|---|---|---|
| | Cerebellum | Spinal Cord |
| CONTROLS | | |
| Morphologic | 11 | 12 |
| Incubation | 21 | 14 |
| Pressure | 5 | 9 |
| HBO TREATED* | | |
| 5 ATA of $O_2$ X 3 h | 23 | 75 |
| 5 ATA of $O_2$ X 5 h | 28 | 9 |

* The explants were fixed at varying intervals of immediately 1 h, 4 h, 8 h, 24 h, and 3 days after hyperbaric oxygen exposure.

TABLE V

Rate of Necrosis in Explants*

| Conditions | Spinal Cord | Cerebellum |
|---|---|---|
| CONTROLS | | |
| Morphologic | 1/12 | 3/12 |
| Incubation | 5/14 | 8/21 |
| Pressure | 2/9 | 1/5 |
| Totals | 8/35 | 12/38 |
| HBO TREATED | | |
| *Intervals* | *Dose* | |
| | a) 5 ATA × 3 h | |
| 0 | 10/22 | 5/8 |
| 1 h | — | 9/11 |
| 4 h | 5/15 | 3/4 |
| 8 h | 11/12 | — |
| 24 h | 7/12 | — |
| 3 days | 8/14 | — |
| Totals | 41/75 | 17/23 |
| | b) 5 ATA × 5 h | |
| 0 | — | 3/4 |
| 1 h | — | 4/4 |
| 4 h | — | 7/10 |
| 8 h | — | 4/4 |
| 24 h | — | 5/6 |
| 3 days | 9/9 | — |
| Totals | 9/9 | 23/28 |

*Number of cultures with necrosis (+1 to +4)/total number.

20/173 or 27%. This is less than the spontaneous rate of necrosis previously reported for CNS explants from this laboratory (14). The degree of necrosis was usually in the range of +1 to +2 and only rarely +4. The rate of necrosis in controls was slightly greater in cerebellar (13/37) than in spinal cord (8/35) explants in general, with the exception of incubation controls. The overall rate of necrosis for hyperoxic-treated explants was significantly ($P < 0.001$) greater (90/135 or 66.6%) than for controls.

The degrees of necrosis were more severe in the experimental explants, especially in the spinal cord cultures treated for 5 h with hyperbaric oxygen (HBO). The latter, as in the study of Detrisac et al. (12; Fig. 2), consistently revealed complete (+4) necrosis.

Autophagocytosis (Fig. 3) and increased pleomorphic dense structures interpreted as lipofuscin (Fig. 4) were frequently observed within neurons and neuroglia in the HBO-treated spinal cord and cerebellar explants, even in the

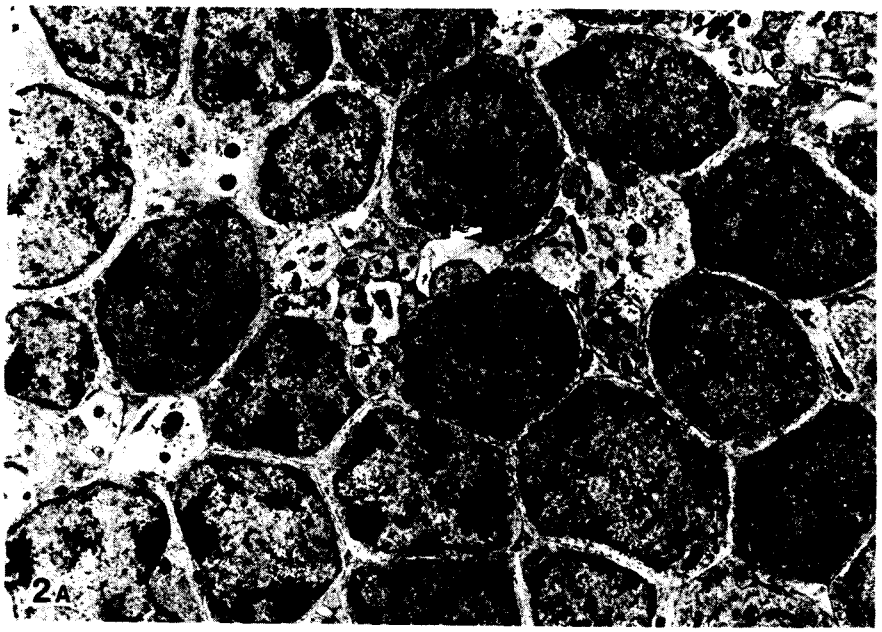



Fig. 2*A*. Normal granular cells of mature cerebellar explant. Pressure control. × 6750.

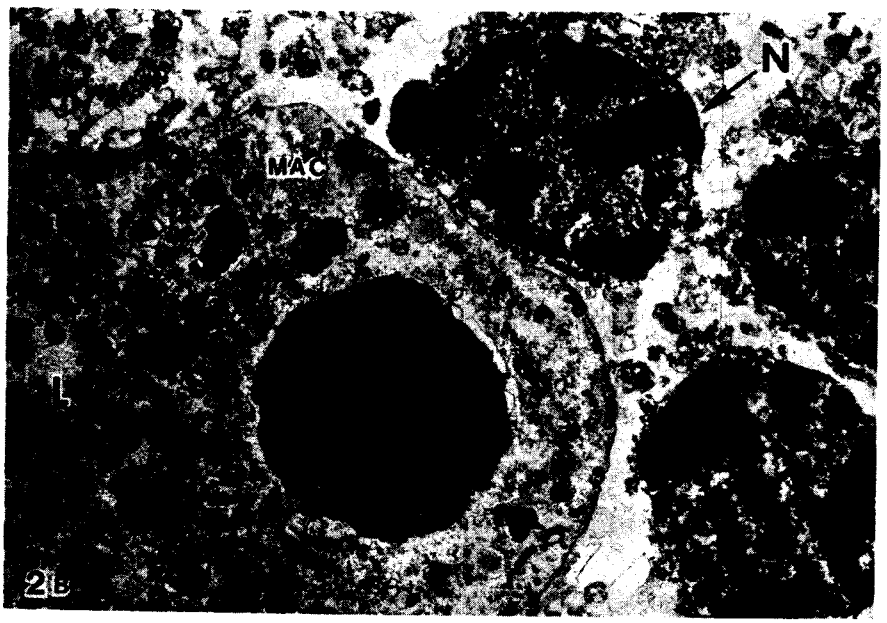



Fig. 2*B*. Necrosis (N) of granular cells in mature cerebellar explant exposed to 6 ATA of oxygen for 5 h. Five days after exposure. A macrophage (MAC), containing numerous phagolysosomes (L), is noted engulfing a necrotic cell. × 11,550. Data from Detrisac et al. (12).

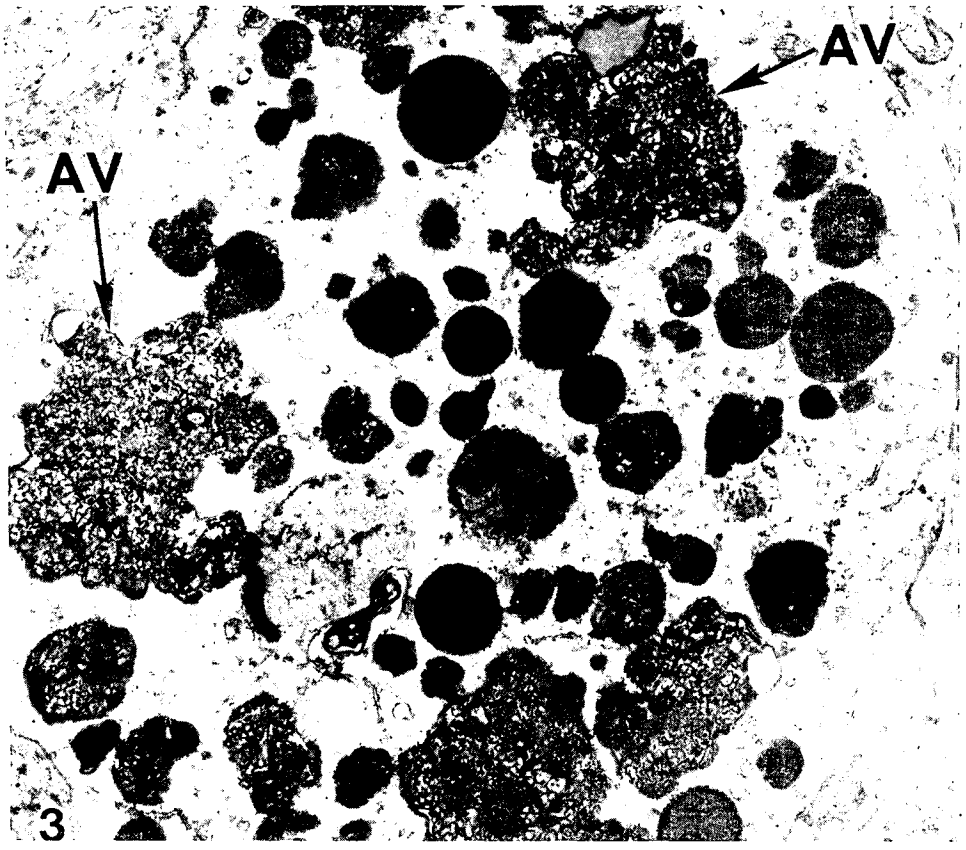



Fig. 3. Close up of neuronal cytoplasm in mature spinal cord explant exposed to 5 ATA of $O_2$ for 3 h and fixed 4 h later. Note numerous pleomorphic lysosomes, including large structures containing compactions of membraneous and vesicular debris interpreted as autophagic vacuoles (AV). The compactions of debris are interpreted as mitochondria with vesiculated cristae and are similar to those observed (15) in oxygen toxicity in vivo. × 18,800.

early postexposure time intervals, by electron microscopy. Similar changes were observed but less frequently in all categories of controls. The ultrastructural features of necrosis were the same in both experimental and control groups. Increased autophagocytosis of mitochondria was observed much more frequently in the oxygen-treated groups compared to the controls. In addition, other mitochondrial (swelling, hypertrophy, vesiculation of cristae) and cellular (myelin figures, lipid bodies) changes were more common in experimental than in control cultures.

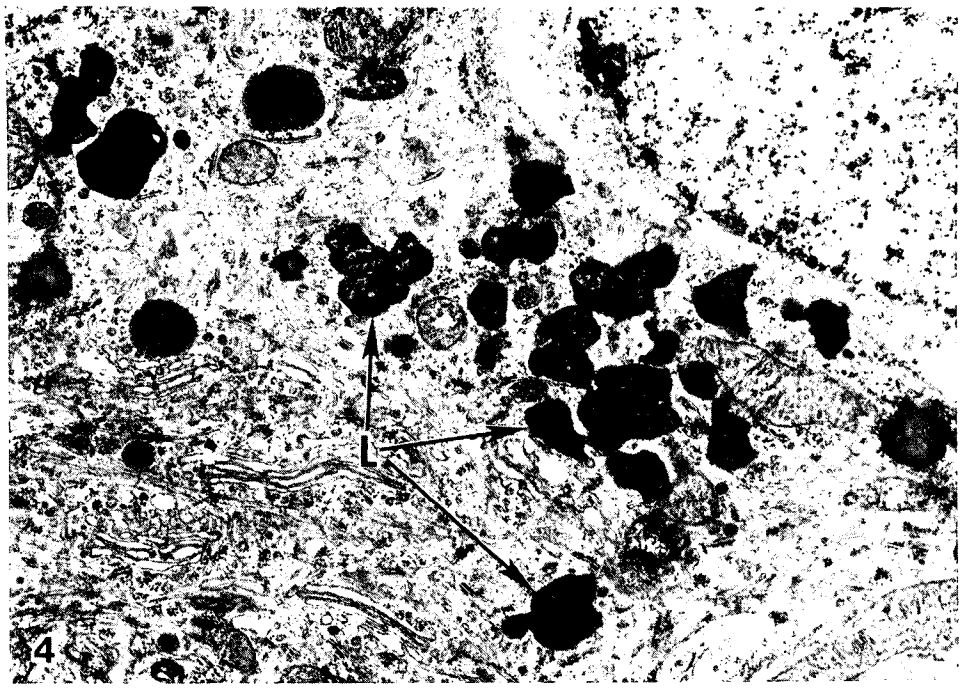



Fig. 4. Neuronal cytoplasm in mature spinal cord explant exposed to 5 ATA of $O_2$ for 3 h and fixed 4 h after exposure. Note numerous pleomorphic dense lysosomal structures interpreted as lipofuscin (L). × 17,300.

## DISCUSSION

This study indicates that hyperbaric oxygen induces significant necrosis in organotypic CNS explants and enhances autophagocytosis and lipofuscin formation within surviving cells, beginning in the earliest time intervals after exposure. The rapid autophagocytosis of mitochondria in nerve cells observed is comparable to that reported in CNS oxygen toxicity in vivo (15).

Lipofuscin has been observed in cultured cells, including neurons (16,17), as a function of time. These observations, along with studies of superoxide dismutase in vitro systems (18), indicate that cells in culture provide an ideal system for studying certain aspects of aging. The enhancement of lipofuscin production in vitro by hyperoxia supports the general membrane theory of oxygen toxicity and the idea that oxygen metabolites may play a significant role in aging.

## Acknowledgment

Supported in part by grant #2P01 NS 11066 from the NINCDS.

## References

1. Balentine JD. Pathology of oxygen toxicity. New York: Academic Press, 1982.
2. Bert P. La Pression Barométrique. Recherches de Physiologie Expérimentale. Masson, Paris, 1878. English version republished by the Undersea Medical Society, Inc., Bethesda, MD, 1978.
3. Gerschman R. Biological effects of oxygen. In: Dickens F, Neil E, ed. Oxygen in the animal organism. New York: MacMillan, 1964:475–494.
4. Chance B. Boveris A. Hyperoxia and hydroperoxide metabolism. In: Robin ED, ed. Extrapulmonary manifestations of respiratory disease. New York: Marcel Dekker 1978:185–237. (Lung biology in health and disease ser, vol. 8).
5. Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev 1979;59:527–589.
6. Greene WB, Balentine JD, Hennigar, GR. Selective mitochondrial degeneration in renal tubules following hyperbaric oxygen exposure. Am J Pathol 1979;96:737–752.
7. Wittner M, Rosenbaum RM. Inhibition and reversal of intranuclear RNA synthesis by hyperatmospheric oxygen. J Appl Phys 1972;33:820–824.
8. Yammamoto E, Wittner M, Rosenbaum RM. Resistance and susceptibility to oxygen toxicity by cell types of the gas-blood barrier of the rat lung. Am J Pathol 1970;59:409–436.
9. Brown OR, Yein F. Dihydroxyacid dehydratase: The site of hyperbaric oxygen poisoning in branch-chain amino acid biosynthesis. Biochem Biophys Res Commun 1978;85:1219–1224.
10. Mengel CE, Kann HE. Effects of in vivo hyperoxia on erythrocytes. J Clin Invest 1966;45:1150–1158.
11. Kellogg EW, Fridovich I. Liposome oxidation and erythrocyte lysis by enzymically generated superoxide and hydrogen peroxide. J Biol Chem 1977;252:6721–6728.
12. Detrisac C, Greene WB, Balentine JD. Ultrastructural pathology of CNS oxygen toxicity, in vitro. Lab Invest 1982;46(1):18A–19A.
13. Bornstein M. Organotypic mammalian central and peripheral nerve tissue. In: Kruse PF, Patterson MK, eds. Tissue culture. New York: Academic Press, 1973:86–92.
14. Dean DL Jr, Balentine JD. Chlorpromazine protects against CNS calcium toxicity in vitro. Fed Proc 1983;42:844.
15. Balentine JD. Ultrastructural pathology of hyperbaric oxygenation in the central nervous system. Lab Invest 1974;31:580–592.
16. Brunk UT, Collins VP. Lysosomes and age pigments in cultured cells. In: Sohal RS, ed. Age pigments. Amsterdam: Elsevier-North Holland, 1981:243–264.
17. Chang H-T, Pao X. Lipofuscin pigment formation in cultured neurons. In: Terry RD, Bolis CL, Toffano G, eds. Aging. New York: Raven Press, 1982:23–31. (Neural aging and its implications in human neurological pathology, vol. 18).
18. Duncan MR, Dell'Orco RT, Kirk KD. Superoxide dismutase specific activities in cultured human diploid cells of various donor ages. J Cell Phys 1979;98:437–441.

# CNS OXYGEN TOXICITY IN CLOSED-CIRCUIT SCUBA DIVERS

*F. K. Butler, Jr., and E. D. Thalmann*

The toxic effects of oxygen on the central nervous system (CNS) have been recognized since the latter half of the 19th century. Paul Bert is given credit for the earliest investigations in this area. In 1878 he subjected larks to 15-20 ATA of air and noted that they convulsed and then died. Further studies by Bert implicated oxygen rather than the nitrogen in air. In 1899, Lorrain Smith described additional toxic effects of oxygen on the lungs. Since these early investigations, oxygen has been shown to have toxic effects on many tissues in the body when present in high partial pressures (1). The precise mechanism through which oxygen exerts its detrimental effect on the tissues and organs of the body is the subject of continuing research and controversy. Among the numerous biochemical and biophysical explanations which investigators have identified as possible causes are (2):

1) Inactivation of enzymes, especially those containing sulfhydryl groups.

2) The formation of powerful oxidizing radicals.

3) Reduction of the amount of GABA (gamma aminobutyric acid), a transmitter at CNS inhibitory synapses.

4) Disruption of cellular membrane function.

The toxic effects of oxygen are of major interest in diving because of the wide use of oxygen in the conduct of diving operations. Hyperbaric oxygen is employed as a component of the breathing mixture in deep He-$O_2$ dives to reduce decompression time in mixed-gas diving and as a therapeutic agent in the treatment of decompression sickness and air embolism. Although the toxic effects of oxygen on the pulmonary system are encountered in these applications, they do not pose the same degree of danger as do CNS symptoms. In addition to these well-known applications, oxygen is also used as the sole breathing medium for combat swimmers who conduct clandestine operations

utilizing close-circuit oxygen Underwater Breathing Apparatuses (UBA). Central nervous system oxygen toxicity is a major concern during all of the above activities and presently constitutes the major limiting factor in combat swimmer operations. Because of the relatively short exposure times in closed-circuit oxygen UBA swimming, pulmonary oxygen toxicity is not encountered.

The current U.S. Navy depth/time limits for closed-circuit oxygen diving are given in Table I. These limits were based largely on the work of Lanphier, done at the Navy Experimental Diving Unit (NEDU) in 1954 (3). Recent studies at NEDU (4), as well as interaction with allied Naval forces, have suggested that these limits are perhaps too conservative. These considerations led us to conduct the study described here not only to investigate the feasibility of lengthening current oxygen exposure times but also to determine the cumulative effect of previous shallower oxygen exposures on subsequent excursions to increased depth.

## METHODS

All dive subjects were active duty military divers from NEDU, U.S. Navy EOD and UDT/SEAL communities, and U.S. Army Special Forces. All divers were in good physical condition and familiar with the use of closed-circuit oxygen UBA's. All divers, standby divers, and chamber personnel were carefully briefed on the recognition of the signs and symptoms of CNS oxygen toxicity, and emergency procedures in the event of a convulsion were thoroughly rehearsed.

All dives were conducted in the wet chamber of the NEDU Ocean Simulation Facility complex in Panama City, Fla. A special platform was built for the 15-ft diameter by 46-ft long wet chamber such that the platform was 4 ½ fsw below the water surface. An pedal-mode ergometer box (5) that was electrically braked was mounted on a specially built frame which allowed the

TABLE I

Oxygen Depth-Time Limits for Closed-Circuit Scuba Divers

| | Depth (ft) | Time (min) |
|---|---|---|
| Normal Operations | 10 | 240 |
| | 15 | 150 |
| | 20 | 110 |
| | 25 | 75 |
| Exceptional Operations | 30 | 45 |
| | 35 | 25 |
| | 40 | 10 |

From *U.S. Navy Diving Manual* (5).

diver to pedal in the prone position approximately 2 fsw below the water surface. This shallow depth precluded the development of air embolism should a convulsion occur and the stricken diver suddenly ascend to the water surface. In addition, all divers wore safety body harnesses that could be rapidly clipped to an overhead winch, which ensured they could be kept out of the water and easily hauled from the wet chamber should a convulsion occur.

Each dive was done using a single diver-subject and standby diver who wore a U.S. Navy MK I full face mask and who was always in direct visual contact with the diver-subject's face. Diver-subjects all wore Draeger LAR V (Dragerwerk, Lubeck, Federal Republic of Germany) closed-circuit oxygen UBA's with full face mask. The full face mask was worn in lieu of a mouthpiece for ease of sampling the breath-by-breath gas tensions at the mouth. The normally self-contained Draeger UBA had its oxygen bottle removed and replaced with an umbilical to a 72-$ft^3$ scuba cylinder equipped with Validyne DP-15, 3000 psi (± 0.5%) pressure transducer (Validyne Engineering, Northridge, CA). The scuba cylinder was placed in the 71°F water in the wet pot to minimize thermal transients during pressure changes. Pressure recordings were made every 10 s by an HP-1000 computer (Hewlett Packard, Cupertino, CA); they were stored and plotted after each dive. The slope of the staircase plot was determined by hand and the mean oxygen consumption was determined from this slope.

Continuous gas samples from the $CO_2$ absorbent canister effluent and oronasal mask were removed from the UBA at 200–250 cc/min and analyzed for $CO_2$, $O_2$, and $N_2$ by either a Perkin Elmer MGA 1100 (Perkin Elmer Aerospace Division, Pomona, CA) or Chemetron Medspect 2 (Chemetron Medical Products, St. Louis, MO) Mass Spectrometer. Gas sample flow rate was controlled by a micro-valve located on the oronasal mask or canister effluent, and gas samples were routed through a penetrator in the chamber wall to the mass spectrometer through a 70-ft long by 0.078-inch i.d. continuous unbroken sample line. This minimized deadspace in the sample line and gave a delay time of 3–5 s with little mixing in the line and good frequency response (5). Gas samples were plotted continuously on a strip chart recorder. The oronasal $CO_2$ tracing was used to estimate end tidal $P_{CO_2}$, and the $CO_2$ absorbent canister effluent $CO_2$ level was used as an estimate of inspired $P_{CO_2}$. If canister effluent $P_{CO_2}$ exceeded 3.8 mmHg, a fresh canister was installed in the UBA. In addition to gas sampling, diver rectal temperature on the long dive profiles was monitored continuously by a YSI 702-A rectal probe (Yellow Springs Instrumentation, Yellow Springs, OH).

The diver-subject inserted his rectal probe and dressed in a "shorty" wet suit or a wetsuit top (⅛-in. or 3/16-in. thickness) before putting on the UBA. Just before entering the water the diver performed a *purge* procedure to raise the oxygen tension in the UBA and his lungs to at least 95%. The oxygen tension was measured after the purge and if it was below 95%, another purge was performed. The diver then entered the water, mounted the ergometer, and began his prescribed work cycle. If at any time during the study, inspired $O_2$ percentage fell below 95%, the diver was required to repurge. All exercise

was done with the ergometer set at 50 W. During the long 1- or 2-h period at the shallow 25 fsw depths, intermittent exercise consisting of 4 min of rest followed by 6 min of work at 50 W was performed. Divers pedalled continuously at 50 W during all excursions to 40 fsw and during the single-depth 40 fsw dives. The water temperature of 22°C combined with the shorty wetsuit insulation were selected to produce an approximate ¼°C/h temperature drop, which approximates the expected rate of core cooling in a diver wearing passive thermal garments (wet suit or dry suit with insulative undergarments) appropriate for the ambient water temperature.

Four separate profiles were studied during this dive series. Two were directly from the surface to 40 fsw; the remaining two incorporated a brief excursion to 40 fsw into a longer dive at 25 fsw. The specific depth-time combinations were

*Profile A*—40 fsw for 20 min
*Profile B*—40 fsw for 15 min
*Profile C*—25 fsw for 60 min
40 fsw for 15 min
25 fsw for 60 min
*Profile D*—25 fsw for 120 min
40 fsw for 15 min

Depths were achieved by compressing the wet chamber 2 fsw shallower than the depths shown because the diver was 2 fsw below the wet pot water surface.

## RESULTS

All signs or symptoms noted by divers during the study which could possibly have been attributed to CNS oxygen toxicity were recorded and are presented in Table II. Each suspected episode of oxygen toxicity, which may have consisted of a single symptom or a number of symptoms, was evaluated and assigned to one of three categories: *Convulsion, Definite, and Probable*.

The *Convulsion* category requires no further elaboration except to say that all three convulsive episodes were of the classic grand mal, tonic-clonic type. *Definite* refers to toxicity episodes that were strongly felt to represent actual oxygen toxicity episodes. Symptoms that would likely result in an episode being classified as a *Definite* hit included tinnitus, blurred or tunnel vision, disorientation, asphasia, dysphasia, nystagmus, muscle twitching, or incoordination. It should be emphasized, however, that other factors were also considered in deciding how to classify a suspected toxicity episode. Among these factors were the condition of the diver as noted by observers, whether or not the dive was stopped when the symptoms occurred, and the duration and severity of the symptoms. The term *Probable* was used to describe episodes that may have been caused by CNS oxygen toxicity but which were more

TABLE II

Symptoms of Oxygen Toxicity and Their Frequency of Occurrence

| Sign/Symptom | Number of Times Reported or Observed | % of Total Symptoms | % of Toxicity Episodes Noted on |
|---|---|---|---|
| Light-Headedness | 6 | 21 | 46 |
| Convulsion | 3 | 11 | 23 |
| Tinnitus | 3 | 11 | 23 |
| Apprehension | 3 | 11 | 23 |
| Dysphoria | 3 | 11 | 23 |
| Blurred Vision | 2 | 7 | 15 |
| Tunnel Vision | 1 | 4 | 8 |
| Disorientation | 1 | 4 | 8 |
| Lethargy | 1 | 4 | 8 |
| Dysphasia | 1 | 4 | 8 |
| Aphasia | 1 | 4 | 8 |
| Eye Twitching | 1 | 4 | 8 |
| Nystagmus | 1 | 4 | 8 |
| Incoordination | 1 | 4 | 8 |
| | 28 TOTAL | | |

equivocal than those placed in the *Definite* category. Symptoms likely to result in this classification were "light-headedness", apprehension, dysphoria (used to describe episodes where the divers reported that they "just didn't feel right"), and lethargy.

A complete listing of the dives made according to profile, the number and types of oxygen toxicity episodes occurring with that profile, and the percentage of dives on each profile resulting in toxicity episodes is shown in Table III. A more detailed listing of these episodes is available in Table IV, which also provides the time into the dive at which signs or symptoms occurred.

TABLE III

Oxygen Toxicity Symptoms by Dive Profile

| *Profile* | Number of Dives | Total Number of Symptoms | *Probable* | *Definite* | *Convulsion* | % of Dives With Symptoms |
|---|---|---|---|---|---|---|
| *A* | 17 | 4 | 2 | 0 | 2 | 24 |
| *B* | 24 | 5 | 5 | 0 | 0 | 21 |
| *C* | 13 | 1 | 0 | 1 | 0 | 8 |
| *D* | 14 | 3 | 0 | 2 | 1 | 21 |
| TOTALS | 68 | 13 | 7 | 3 | 3 | 19 |

TABLE IV

Oxygen Toxicity Episodes by Dive

| Profile | Dive Number | Subject Number | Class of Toxicity | Signs/Symptoms Noted (in order of occurrence) | Time into Dive | Comments |
|---|---|---|---|---|---|---|
| *PROFILE A* 40 ft for 20 min | *A-5* | 10 | *Probable* | Light-Headedness | Ascent | 1) Occurred at 12 ft on ascent.<br>2) Cleared quickly. |
| | *A-10* | 16 | *Convulsion* | Apprehension<br>Convulsion | 20:00 | 1) Diver was able to stand up in water and remove face mask, but convulsion followed quickly. |
| | *A-11* | 12 | *Probable* | Light-Headedness | 12:00 | 1) Symptom reported after dive.<br>2) Lasted approx. 45 s.<br>3) Dive not aborted. |
| | *A-17* | 1 | *Convulsion* | Tinnitus<br>Convulsion | 19:33 | 1) Tinnitus noted approx. 1 min before convulsion. |
| *PROFILE B* 40 ft for 15 min | *B-2* | 4 | *Probable* | Tunnel Vision<br>Light-Headness | Upon Surfacing | 1) S/S occurred just prior to exiting wet chamber. |
| | *B-6* | 4 | *Probable* | Dysphoria<br>Blurred Vision<br>Disorientation | 12:00 | 1) Symptoms transient. Reported postdive.<br>2) Dive not aborted. |
| | *B-21* | 7 | *Probable* | Dysphoria<br>Lethargy | 15:00 | 1) Symptoms reported postdive.<br>2) Dive not aborted. |
| | *B-22* | 5 | *Probable* | Dysphoria | 15:00 | 1) Symptoms reported postdive.<br>2) Dive not aborted. |
| | *B-24* | 5 | *Probable* | Light-Headness | Upon Surfacing | |

TABLE IV (Continued)

Oxygen Toxicity Episodes by Dive

| Profile | Dive Number | Subject Number | Class of Toxicity | Signs/Symptoms Noted (in order of occurrence) | Time into Dive | Comments |
|---|---|---|---|---|---|---|
| *PROFILE C*<br>25 ft/60 min<br>40 ft/15 min<br>25/60 min | *C-3* | 2 | *Definite* | Blurred Vision<br>Light-Headness<br>Dysphoria<br>Nystagmus | After 14:32<br>at 40 ft | |
| *PROFILE D*<br>25 ft for<br>120 min<br>40 ft for<br>15 min | *D-2* | 1 | *Definite* | Incoordination<br>Apprehension<br>Tinnitus | After 8:00<br>at 40 ft | 1) Dive aborted as soon as tinnitus began. Had incoordination for 4 min and apprehension 30 s previous to onset of tinnitus. |
| | *D-8* | 16 | *Convulsion* | Apprehension<br>Tinnitus<br>Aphasia<br>Convulsion | 72 Min | 1) Apprehension and tinnitus lasted 30 s before diver removed mask.<br>2) Aphasic at foot of ladder 2–3 min.<br>3) Convulsions occurred 3 min post-$O_2$. |
| | *D-15* | 7 | *Definite* | Light-Headness<br>Eye Twitching | After 14 min<br>at 40 ft | |

All dives were aborted at the onset of symptoms unless otherwise noted.

There were 28 total symptoms or signs reported in 13 separate episodes. Some divers experienced only one sign or symptom while others reported as many as four. The most common symptom was light-headedness, which was reported on 46% of the toxicity episodes and accounted for 21% of all symptoms. Convulsion, tinnitus, apprehension, and dysphoria were the next most frequent symptoms; each was seen on 23% of toxicity episodes.

Oxygen consumptions were calculated for 4 dives at 40 fsw (continuous pedalling) and 5 dives at 25 fsw (intermittent work/rest cycles). The average $O_2$ consumption measured for these dives was 1.89 L/min and 1.34 L/min at STPD respectively. No significant difference in oxygen consumption was noted between those dives which resulted in oxygen toxicity and those which did not. No diver exceeded 3.8 mmHg $CO_2$ at any time; average values were 0–1.5 mmHg with only one diver having to change canisters because of a $CO_2$ of 2.7 mmHg which was rising. No oxygen toxicity was encountered on that dive. Rectal temperature drop ranged from 0–1°F during *Profiles C* and *D* and did not appear to correlate with symptoms of oxygen toxicity.

A total of 17 dives was conducted on *Profile A* (40 fsw for 20 min). On *Dive A–10*, a grand mal convulsion occurred after the end of the 20 min stay at depth, during the ascent. Another convulsion was experienced on *Dive A–17* at 19 min and 33 s. Two *Probable* episodes of $O_2$ toxicity also occurred on *Profile A* for a toxicity percentage of 24%. As a result of the high incidence of CNS toxicity symptoms, the exposure at 40 fsw was shortened to 15 min and relabelled *Profile B*. This profile was used for 24 dives. No convulsions or *Definite* toxicity episodes occurred, but five *Probable* symptoms were reported. Of these symptoms, two were reported during ascent or upon surfacing. Two others were experienced at the end of the 15 min at 40 fsw and not reported until postdive. The last symptom occurred at 12 min into the dive but was not reported until after the dive because the symptoms were transient (lasting only about 45 s).

*Profile C* had a single instance of toxicity among 13 dives. This profile consisted of 60 min at 25 fsw followed by 15 min at 40 fsw, and then an additional 60 min at 25 fsw. The toxicity episode included blurred vision, light-headedness, dysphasia, and nystagmus; it was considered in the *Definite* category. Onset of symptoms was noted after 14 min and 32 at 40 fsw.

*Profile D* consisted of a 2-h dive at 25 fsw followed by a 15-min excursion to 40 fsw. Two *Definite* symptoms were encountered during the excursion (at 8 and 14 min) and one convulsion occurred after 72 min at 25 fsw.

Table V presents the initial manifestations of oxygen toxicity as experienced by our divers. As shown, the subjective complaints of light-headedness, dysphoria, and apprehension accounted for 69% of the initial symptoms observed. These complaints occurred both as the only symptom or as a precursor to more serious problems. The other four initial symptoms observed were tinnitus, incoordination, blurred vision, and tunnel vision. Convulsion never occurred without a preceding aura and therefore was never the presenting symptom in this study.

TABLE V

Initial Symptoms of Oxygen Toxicity

| Symptom | Number of Times Reported As Initial Symptom | % of Total |
|---|---|---|
| Light-Headedness | 4 | 31 |
| Dysphoria | 3 | 23 |
| Apprehension | 2 | 15 |
| Tinnitus | 1 | 8 |
| Incoordination | 1 | 8 |
| Blurred Vision | 1 | 8 |
| Tunnel Vision | 1 | 8 |

## DISCUSSION

One observes that as the depth of the oxygen exposure increases, the duration allowed decreases. This relationship reflects the fact that CNS oxygen toxicity is directly related to the partial pressure at which oxygen is breathed. The factors that are known to affect the onset of oxygen toxicity are listed:

1) *Partial pressure of oxygen*. The greater the depth or pressure, the greater the likelihood of developing oxygen toxicity (1–4,6,7).

2) *Duration of exposure*. Toxicity increases as time of exposure increases (1–4,6,7).

3) *Individual variation in susceptibility*. This variation applies not only to a difference in susceptibility between different individuals but also to differences in the same individual at different times (2,7).

4) *Immersion in water*. The onset of toxicity is increased by immersion. Divers under treatment for air embolism or decompression sickness routinely breathe oxygen in the recompression chamber at 60 fsw. Breathing oxygen at a depth of 60 fsw in the water for an equal amount of time would result in a high incidence of CNS $O_2$ toxicity (2,7).

5) *Hypercarbia*. Divers breathing an increased partial pressure of $CO_2$ are rendered more susceptible to CNS oxygen toxicity (1,2,7).

6) *Exercise*. Even in the absence of an exercise-induced hypercarbia, the exercising diver is more likely to encounter toxic symptoms than the diver at rest (7).

In 1942 and 1943, a British investigator, H. K. Donald (7), conducted over 2000 man-dives for the purpose of establishing guidelines regarding oxygen toxicity for proposed use by British frogmen in World War II. His research remains the largest dive series conducted to date investigating CNS $O_2$ toxicity. In one of his experiments, Donald immersed 100 volunteer subjects in a Davis submarine escape apparatus in a wet chamber (water tem-

perature 65°F) at 50 fsw (2.5 ATA) for a maximum time of 30 min. Of these 100 divers, 26 convulsed, 24 had other symptoms of oxygen toxicity, and 50 had no signs or symptoms. Donald also attempted to find the depth at or about which convulsions would not be observed. After doing 2-h resting dives at 40, 35, 30, and 25 fsw, he concluded that 25 fsw was the depth at or above which convulsions would not occur.

A U.S. Navy Experimental Diving Unit (NEDU) study published in 1947 by Yarborough et. al. (8) tested oxygen exposures at various depths ranging from 30 to 100 fsw. In a resting dive underwater at 60 fsw, 32 out of 107 exposures were terminated prior to 60 min, which was the maximum time for the dive. The average time to termination was 32 min (range 8 to 58 min). Two convulsions were noted: one at 13 min and the other at 24 min. In their other depths tested, they do not mention the time elapsed before the onset of convulsions.

Further work done at NEDU in 1953 by Lanphier (3) used a continuous-flow $O_2$ face mask apparatus in 80°F water. Work rate was set at "greater than a man would voluntarily sustain under diving conditions, although less than the maximum possible." No convulsions were obtained at depths less than 35 fsw. One convulsion was seen at 35 fsw after 42 min (out of a total of 5 dives to a maximum time of 43 min). One convulsion was also noted at 40 fsw after 31 min (total: 13 dives to a maximum time of 30 min). No convulsions were noted in 5 dives to 45 fsw for a maximum time of 19 min.

Results from these and other researchers (4) are shown in a tabular form according to exposure depth in Table VI. Exposures deeper than 60 fsw are not included. In evaluating these results, care must be taken to note whether the dive in question was performed under working or resting conditions.

The present study eventually resulted in 68 man-dives with a total of 13 toxicity episodes, 3 of which were convulsions. Testing of *Profiles A* and *B* at 40 fsw provided a total of 41 dives at this depth and indicated that 15 min is the time beyond which severe symptoms of $O_2$ CNS toxicity begin to occur. The two convulsions observed of 20 and 19:23 min make it clear that a 20-min exposure exceeds safe operating limits. The 15-min exposure at 40 fsw, although it resulted in a 21% symptom rate, produced no symptoms that we felt were unequivocal manifestations of $O_2$ toxicity. All 5 divers completed the 15-min dive; 4 had their symptoms at the very end of the dive or just after reaching the surface. Most of the symptoms reported were mild, subjective feelings of abnormality, which may or may not have been a result of $O_2$ toxicity.

The results compiled from other studies shown in Table VI might initially lead one to assume that a much longer time than 15 min could be spent at 40 fsw. The studies by Donald and Yarborough (7,8), while aspiring for a 2-h exposure time, did not indicate at what times their reported symptoms occurred. (It should also be noted that Donald's 40-ft exposures were at rest.) Lanphier's study showed a convulsion occurring after 31 min and this combined with the data from the present study would lead one to the conclusion

TABLE VI

Results of Dives Reported by Other Researchers

| Depth | Researcher | Year | Dive Activity | Water Temperature | Number of Dives | Length of Dive | Results |
|---|---|---|---|---|---|---|---|
| 25 fsw | DONALD | 1942 | Rest | 65°F | 28 | 2 h | No Symptoms |
| | DONALD | 1942 | Work | 65°F | 18 | Not stated | 5 Symptoms<br>No Convulsions |
| | LANPHIER | 1953 | Work | 80°F | 5 | 81 min | No Symptoms |
| | PIANTADOSI | 1980 | Work | 70°F | 6 | 180 min<br>239 min<br>249 min<br>271 min<br>178 min<br>252 min | 1 Symptom at 178 min<br>Admin stop at 180 min<br>4 dives to 240 min + |
| | PIANTADOSI | 1980 | Work | 40°F | 6 | 194 min<br>179 min<br>167 min<br>157 min<br>139 min<br>139 min | No Symptoms |
| 30 fsw | DONALD | 1942 | Rest | 65°F | 20 | 2 h | 3 Symptoms<br>2 Convulsions |
| | YARBOROUGH | 1942 | Work | 90°F | 35 | 2 h | 2 Symptoms; 87 min, 111 min<br>? Convulsions |
| | LANPHIER | 1953 | Work | 80°F | 11 | 57 min | No Symptoms |
| 35 fsw | DONALD | 1942 | Rest | 65°F | 21 | 2 h | 6 Symptoms<br>1 Convulsion |
| | LANPHIER | 1953 | Work | 90°F | 5 | 43 min | 2 Symptoms<br>1 Convulsion (42 min) |

TABLE VI (Continued)

Results of Dives Reported by Other Researchers

| Depth | Researcher | Year | Dive Activity | Water Temperature | Number of Dives | Length of Dive | Results |
|---|---|---|---|---|---|---|---|
| 40 fsw | DONALD | 1942 | Rest | 65°F | 29 | 2 h | 15 Symptoms<br>4 Convulsions |
| | LANPHIER | 1953 | Work | 80°F | 13 | 30 min | 3 Symptoms<br>1 Convulsion (31 min) |
| | YARBOROUGH | 1947 | Work(Closed Circuit) | 90°F | 48 | 2 h | 11 Symptoms<br>? Convulsions |
| | YARBOROUGH | 1947 | Work(Open Circuit) | 90°F | 23 | 2 h | 8 Symptoms<br>? Convulsions |
| 50 fsw | DONALD | 1942 | Rest | 65°F | 100 | 30 min | 50 Symptoms<br>26 Convulsions |
| | YARBOROUGH | 1947 | Work | 90°F | 5 | 2 h | 3 Symptoms<br>? Convulsions |
| | LANPHIER | 1953 | Work | 80°F | 2 | 15 min | 1 Symptom<br>No Convulsions |
| 60 fsw | DONALD | 1942 | Rest | 65°F | 6 | 12 min<br>19 min<br>25 min<br>37 min<br>61 min<br>76 min | ? Convulsions |
| | YARBOROUGH | 1947 | Rest | 90°F | 107 | 2 h | 11 Divers Completed 2 h<br>75 Divers Completed 1 h<br>2 Convulsions; 13 & 24 min |

Where times not noted, time to onset of symptom unknown.

that symptoms begin occurring well before 2 h in divers exercising underwater.

*Profiles C* and *D* showed that it is possible to allow a diver a prolonged $O_2$ exposure at 25 fsw and still be able to make an excursion to 40 fsw for a period almost as long as would be expected without any previous $O_2$ exposure. *Profile C* had only one toxicity episode which occurred after 14 1/2 min at 40 fsw following a 60-min period at 25 fsw. The occurrence of symptoms near the 15-min mark was consistent with the results obtained on *Profile B* (40 fsw for 15 min) although the symptoms were more definite than those noted on *Profile B*. Still, one symptom in 13 dives was the lowest incidence of toxicity of any of the profiles studied. None of the divers on *Profile C* had any symptoms at 25 fsw, either before or after the 40-fsw excursion. *Profile D* produced one convulsion after a surprisingly short 72-min exposure at 25 fsw. More will be said about this later. Of the two symptoms occurring during the excursion to 40 fsw, one occurred at 8 min and the other at 14 min. Again, the episodes were both believed to be definite cases of $O_2$ toxicity. This fact, plus the fact that one episode occurred as early as 8 min, suggest a cumulative increase in susceptibility due to the preceding 120-min exposure at 25 fsw. However, it appears that a previous 60-min exposure at 25 fsw did not significantly reduce the safe exposure limit at 40 fsw. The relatively small number of exposures done on *Profiles C* and *D* (13 and 14 respectively) make it difficult to make any statistically sound conclusion as to expected incidence. If one assumes that the occurrence of oxygen toxicity symptoms is random and follows the binomial distribution, then at the 95% confidence limit all one can say is that on *Profile C* one could expect less than a 35% incidence of symptoms and less than a 22% incidence of convulsions and on *Profile D* one could expect less than a 52% incidence of symptoms and less than a 35% incidence of convulsions. Clearly, more work needs to be done in this area.

We noted in our series that the relative frequency of the various signs and symptoms of oxygen toxicity was significantly different from previous studies. Donald noted facial twitching as the most common symptom occurring in underwater hyperbaric oxygen exposures (60.6%) (4). Yarborough et al. found that nausea was the most frequent symptom encountered in their combination of wet and dry exposures (7). Lanphier noted nausea occurring three times and convulsions twice out seven symptoms in his underwater exposures (3). In this study, the most frequent symptoms were those pertaining to altered mental status (Table II). Light-headedness was reported six times; dysphoria (''just didn't feel right'') and apprehension were each reported three times. Convulsion and tinnitus were also noted three times apiece. Blurred vision was noted twice and a number of other symptoms were seen on a single occasion.

When only the initial symptoms of oxygen toxicity are considered, altered mental status was also predominant. The symptoms of light-headedness, dysphoria, and apprehension accounted for 69% of all initial manifestations of oxygen toxicity. It should be noted that a convulsion was never an initial symptom of oxygen toxicity, but, when it occurred, followed within 30 s to 4 min from the onset of symptoms. In two of the three convulsive episodes,

oxygen breathing was discontinued before the onset of the convulsion. In these cases, a return to breathing a normoxic mixture did not prevent the progression of the CNS toxicity to a convulsion. One of the divers who experienced a convulsion on one dive, however, had another toxicity episode in which the initial symptom (tinnitus) was identical to the prodrome which preceded his previous convulsion. On this occasion, however, he discontinued oxygen breathing as soon as he noted the tinnitus. This resulted in the abatement of his tinnitus and he recovered without further symptoms of toxicity. There were no instances in which a symptom present for more than 4 min progressed on to a convulsion.

No correlation was noted between the magnitude of core temperature drop and the susceptibility to oxygen toxicity. Although hypothermia is often mentioned as a possible contributing factor in oxygen toxicity, none of the studies mentioned addressed this question experimentally except for Donald (7). Most of his dives were done in water maintained at 65°F. In an effort to ascertain the effect of thermal stress on oxygen toxicity, he conducted a number of dives in both colder (45°F) and warmer (87°F) water. While he does not elaborate on the number of dives or specifics regarding the profiles used, he does say that "oxygen tolerance was equally affected by heat and cold. Although the performances below 30 minutes were only slightly impaired, all outstanding performances were eliminated." The exact mechanism by which hypothermia would increase susceptibility cannot be ascertained from any of the studies cited here (including the present study) but it could simply be a reflection of the increased oxygen consumption associated with cold stress. Thus, a cold water exposure at rest could well increase the diver's susceptibility the same amount as an exercising diver in warmer water providing the oxygen consumptions are the same in both cases.

Perhaps the most unexpected finding in this dive series was the occurrence of an oxygen convulsion at 25 fsw. No convulsions at this depth had been reported in the five previous controlled studies we examined. A total of 63 dives at 25 fsw had been conducted with exposures ranging from 81 to 252 min. Based on these results, our expectation was the exposures at 25 fsw would perhaps result in less serious $O_2$ toxicity symptoms, but would not produce any convulsions. We found that of 26 exposures at 25 fsw for 2 h, 25 were completely symptom-free. Only one diver experienced any problems; unfortunately, the problem was a grand mal seizure.

The interesting thing about this episode was that it occurred after only a 72-min exposure at 25 fsw (1.76 ATA), which is within the currently accepted U.S. Navy maximum exposure limit for 25 fsw. The individual who experienced the convulsion had also convulsed on an earlier dive after 20 min at 40 fsw. This suggests that perhaps this individual was one of those who are very sensitive to oxygen. The diver involved, however, had previously passed two standard Navy oxygen tolerance tests (30 min of 100% $O_2$ at rest in a dry chamber at 60 fsw), the first in 1971 and the second in 1981. Since then, eight additional oxygen tolerance tests have been conducted on this diver. No symptoms were observed on any of the tests. In addition, although the diver

was serving as a Diving Medical Officer at the time of these tests, he had previously been a Navy Special Warfare (UDT/SEAL) operator who had made numerous training and operational dives using closed-circuit $O_2$ equipment with no incidents of oxygen toxicity. There was no indication at all of hypercarbia or other departures from normal control conditions with regard to his breathing mixture. Failure of the oxygen tolerance test to identify this individual as being sensitive to $O_2$ raises the question of whether this test is an effective screening device.

One of the characteristics desirable in a screening test is that the results should be consistent within the same individual. This is not the case with oxygen tolerance as shown by Donald (7) in his classic work. In this study, a single diver was compressed in a wet chamber to 70 fsw 20 separate times over a period of 90 days. He was allowed to remain at depth breathing 100% $O_2$ until the onset of symptoms on each occasion. His average time at depth before the onset of symptoms was 55.4 min, but the times for each exposure ranged from a low of 7 min to a high of 148 min. This remarkable variation shows a maximum exposure tolerated 21 times longer than his shortest time to symptoms. Another subject convulsed after 12 min at 50 fsw but 16 days later completed 100 min at 50 fsw without symptoms. It should be emphasized that these exposures were under controlled conditions and do not involve the additional considerations of variation in exercise levels and thermal stress. These examples illustrate the inability of a single screening test to predict accurately an individual's susceptibility to oxygen toxicity on any given dive.

In summary, the observations made during this dive series include:

1) Oxygen toxicity convulsions may be seen during what are usually considered safe exposures, as evidenced by the convulsion that occurred after only a 72-min exposure at 25 fsw.

2) The current limit of 10 min at 40 fsw in the *U.S. Navy Diving Manual* will certainly not be extended beyond 15 min. Further dives at 40 fsw for 15 min are necessary to determine whether or not this profile is safe for operational use.

3) Brief excursions to 40 fsw were possible after a prolonged exposure to oxygen at 25 fsw. Pre-exposures of 60 min at 25 fsw do not seem to influence the safe 40-fsw exposure time, but a 2-h pre-exposure did seem to decrease the safe 40-fsw exposure time somewhat.

## References

1. Lambertsen CJ, Effects of hyperoxia on organs and their tissues. In: Robin ED, ed. Extrapulmonary manifestations of respiratory disease: Vol. 8 of Lenfant C, ed. Lung biology in health and disease. New York: Marcel Dekker, 1978.
2. Edmonds C, Lowry L, Pennefather J: Diving and subaquatic medicine, 2nd edition. Mosman, Australia: Diving Medical Center, 1981.
3. Lanphier EH, Dwyer JV: Diving with self-contained underwater operating apparatus. Washington, DC: Navy Experimental Diving Unit, 1954. (NEDU report no. 11–54)

4. Piantadosi CA: Prolonged oxygen exposures in immersed exercising divers at 25 fsw. Washington, DC: Navy Experimental Diving Unit, 1980. (NEDU report no. 4–80)
5. Thalmann, ED, Sponholtz, DK, Lundgren, CEG. Chamber-based system for physiological monitoring of submerged exercising subjects. Undersea Biomed Res 1978;5:293–300.
6. U.S. Navy diving manual. Washington: Navy Department, 1979. (NAVSEA 0994-LP-001-9010) Distributed by Best Bookbinders, Carson, CA.
7. Donald KW: Oxygen poisoning in man, I & II. Br Med J 1947;1:667–672, 712–717.
8. Yarborough OD, Welham W, Brinton ES, Behnke AR. Symptoms of oxygen poisoning and limits of tolerance at rest and at work. Washington, DC: Navy Experimental Diving Unit, 1947. (NEDU report no. 01–47)

# EFFECTS OF INSPIRED OXYGEN PRESSURE ON THE NATURE AND DEGREE OF OXYGEN TOLERANCE MODIFICATION

*J. M. Clark and C. J. Lambertsen*

Many factors that alter susceptibility to oxygen poisoning have been studied in efforts either to identify detrimental effects that may significantly decrease the tolerance of a patient to therapeutic oxygen administration or to provide protective influences that extend tolerance to hyperoxia and permit greater utilization of its beneficial effects (1,2). In the interpretation or application of these results, however, it is important to recognize that effects on oxygen tolerance may vary significantly at different oxygen pressures or conditions of exposure. In illustration of this principle, several examples that are relevant to therapeutic and diving operational uses of hyperoxia are described below.

## OXYGEN TOLERANCE AFTER ADAPTATION TO CHRONIC HYPOXIA

Many patients who eventually require oxygen therapy for chronic pulmonary insufficiency are exposed to prolonged periods of hypoxemia during the progression of their disease. A laboratory model for some of the effects of prolonged hypoxemia can be produced in rats by exposure for several days to an atmosphere with a low inspired $P_{O_2}$. Previous studies have shown that hypoxia-adapted rats are extremely tolerant to oxygen exposure at 1.0 ATA (3,4), but that adapted rats (3) or mice (5) are unusually susceptible to oxygen poisoning at 4.0 ATA or higher. Recent extension of these studies to intermediate oxygen pressures indicates that the protective influence of hypoxia adaptation is also prominent at 1.5 ATA, where exposure durations for 50%

mortality ($LD_{50}$) are 27 and 63 h in normal and adapted rats, respectively (Fig. 1). At 2.0 ATA, hypoxia-adapted rats still have a small but statistically significant increment in $LD_{50}$ from 17 to 22 h. During oxygen exposure at 3.0 ATA, $LD_{50}$ values of 9.0 and 7.9 h are not significantly different in normal and adapted rats, respectively. However, at 4.0 ATA, normal rats live significantly longer than adapted rats, with respective $LD_{50}$ values of 6.4 and 3.8 h.

The observed effects of hypoxia adaptation on tolerance to oxygen pressures of 1.0 to 4.0 ATA are consistent with the interpretation that pulmonary oxygen tolerance is enhanced, while neurologic tolerance is diminished. In agreement with the latter conclusion is the finding that hypoxia-adapted rats have extremely violent convulsions almost immediately upon exposure to oxygen at 4.0 ATA (Fig. 1). Oxygen tolerances of the endocrine organs, liver, kidney, and myocardium also may be altered by hypoxia adaptation, but these effects have not been studied to date.

## EFFECTS OF ACUTE AND CHRONIC HYPERCAPNIA ON OXYGEN TOLERANCE

It is well known that acute hypercapnia accelerates the development of oxygen poisoning at pressures that are above the convulsive threshold (1,6). For example, the $LD_{50}$ of rats exposed to oxygen at 4.0 ATA is decreased from 6.4 to 2.0 h when inspired $P_{CO_2}$ ($P_{I_{CO_2}}$) is increased from 0 to 60 Torr. At 3.0 ATA the corresponding $LD_{50}$ values are 9.0 and 2.9 h in normal and hypercapnic rats. Onset of convulsions during oxygen breathing at 4.0 ATA is also hastened by acute hypercapnia (Fig. 2). However, the effects of acute hypercapnia on tolerance to lower oxygen pressures are not statistically significant. Respective $LD_{50}$ values for normal and hypercapnic rats are 17 and 16 h at 2.0 ATA, 27 and 25 h at 1.5 ATA, and 76 and 75 h at 1.0 ATA (Fig. 2).

The adverse effects of acute hypercapnia on tolerance to convulsive oxygen pressures are largely, if not completely, caused by cerebral vasodilation with concomitant increments in brain oxgyen dose and rate of intoxication (Fig. 3)(7). The unmeasurable effects of acute hypercapnia at oxygen pressures of 2.0 ATA or less are consistent with less prominent influences on the onset and progression of pulmonary oxygen poisoning.

The adaptive changes that occur during chronic exposure to hypercapnia include diminished responsiveness of the cerebral vasculature to elevation of arterial $P_{CO_2}$ (8,9). Rats exposed to oxygen with 60 Torr $P_{I_{CO_2}}$ ($O_2$-$CO_2$) after adaptation to chronic hypercapnia would therefore have a lower brain $P_{O_2}$ than rats exposed acutely to the same inspired gas. When hypercapnia-adapted rats are exposed to $O_2$-$CO_2$ at 4.0 and 3.0 ATA, $LD_{50}$ values are increased from 2.0 to 4.1 h and from 2.9 to 7.5 h, respectively. Convulsions during $O_2$-$CO_2$ exposure at 4.0 ATA are also delayed (Fig. 2). However, exposures to $O_2$-$CO_2$ at 2.0 and 1.5 ATA again yield no significant differences in $LD_{50}$ values for normal and adapted rats at either pressure. Surprisingly, rats adapted to hypercapnia are actually less tolerant to $O_2$-$CO_2$ exposure at 1.0 ATA than are

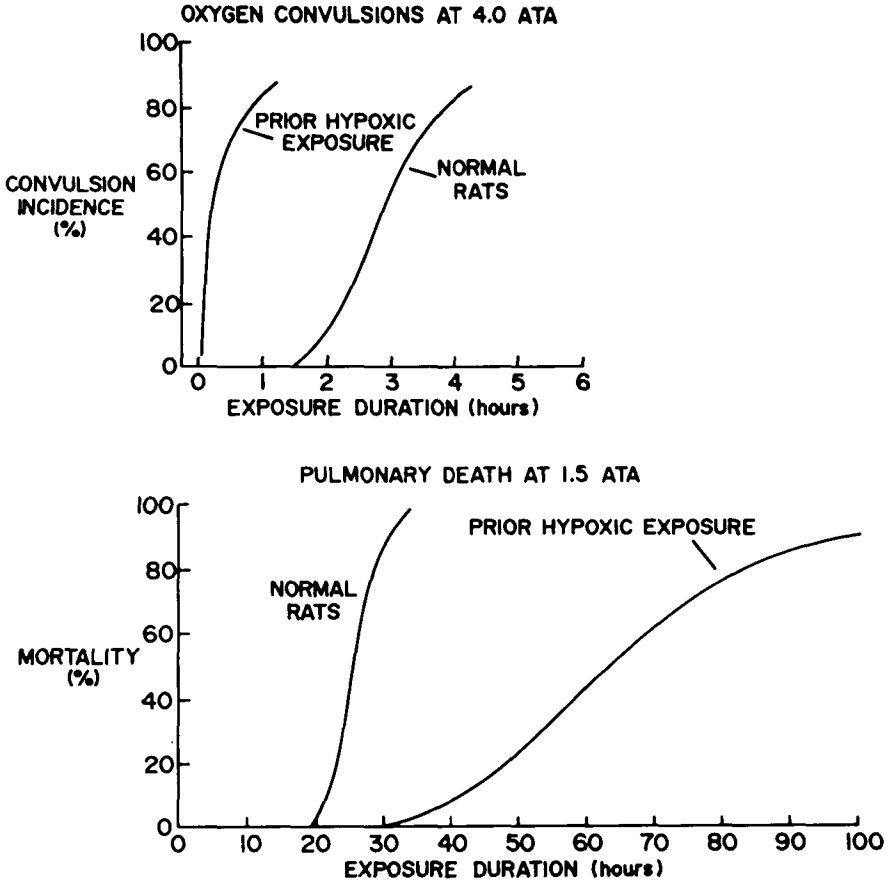



Fig. 1. Effects of prior adaptation to hypoxia on oxygen tolerance in rats at 4.0 and 1.5 ATA. Groups of at least 20 normal and hypoxia-adapted rats were exposed to $O_2$ at 4.0 and 1.5 ATA. Rats were adapted to hypoxia by a 5-day exposure to an inspired $P_{O_2}$ of 70 Torr. Sigmoid convulsion and mortality curves were obtained by linear translation of regression lines fitted to data plotted on probability-log coordinates. Not all rats convulsed prior to death at 4.0 ATA.

normal rats (Fig. 2). Under such conditions, adapted rats have 100% mortality with an $LD_{50}$ of 65 h, as compared to 70% mortality with an $LD_{50}$ of 75 h in normal rats. These results are consistent with an adverse effect of hypercapnia adaptation on pulmonary tolerance to $O_2$-$CO_2$ in direct contrast to its beneficial effects on CNS tolerance to the same condition.

## EXTENSION OF OXYGEN TOLERANCE

Many investigators have studied pharmacological agents that delay oxygen poisoning in attempts to provide protection against the deleterious effects

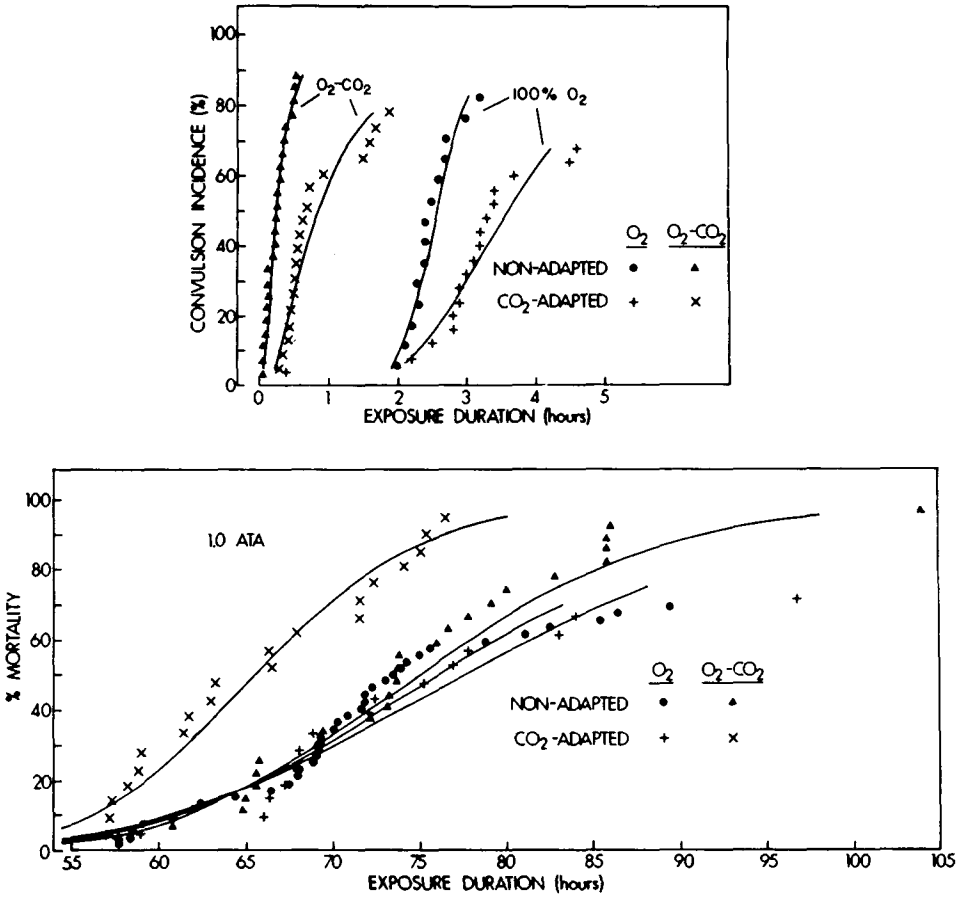



Fig. 2. Effects of acute and chronic hypercapnia on oxygen tolerance in rates at 4.0 and 1.0 ATA. Normal or hypercapnia-adapted rats were exposed in groups of 20 to $O_2$ or $O_2$-$CO_2$ (60 Torr $Pt_{CO_2}$) at 4.0 and 1.0 ATA. Rats were adapted to hypercapnia by a 5-day exposure to an inspired $PCO_2$ of 60 Torr. Rat groups and exposure conditions are indicated by 4 different symbols. Each point represents the time of the first convulsion at 4.0 ATA *(top)* or survival time at 1.0 ATA *(bottom)* in one rat. Sigmoid curves were derived as in Fig. 1. Not all rats convulsed prior to death at 4.0 ATA, and some rats survived exposure to $O_2$ or $O_2$-$CO_2$ at 1.0 ATA for up to 2 weeks. Figure modified from Clark (6).

of oxygen while taking advantage of its beneficial properties (Table I). However, it cannot be assumed that an equivalent degree of protection will be provided by a given agent over a wide range of oxygen pressures. It is even true that the same agent may extend overall tolerance to one oxygen pressure while decreasing tolerance to a different level of hyperoxia.

## Effects of Disulfiram Administration on Oxygen Tolerance

Data obtained in three different laboratories indicate that administration of disulfiram has an overall protective influence in animals exposed to oxygen

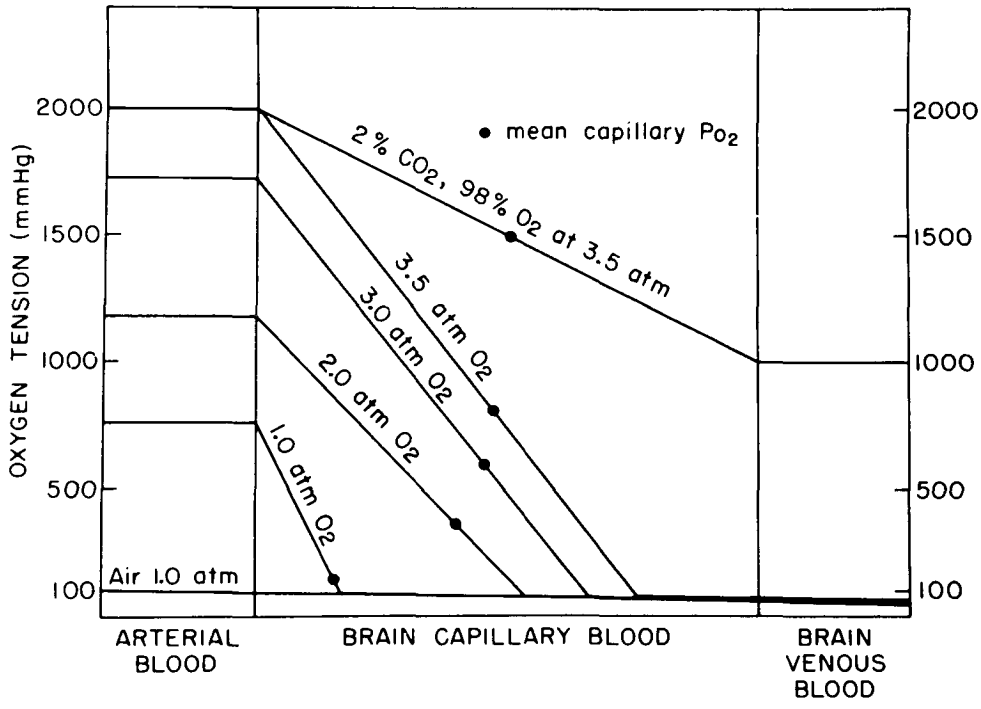



Fig. 3. Effect of acute hypercapnia on brain oxygen tension in man. The indicated changes in brain capillary $P_{O_2}$ were calculated from measurements of arterial and brain venous blood $P_{O_2}$, on the assumption of uniform oxygen loss along the capillary. Administration of 2% $CO_2$ in $O_2$ at 3.5 ATA caused more than a 10-fold increment in brain venous $P_{O_2}$. Figure from Lambertsen (7).

pressures that are above the convulsive threshold, but it enhances the progression of oxygen intoxication at lower pressures (Table II). Early studies of Faiman et al. (20,21) showed that pretreatment with disulfiram greatly delays the occurrence of convulsions and lung damage in mice and dogs exposed to oxygen at 4.0 ATA. For example, the convulsion time for 50% of the dogs exposed at 4.0 ATA was extended from 6 to 53 min by prior intraperitoneal administration of disulfiram in a dose of 200 mg/kg.

However, when the same dose of disulfiram was given to rats prior to oxygen exposure at 1.0 ATA, the occurrence of pulmonary pathology was greatly accelerated, and all rats died within 48 h of exposure as compared to 5% mortality in untreated rats (31). Similar results were obtained at 2.0 ATA where all disulfiram-treated rats died by 16 h of exposure, while untreated rats had only 6% mortality (30). These results are consistent with the interpretation that administration of disulfiram prior to oxygen exposure delays neurologic effects of oxygen toxicity, but exacerbates pulmonary effects.

## Effects of Systematic Alternation of Oxygen and Normoxic Exposure

An effective and practical means for extending oxygen tolerance is by the systematic alternation of oxygen and normoxic exposure periods. Although the

TABLE I

Pharmacological Agents That Slow Rate of Development of Oxygen Poisoning

| Agent | Reference |
|---|---|
| Adrenergic Blocking Drugs | 10,11 |
| Anesthesia | 12–14 |
| Antioxidants | 15–17 |
| Chlorpromazine | 18,19 |
| Disulfiram | 20,21 |
| Gamma-aminobutyric acid | 22,23 |
| Ganglionic Blocking Drugs | 11 |
| Glutathione | 15,16 |
| Reserpine | 18 |
| Succinate | 24,25 |
| Tris-aminomethane | 26,27 |
| Vitamin E | 28,29 |

effectiveness of this procedure has been validated over a wide range of oxygen pressures (32–39), the same intermittent exposure schedule does not provide equivalent extension of oxygen tolerance at different pressures. For example, the data in Fig. 4 show survival time extensions in rats for the same intermittent oxygen exposure patterns at 4.0 and 2.0 ATA. At each pressure oxygen exposure periods of 60 min are alternated with normoxic intervals of 15, 30, 60, or 180 min. Relative increments in $LD_{50}$ values for the four normoxic intervals at 4.0 ATA are 1.4, 1.6, 1.7, and 2.4 times the $LD_{50}$ for continuous exposure, and the corresponding ratios at 2.0 ATA are 1.2, 1.4, 2.1, and more than 3.8. These data are consistent with the interpretation that intermittency schedules with short normoxic intervals are equally or more effective at

TABLE II

Effects of Prior Administration of Disulfiram on Neurologic and Pulmonary Oxygen Poisoning

| Species | Oxygen Pressure (ATA) | Toxicity Index | Control (vehicle) | | Treated (200 mg/kg) | | Reference |
|---|---|---|---|---|---|---|---|
| Mouse | 4.0 | $CT_{50}$ (min) | 126±10 | (14) | >360 | (15) | 20 |
| Dog | 4.0 | $CT_{50}$ (min) | 6±0.5 | ( 8) | > 60 | (10) | 21 |
| Rat | 2.0 | 16 h Mortality | 6% | (16) | 100% | ( 8) | 30 |
| Rat | 1.0 | 48 h Mortality | 5% | (42) | 100% | (20) | 31 |

( ) indicates number of exposed animals.

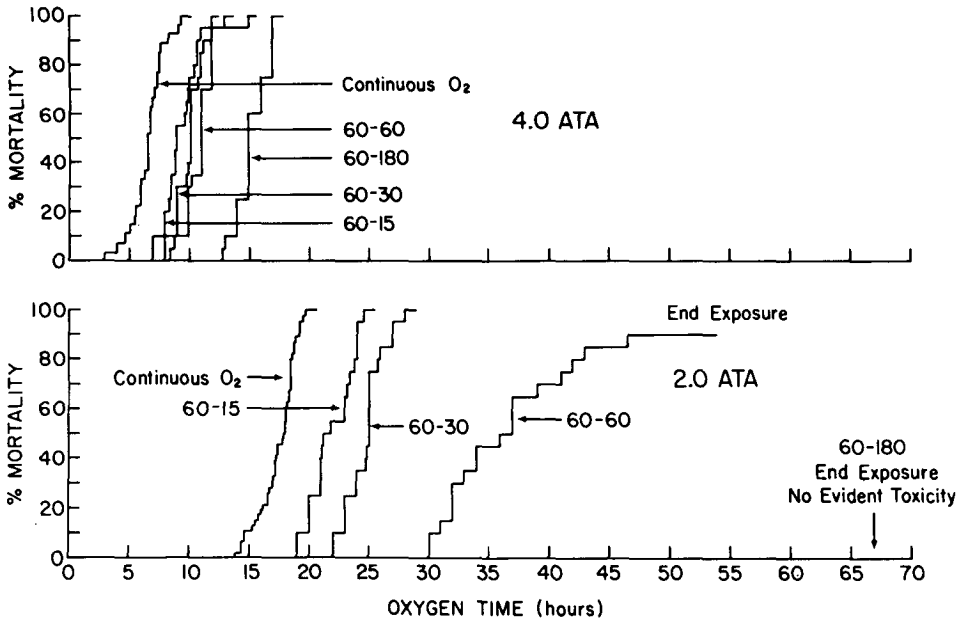



Fig. 4. Effects of intermittent hyperoxic exposure on oxygen tolerance in rats at 4.0 and 2.0 ATA. Groups of at least 20 rats were exposed continuously or intermittently to $O_2$ at 4.0 or 2.0 ATA. Each step represents the death of one or more rats. *Numbers* indicate durations (min) of the alternating oxygen-normoxic intermittent exposure periods. Oxygen time on the *abscissa* represents duration of oxygen exposure and does not include the normoxic periods.

4.0 ATA, while schedules with longer normoxic intervals are more effective at 2.0 ATA.

Different responses to the same intermittent exposure patterns at 4.0 and 2.0 ATA are particularly striking for the 60–180 schedule. On this schedule the $LD_{50}$ at 4.0 ATA increased by a factor of 2.4, whereas at 2.0 ATA, all 20 rats survived an intermittent oxygen exposure that is nearly 4 times the $LD_{50}$ for continuous exposure. Furthermore, electron microscopic examination revealed normal morphology and ultrastructural constituents in lungs removed from randomly selected surviving rats.

## FACTORS THAT DETERMINE TOLERANCE LIMITATIONS AT DIFFERENT OXYGEN PRESSURES

Although exposure to any toxic level of hyperoxia will ultimately poison every cell in the body, many interacting factors determine which specific organ or function will limit the safe duration of oxygen exposure at a given pressure. These factors include inherent susceptibility to oxygen poisoning and the

oxygen dose in specific cells and tissues, as well as the nature and degree of functional impairment caused by toxic effects on a specific organ (7). In vitro experiments have shown that susceptibility to oxygen toxicity varies among different tissues, with brain homogenates being the most sensitive of the preparations that were studied (2,40). However, inter-organ differences in the rates of development of oxygen poisoning in vivo are probably influenced less by inherent differences in susceptibility than they are by the wide range of oxygen pressures existing in different organs at a constant inspired $P_{O_2}$ (Fig. 5). The magnitude of range in tissue $P_{O_2}$ is determined by local differences in circulatory supply and metabolic utilization of oxygen. The net influences of these factors are such that tissues in the lung and carotid body, for example, are exposed to the highest $P_{O_2}$ levels, while tissues at the venous end of capillaries in the brain and heart are exposed to relatively low levels of $P_{O_2}$.

## Interactions Among Pulmonary, Neurologic, and Other Effects of Oxygen Toxicity

Prolonged exposures to oxygen at pressures of 1.0 to 2.0 ATA are usually limited by progressive pulmonary intoxication, while prominent neurologic effects are relatively infrequent (1,2). At oxygen pressures above 3.0 ATA, on the other hand, convulsions usually occur before pulmonary intoxication becomes evident (2,41). These well-known characteristics of oxygen toxicity indicate that the high pulmonary dose of oxygen is a dominant factor in the development of oxygen poisoning at 1.0 to 2.0 ATA, whereas the greater inherent susceptibility of brain tissue has a more dominant influence at pressures above 3.0 ATA. Any agents or procedures having effects on pulmonary or neurologic oxygen tolerance that vary in nature or degree would therefore be expected to have variable influences on overall oxygen tolerance over a range of oxygen pressures.

The observed effects of hypoxia adaptation, hypercapnia, and disulfiram on survival times over a range of oxygen pressures can all be interpreted as manifestations of different effects on pulmonary and neurologic oxygen tolerance. Comparing the magnitudes of increments in survival time provided by the same patterns of intermittent exposure at 2.0 and 4.0 ATA indicates that a single pattern will not optimally extend both pulmonary and neurologic oxygen tolerance. It is important to recognize that additional effects of oxygen toxicity on organs other than the lungs and brain, although more subtle and difficult to assess, must certainly occur during toxic exposures and may have slowly reversible or permanent consequences. Any agent or procedure that appears to provide a measure of protection against oxygen toxicity must therefore be evaluated over a range of oxygen pressures and under a variety of experimental conditions to determine its efficacy against the multiple and diverse manifestations of oxygen poisoning.

## References

1. Clark JM, Lambertsen CJ. Pulmonary oxygen toxicity: a review. Pharm Rev 1971;23:37–133.

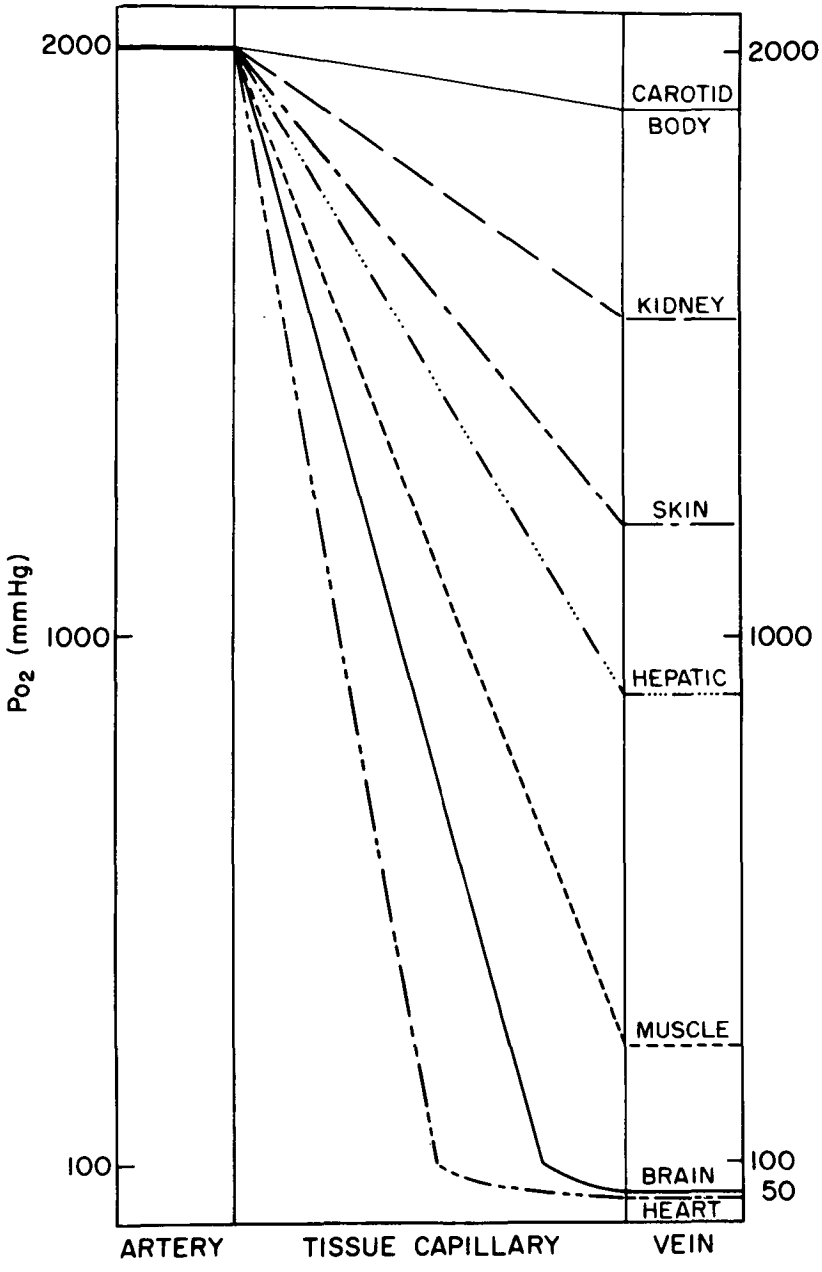



Fig. 5. Range of oxygen pressure in different organs and tissues during oxygen breathing at 3.5 ATA. The curve for brain represents average measurements of arterial and internal jugular venous blood $P_{O_2}$ in 16 conscious men. Venous values and capillary $P_{O_2}$ changes for other organs and tissues were calculated from measured arterial values and tabulated values of tissue oxygen consumption and blood flow in man. Even within an organ or tissue, inequalities of metabolic rate and blood flow should cause local differences in $P_{O_2}$. Cells near the arterial end of a capillary are exposed to much higher $P_{O_2}$ levels than other cells near the venous end. Pathological states and drug effects on circulation or metabolism should be expected to alter the patterns shown here. Figure from Lambertsen (7).

2. Clark JM. Oxygen toxicity. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving. London: Baillière Tindall, 1982:200–238.
3. Brauer RW, Parrish DE, Way RD, Pratt PC, Pessotti RL. Protection by altitude acclimatization against lung damage from exposure to oxygen at 825 mmHg. J Appl Physiol 1970;28:474–481.
4. Brashear RE, Sharma HM, DeAtley RE. Prolonged survival breathing oxygen at ambient pressure. Am Rev Resp Dis 1973;108:701–704.
5. Hall P, Schatte CL, Fitch JW. Relative susceptibility of altitude-acclimatized mice to acute oxygen toxicity. J Appl Physiol 1975;38:279–281.
6. Clark JM. Effects of acute and chronic hypercapnia on oxygen tolerance in rats. J Appl Physiol: Respirat Environ Exercise Physiol 1981;50:1036–1044.
7. Lambertsen CJ. Effects of hyperoxia on organs and their tissues. In: Robin ED, ed. Extrapulmonary manifestations of respiratory disease. New York: Marcel Dekker, 1978:239–303.
8. Raichle ME, Stone HL. Cerebral blood flow autoregulation and graded hypercapnia. Eur Neurol 1971/72;6:1–5.
9. Stone HL, Raichle ME, Hernandez M. The effect of sympathetic denervation on cerebral $CO_2$ sensitivity. Stroke 1974;5:13–18.
10. Gerschman R, Gilbert DL, Nye SW, Price WE, Fenn WO. Effects of autonomic drugs and of adrenal glands on oxygen poisoning. Proc Soc Exp Biol Med 1955;88:617–621.
11. Johnson PC, Bean JW. Effect of sympathetic blocking agents on the toxic action of oxygen at high pressure. Am J Physiol 1957;188:593–598.
12. Bean JW, Zee D. Influence of anesthesia and carbon dioxide on CNS and pulmonary effects of oxygen at high pressure. J Appl Physiol 1966;20:521–526.
13. Bean JW, Zee D. Metabolism and the protection by anesthesia against toxicity of oxygen at high pressure. J Appl Physiol 1965;20:525–530.
14. Jamieson D. The effect of anaesthesia and carbon dioxide on survival time and lung damage in rats exposed to high pressure oxygen. Biochem Pharmacol 1966;15:2120–2122.
15. Gerschman R, Gilbert DL, Caccamise D. Effects of various substances on survival times of mice exposed to different high oxygen tensions. Am J Physiol 1958;192:563–571.
16. Gerschman R, Gilbert DL, Nye SW, Dwyer P, Fenn WO. Oxygen poisoning and x-irradiation: a mechanism in common. Science 1954;119:623–626.
17. Jamieson D, Van Den Brenk HAS. The effects of antioxidants on high pressure oxygen toxicity. Pharmacol 1964;13:159–164.
18. Bean JW. Reserpine, chlorpromazine and the hypothalamus in reactions to oxygen at high pressure. Am J Physiol 1956;187:389–394.
19. Van Den Brenk HAS, Jamieson D. Studies of mechanisms of chemical radiation protection in vivo and its relationship to oxygen poisoning. Int J Radiat Biol 1962;4:379–402.
20. Faiman MD, Mehl RG, Oehme FW. Protection with disulfiram from central and pulmonary oxygen toxicity. Biochem Pharmacol 1971;20:3059–3067.
21. Faiman MD, Nolan RJ, Oehme FW. Effect of disulfiram on oxygen toxicity in beagle dogs. Aerosp Med 1974;45:29–32.
22. Wood JD, Stacey NE, Watson WJ. Pulmonary and central nervous system damage in rats exposed to hyperbaric oxygen and protection therefrom by gamma-aminobutyric acid. Can J Physiol Pharmacol 1965;43:405–410.
23. Wood JD, Watson WJ, Clydesdale FM. Gamma-aminobutyric acid and oxygen poisoning. J Neurochem 1963;10:625–633.
24. Currie WD, Gelein RM Jr, Sanders AP. Comparison of protective agents against hyperbaric oxygen in large animals. Aerosp Med 1973;44:996–998.
25. Currie WD, Kramer RS, Sanders AP. Effects of hyperbaric oxygenation on metabolism. VII. Succinate protection against oxygen toxicity in large animals. Proc Soc Biol Med 1971;136:630–631.
26. Bean JW. Tris buffer, carbon dioxide, and sympatho-adrenal system in reactions to oxygen at high pressure. Am J Physiol 1961;201:737–739.
27. Sanger C, Nahas GG, Goldberg AR, D'Allesio GM. Effects of 2-amino-2-hydroxymethyl-1-3-propanediol on oxygen toxicity in mice. Ann NY Acad Sci 1961;92:710–723.
28. Kann HE Jr, Mengel CE, Smith W, Horton B. Oxygen toxicity and vitamin E. Aerosp Med 1964;35:840–844.

29. Taylor DW. The effects of vitamin E and methylene blue on the manifestations of oxygen poisoning in the rat. J Physiol London 1956;131:200–206.
30. Forman HJ, York JL, Fisher AB. Mechanism for the potentiation of oxygen toxicity by disulfiram. J Pharmacol Exp Ther 1980;212:452–455.
31. Deneke SM, Bernstein SP, Fanburg BL. Enhancement by disulfiram (antabuse) of toxic effects of 95%–97% $O_2$ on the rat lung. J Pharmacol Exp Ther 1979;208:377–380.
32. Soulie P. Modifications experimentales de la resistance individuelle de certains animaux a l'action toxique de l'oxygene. CR Seances Soc Biol 1939;130:541–542.
33. Paine JR, Lynn D, Keys A. Observations on the effects of prolonged administration of high oxygen concentration to dogs. J Thorac Cardiovasc Surg 1941;11:151–168.
34. Lambertsen CJ. Respiratory and circulatory actions of high oxygen pressure. In: Goff LG, ed. Proceedings of the underwater physiology symposium. Washington, DC: National Academy of Sciences (National Research Council Publ. 377) 1955;25–38.
35. Penrod KE. Effect of intermittent nitrogen exposure on tolerance to oxygen at high pressure. Am J Physiol 1956;186:149–151.
36. Ackerman NB, Brinkley FB. Cyclical intermittent hyperbaric oxygenation: a method for prolonging survival in hyperbaric oxygen. In: Brown IW Jr, Cox BG, eds. Proceedings of the third international conference on hyperbaric medicine. Washington, DC: National Academy of Sciences-National Research Council 1966;200–207.(NAS-NRC publ. no. 1404)
37. Wright RA, Weiss HS, Hiatt EP, Rustagi JS. Risk of mortality in interrupted exposure to 100 percent $O_2$: role of air vs. lowered $P_{O_2}$. Am J Physiol 1966;210:1015–1020.
38. Hall DA. The influence of the systematic fluctuation of $P_{O_2}$ upon the nature and rate of the development of oxygen toxicity in quinea pigs. Philadelphia: University of Pennsylvania, 1967. (Master of Science Thesis).
39. Hendricks PL, Hall DA, Hunter WL Jr, Haley P. Extension of pulmonary $O_2$ tolerance in man at 2 ATA by intermittent $O_2$ exposure. J Appl Physiol 1977;42:593–599.
40. Dickens F. The toxic effects of oxygen on brain metabolism and on tissue enzymes. Biochem 1946;40:145–186.
41. Donald KW. Oxgyen poisoning in man. I and II. Br Med J 1947;1:667–672, 712–717.

# SERUM ANGIOTENSIN-CONVERTING ENZYME ACTIVITY IN DOGS AND MAN EXPOSED TO INCREASED LEVELS OF OXYGEN

*A. L. Harabin, R. G. Eckenhoff, and M. E. Bradley*

Angiotensin-converting enzyme (ACE) is a constituent of vascular endothelium in most or all organs (1). Because the lung is so rich in endothelium and receives the entire cardiac output, it probably plays a major role in angiotensin metabolism. Plasma also has ACE activity and while the exact relationship of this enzyme to the endothelial enzyme is not clear, they are molecularly very similar (2). Since Lieberman showed that serum ACE activity increases in patients with sarcoidosis (3), interest has grown in determining whether this activity might be a useful index of the severity of pulmonary injury. Serum ACE activity has been found to be altered in a variety of lung diseases, including those caused by bleomycin (4), paraquat (5), thiourea (6), hypoxia (7), and the adult respiratory distress syndrome (8,9,10). Because prolonged exposure to increased levels of oxygen causes pulmonary damage, we measured serum ACE activity in men breathing the equivalent of 1 ATA $O_2$ for 48 h as a part of a 5-ATA air exposure, and in dogs continuously exposed to normobaric hyperoxia.

## METHODS

Serum ACE activity was determined using the radiochemical assay of Rohrbach (11), based on quantitation of [glycine-1$^{14}$C]hippuric acid liberated from the ACE substrate [glycine-1$^{14}$C]hippuryl-L-histidyl-L-leucine (Hip-His-Leu; New England Nuclear). Ten microlitres of serum were used and hydrolysis was carried out at 37°C for 60 min. Substrate concentration was 5 μM at a final specific radioactivity of 0.8 mCi/mM. Captopril (a gift of S.J. Lucania,

E. R. Squibb & Sons), at a final concentration of 1 μM, was added to some samples and blanks. ACE activity is expressed as nmoles of substrate hydrolyzed per minute per millilitre of serum. Each sample was measured at least in triplicate. Analysis of variance with repeated measures and Dunnett's multiple comparison procedure (12) were used to test the effect of time.

Human samples were obtained from nine healthy male subjects participating in a 5-ATA air exposure, conducted at the Naval Submarine Medical Research Laboratory. The profile of the exposure is shown in Fig. 1. Control venous blood samples were drawn 2 days prior to, as well as the morning of, the dive. The men were compressed at 30 ft/min to 5 ATA and breathed an $N_2$-$O_2$ mixture containing a $P_{O_2}$ level of 0.3 ATA for 12 h. The gas mixture was changed to air and maintained for 48 h. At this pressure, air has a $P_{O_2}$ slightly greater than 1 ATA. Decompression occurred over the next 65 h with $P_{O_2}$ held constant at 360 mmHg for the first 35 h. Blood samples were drawn every 24 h during the exposure and every 48 h postexposure, as shown in Fig. 1. Samples drawn at depth were placed on ice, vented, and decompressed at 8 ft/min. Blood was then spun, clotted, and the serum was frozen.

Samples were obtained from 13 male mongrel dogs according to the following procedure. Using aseptic techniques, we catheterized the right carotid artery and jugular vein under nitrous-oxide halothane anesthesia. Catheters were filled with a saline-heparin solution (200 units/cc) and the dogs received penicillin subcutaneously every other day. Following 4 days of recovery, a Swan-Ganz® catheter was inserted into the pulmonary artery through the jugular catheter. The dog was then placed in a polycarbonate exposure cham-

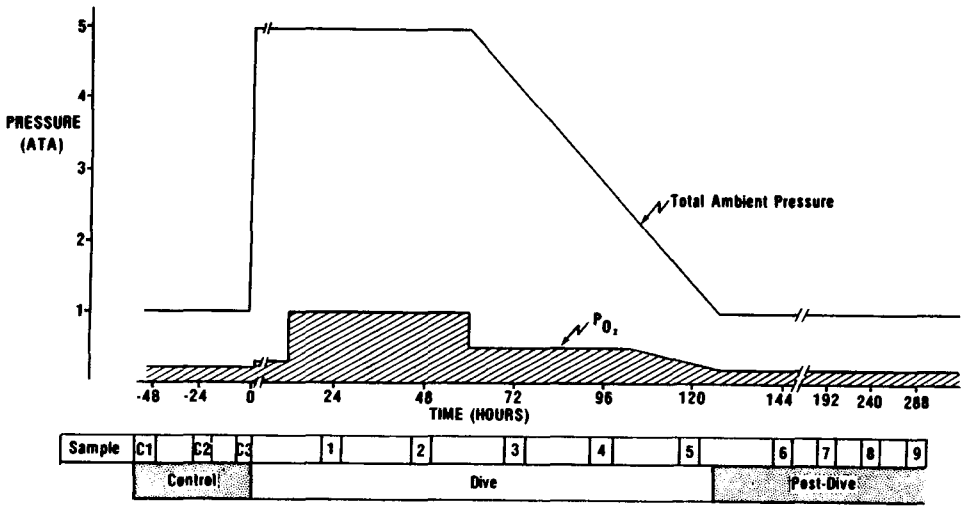



Fig. 1. Exposure profile and sampling schedule for human serum ACE study. The *top curve* shows the total ambient pressure; the *lower curve* shows inspired $P_{O_2}$. Venous blood samples were drawn at times indicated throughout the dive.

ber in which he had been trained to sit quietly. The chamber was large enough to allow complete freedom of movement and was designed to permit housekeeping, feeding, and access to the animal with little atmospheric contamination. A gas flow of approximately 15 L/min, in conjunction with an air-conditioning loop that circulated and cooled the environment, maintained humidity around 50% and a $CO_2$ level less than 0.5%. In all animals, initial measurements were made while the chamber was flushed with air. Control animals ($n = 5$) remained in the box for 96 h breathing air; experimental animals ($n = 8$) breathed an $O_2$ environment until death. Oxygen concentration was monitored continuously and was maintained at 98%. Arterial samples were drawn every 24 h, centrifuged, clotted, and frozen until analysis.

## RESULTS

The coefficient of variation for a given measurement averaged 4.9% for human and 8.4% for canine samples. When captopril, a specific ACE inhibitor, was incubated with samples, activity was reduced to 3.3% of control activity in human samples and to 12.2% in canine samples (Table I).

The results from the human subjects are shown in Fig. 2. The sample numbers refer to the sampling times shown in Fig. 1. Sample 1 was taken after the men had breathed 1 ATA $O_2$ (at a total pressure of 5 ATA) for 12 h, and Sample 2 was taken after 36 h of $O_2$ breathing. All subjects complained of symptoms usually associated with $O_2$ toxicity. Beginning after about 12 h and becoming progressively more severe throughout the 48-h exposure to 5 ATA air, subjects experienced substernal discomfort, coughing, anorexia, and nausea. These symptoms generally began to abate after 12 h of exposure to 0.5 ATA $P_{O_2}$. The experimental protocol significantly decreased serum ACE activity. Analysis of variance on these data resulted in: $F = 2.44$ and $P < 0.025$.

Serum ACE activity as a function of time is shown in Fig. 3 for control dogs breathing air and experimental dogs breathing $O_2$. The mean survival time for experimental dogs was 91.1 h (range = 60–123 h).

TABLE I

Effect of One μM Captopril on Serum ACE Activity

| Subjects | Control Activity | Captopril | % Inhibition |
|---|---|---|---|
| | nM $min^{-1}$ $mL^{-1}$ (±1 SD) | | |
| Human ($n = 7$) | 17.44 (±3.2) | 0.58 (±0.55) | 96.7 |
| Canine ($n = 9$) | 6.65 (±1.16) | 0.81 (±0.749) | 87.8 |

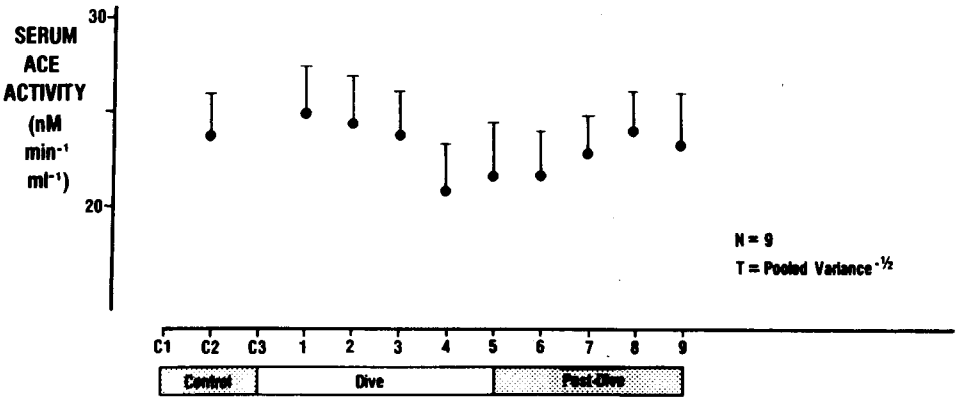



Fig. 2. Serum ACE activity of men participating in 5-ATA air-saturation exposure. Sample numbers correspond to the times outlined in Fig. 1. Results of control samples drawn 2 days prior to and on the day of the dive were combined. A one-way analysis of variance with repeated measures resulted in $F = 2.44$, and $P < 0.025$. The error shown is the square root of the residual mean square error from the ANOVA.

Beginning within 12 h before death, dogs breathing $O_2$ had decreases in both $Pa_{O_2}$ and pHa, with increases in $Pa_{CO_2}$. Given this evidence of terminal pulmonary injury, we could not demonstrate a difference in serum ACE activity whether animals were breathing air or $O_2$.

## DISCUSSION

The nine men exposed to the pressure profile shown in Fig. 1 had significantly altered serum ACE activity. At its lowest point, (Sample 4), serum ACE activity decreased from a control value of 23.8 to 20.8 nM $s^{-1}$ $mL^{-1}$. At this stage in the protocol the men had been exposed to 48 h of 1 ATA $O_2$, and 36 h of 0.5 ATA $O_2$. Upon completion of decompression and on ensuing postexposure days, ACE activity returned to control levels. It is possible that this small but consistent decrease in ACE activity reflects pulmonary injury resulting from $O_2$ exposure.

An index for evaluation of the development of pulmonary injury from $O_2$ exposure has remained elusive. There are very few human studies to allow prediction of man's tolerance to $O_2$ exposure, which makes it difficult to state what sort of dose the present experiment represented. Most mammals survive approximately 72 h when exposed to 100% $O_2$ at 1 ATA (13), although primates appear to be somewhat more resistant. The dose of $O_2$ used here was clearly deleterious, as all subjects experienced symptoms usually associated with pulmonary $O_2$ toxicity.

It is of interest that ACE activity fell rather than rose in the face of exposure to increased $O_2$. In several models of lung disease investigators have

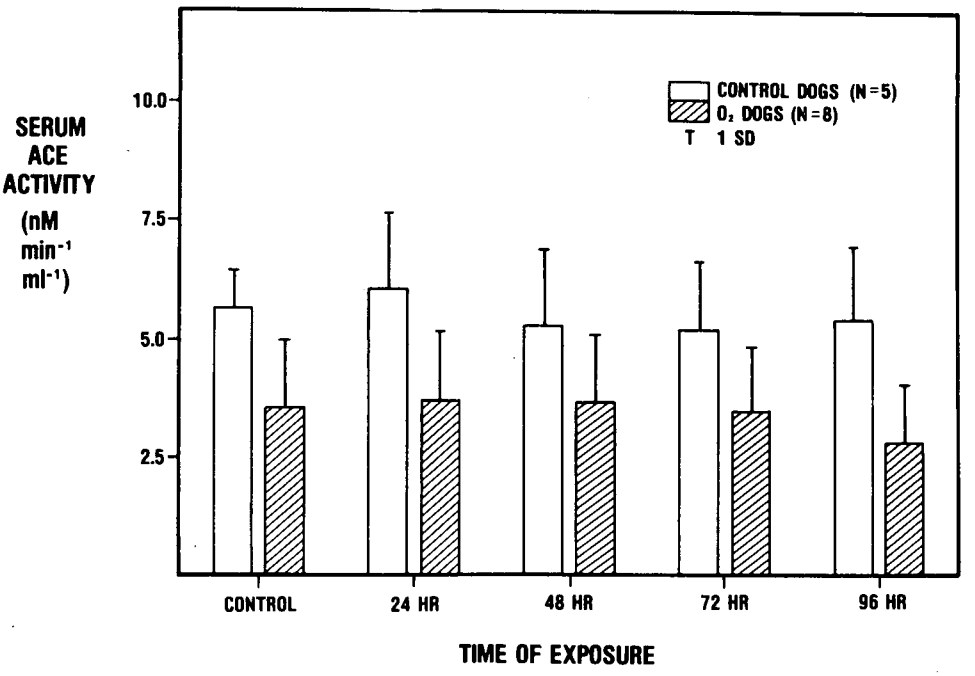



Fig. 3. Effect of normobaric $O_2$ exposure on serum ACE activity in awake dogs. Control dogs were exposed to air for 96 h and experimental dogs to 100% $O_2$ at 1 ATA. Arterial samples were drawn every 24 h. We could not show a significant effect of time of exposure.

found increases in ACE activity, and it has been hypothesized that damaged endothelium sloughs this enzyme into the circulation (2). Patients with the adult respiratory distress syndrome (ARDS), however, have significantly lower ACE activity (8,9,10). Both ARDS and pulmonary $O_2$ poisoning in experimental animals ultimately result in impaired pulmonary transfer $O_2$, increased lung permeability, and edema. In addition, both conditions reveal neutrophil aggregation that is complement-mediated (14,15). Thus there are intriguing similarities between the two forms of lung injury. Whether the decreased serum ACE activity reflects impaired microvascular competence as Hollinger recently suggested (16) or whether it results from increased proteolysis or enzyme inhibition remains to be determined.

We were unable to show an effect of lethal, normobaric $O_2$ exposure on dog serum ACE activity. By using the model substrate Hip-His-Leu, we determined that control dogs had only 20% of the activity measured in human serum. Canine enzyme catalyzes hydrolysis of Hip-His-Leu much less efficiently than its natural substrate, angiotensin I (17), and thus does not necessarily reflect a true difference in the conversion capacity of plasma angiotensin I. This substrate specificity, however, may have precluded our ability to demonstrate an effect of $O_2$ exposure.

To test the specificity of the assay used in this study, we added captopril, a specific ACE inhibitor, to samples. Although captopril abolished nearly all the activity in human samples, the percentage remaining in canine samples was much higher (Table I). The actual remaining activity was nearly the same in both groups, but the differential of nearly 9% is another reflection of the difference in the human and canine capacities for Hip-His-Leu hydrolysis.

In summary, we showed that serum ACE activity decreased significantly in nine healthy human subjects, but not in dogs, exposed to increased levels of $O_2$. At this time, the significance of this small change is uncertain. Results from six more subjects will be obtained when the hyperbaric air exposures pertaining to this study are completed, and these results will be correlated with other measurements of lung function.

## Acknowledgments

The authors wish to thank Dr. K. K. Kumaroo for many helpful discussions and Ms. S. K. Nagle and Ms. K. A. Chenevert for excellent technical assistance. Ms. Maureen Miller, Mrs. Ellen Hughes, and Ms. Janet Gaines graciously provided editorial services.

This work was supported by NMRDC Task No. M0099PN.010.0010. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting views of the Navy Department or the Naval Service at large. The experiments supported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animals Resources, National Research Council, DHEW, Pub. No. (NIH) 78–23.

## References

1. Caldwell PRB, Seegal BC, Hsiu KC, Das M, Soffer RL. Angiotensin-converting enzyme: Vascular endothelial localization. Science 1976;191:1050–1051.
2. Das M, Soffer RL. Pulmonary angiotensin-converting enzyme antienzyme antibody. Biochemistry 1976;15:5088–5094.
3. Lieberman J. Elevation of serum angiotensin-converting enzyme (ACE) level in sarcoidosis. Am J Med 1975;59:365–372.
4. Newman RA, Kimberly PJ, Stewart JA, Kelley J. Assessment of bleomycin lung toxicity using angiotensin-converting-enzyme in pulmonary lavage. Cancer Res 1980;40:3621–3626.
5. Hollinger MA, Patwell SW, Zuckerman JE, Gorin AB, Parsons G, Giri S. Effect of paraquat on serum angiotensin converting enzyme. Am Rev Resp Dis 1980;121:795–798.
6. Hollinger MA, Giri SN, Patwell S, Zuckerman JE, Gorin A, Parsons G. Effect of acute lung injury on angiotensin converting enzyme in serum, lung lavage, and effusate. Am Rev Resp Dis 1980;121:373–376.
7. Mattoli L, Zakheim RM, Mullis K, Molteni A. Angiotensin-I-converting enzyme activity in ideopathic respiratory distress syndrome of the newborn infant and in experimental alveolar hypoxia in mice. J. Pediatr 1975;87:97–100.
8. Bedrossian CW, Woo J, Miller WC, Cannon DC. Decreased angiotensin-converting enzyme in the adult respiratory distress syndrome. Am J Clin Pathol 1978;70:244–247.
9. Casey L, Krieger B, Kohler J, Rice C, Oparil S, Szidon P. Decreased serum angiotensin-converting enzyme in adult respiratory distress syndrome associated with sepsis: A preliminary report. Critical Care Medicine 1981;9:651–654.
10. Fourrier F, Chopin C, Wallaert B, Wattre P, Mangalaboyi J, Durocher A, Dubois D, Wattel F. Angiotensin-converting enzyme in human adult respiratory distress syndrome. Chest 1983;83:593–597.

11. Rohrbach MS. [Glycine-1-$^{14}$C]Hippuryl-L-Histidyl-L-Leucine: A substrate for the radiochemical assay of angiotensin converting enzyme. Anal Biochem 1978;84:272–276.
12. Kirk RE. Experimental design: procedures for the behavioral sciences. Belmont, CA: Brooks/Cole Publishing Co., 1968;94–95:132–139.
13. Clark JM, Lambertsen CJ. Pulmonary oxygen toxicity: A review. Pharmacol Rev 1971;23:37–133.
14. Rinaldo JE, Rogers RM. Adult respiratory-distress syndrome; changing concepts of lung injury and repair. N Engl J Med 1982;306:900–909.
15. Fox RB, Hordal JR, Brown DM, Repine JE. Pulmonary inflammation due to oxygen toxicity: Involvement of chemotactic factors and polymorphonuclear leukocytes. Am Rev Respir Dis 1981;123:521–523.
16. Hollinger, MA. Serum angiotensin-converting enzyme: status report on its diagnostic significance in pulmonary disease. Chest 1983;83:589–590.
17. Conroy JM, Hartley JL, Soffer RL. Canine pulmonary angiotensin-converting enzyme. Physicochemical, catalytic and immunological properties. Biochem Biophys Acta 1978;524:403–412.

# THE ENDOTOXIN-PRETREATED, $O_2$-ADAPTED RAT MODEL IN 2.0 and 4.0 ATA $O_2$

*R. Jackson*

The interest in oxygen demonstrated at previous Symposia reflects the dilemma central to its use: that its biologic necessity and operational utility are accompanied by toxicity directly related to the inspired partial pressure. Levels of oxygen as low as 0.6 ATA have been shown to deleteriously affect pulmonary function in rats (1). The problem of oxygen-induced worsening of respiratory failure is sometimes observed in clinical medicine, where the inspired oxygen may be 100% at sea level. In diving, both decompression and therapy of gas lesion diseases may require oxygen breathing at up to 2.8 ATA. It is now clear that a means to extend oxygen tolerance in humans would make decompression and therapy more efficient. Such means cannot be found in an optimal way empirically; it will require an understanding of the mechanisms by which oxygen affects molecular and cellular systems.

A mechanism by which oxygen-free radicals might damage tissues in hyperoxic environments was postulated by Gerschman (2), and the discovery of the superoxide dismutases by McCord and Fridovitch (3) is taken as evidence that organisms have evolved mechanisms to "detoxify" superoxide anions and their potential reaction products (4). Others have extended the concept of specific cellular defense mechanisms against oxidizing chemical species to include with the superoxide dismutases; enzymes of glutathione metabolism, glucose 6-phosphate dehydrogenase, and the catalases (collectively sometimes referred to as *antioxidant enzymes* (5,6,7).

Frank and Roberts found that rats pretreated with small amounts of gram-negative bacterial lipopolysaccharide (endotoxin) before exposure to >95% $O_2$ at 1.0 ATA develop resistance to pulmonary oxygen poisoning characterized by improved survival and decreased lung injury (8). These changes were associated with increased activities of total superoxide dismutase (SOD), glu-

tathione peroxidase (GP), glucose 6-phosphate dehydrogenase (G6-PD), and catalase (CAT) in whole lung homogenates (8).

These studies were reviewed in detail by Frank et al. in the previous *Proceedings* (9). In addition to the protection from death afforded by endotoxin, endotoxin-pretreated rats surviving hyperoxia for 7 days had less lung fibrosis (after a 6-week recovery period in air) than did untreated rats which also survived the 7-day period in oxygen (9). Such studies were limited to normobaric hyperoxia, and the effect of the same endotoxin-pretreatment on rats in higher partial pressures of oxygen was not investigated.

Based on these observations we designed a study to examine the behavior of this uniquely *oxygen-tolerant* rat model at 2.0 ATA $O_2$, where oxygen-induced lung damage occurs in the absence of seizures, and at 4.0 ATA $O_2$, where there are overt manifestations of both lung and central nervous system toxicity. We found that rats pretreated with endotoxin before pre-exposure to 1.0 ATA $O_2$ for 72 h did *not* have improved survival during subsequent hyperbaric hyperoxia, despite significantly increased activities of lung SOD, GP, and CAT. Even before the hyperbaric oxygen exposure, lungs from these rats had slightly increased wet-weight to dry-weight ratios, indicating that limited but detectable lung injury had occurred during the normobaric oxygen pre-exposure despite the administration of endotoxin.

## METHODS

### Experimental Animals

The animals used in this study were adult male Sprague-Dawley albino rats (strain Crl: CD (SD)BR; Charles River Laboratories, Wilmington, MA), which had been raised under *pathogen-free* conditions. They weighed between 250–350g and were approximately 70 days old.

### Endotoxin-Pretreatment Schedule

Experimental rats received a single intraperitoneal (i.p.) injection of phenol-extracted endotoxin (E) (Sigma Chemical Co., St. Louis, MO) dissolved in phosphate-buffered saline (100 μg E/mL PBS) in a dose of 500 μg/kg immediately before exposure to oxygen. Control rats received a single concurrent 2.0 mL/kg i.p. injection of buffered saline.

### Oxygen Exposures

Those rats used as controls were exposed only to air under a laminar-flow hood. Exposures to $O_2$ at 1.0 ATA took place in plexiglass chambers designed to maintain the desired $O_2$ concentration while allowing manipulation of the animals and access to the interior of the chamber. The interior [$O_2$] was kept >97% and was continuously monitored (Servomex Oxygen Analyser, Ser-

vomex Controls Ltd., Sussex, England). The [$CO_2$] was similarly monitored (Spino Model LB-1, Beckman Instruments, Fullerton, CA) and was maintained less than 0.2% by recirculation of chamber gas over $CO_2$ absorbent (Sodasorb, W. R. Grace and Co., Lexington, MA). The gas also circulated over a cooling coil to dehumidify it and to keep the chamber's internal temperature less than 27°C. The 2.0 and 4.0 ATA $O_2$ exposures took place in a steel pressure vessel (Piersol-Pine Mfg. Co., Oaks, PA) loaded with $CO_2$ absorbent and flushed continuously with $O_2$ to maintain the [$O_2$] >95%, [$CO_2$] <0.5%, and the T <27°C.

## Exposure Schedules

Groups of 8–20 experimental rats received 500 μg/kg endotoxin i.p. immediately before being placed in the 1.0 ATA $O_2$ plexiglass chambers, where they were kept continuously in >97% $O_2$ for 72 h. Any deaths were recorded. At the end of 72 h in 1.0 ATA $O_2$, groups of rats (along with concurrent age and weight-matched, air-exposed controls) were sacrificed for determination of their lung antioxidant enzyme activities or were then placed directly in 2.0 or 4.0 ATA $O_2$ for the successive experiments. Those rats placed in 2.0 or 4.0 ATA $O_2$ were either kept there until death for determination of their survival times, or they were removed after 1 h in 4.0 ATA $O_2$ or 4 h in 2.0 ATA $O_2$ (times chosen arbitrarily, but before any deaths) and then sacrificed for determination of the lung antioxidant enzyme activities. Two separate groups of rats were taken from air, given 500 μg/kg endotoxin i.p., and placed directly into 2.0 or 4.0 ATA $O_2$ for determination of their survival times, since this pretreatment alone would result in prolongation of survival in 1.0 ATA $O_2$ (8).

## Biochemical Studies

Rats used for biochemical measurements were anesthetized with 50 mg/kg pentobarbitol i.p. and then sacrificed by severing the abdominal aorta and vena cava. Their lungs were perfused free of blood with 0.1 M potassium phosphate-0.15 M potassium chloride buffer (pH 7.4, 1°–2°C). removed and homogenized (1:15 wt:vol) in 0.005 M potassium phosphate buffer (pH 7.8, 1°–2°C) using a Brinkmann Polytron (Brinkmann Instruments, Westbury, NY) at high speed. Lung homogenates were centrifuged for 10 min at 1000 xg for SOD measurements, and 15,000 xg for GP and CAT measurements. Enzyme activity was determined in the cell-free supernatant. Total (CuZn and Mn) SOD activity at pH 7.8 was measured by following the rate of reduction of cytochrome C in the presence of a xanthine-xanthine oxidase superoxide generating system as described by McCord and coworkers (3,10). Glutathione peroxidase activity was measured following the rate of oxidation of NADPH in the presence of t-butyl-hydroperoxide (cumene hydroperoxide) and glutathione reductase (11). Catalase activity was measured by following the rate of disappearance of hydrogen peroxide in the presence of lung homogenate supernatant

(12). A concurrently sacrificed group of air control rats were used for all assays.

Wet-weight to dry-weight ratios of a small piece of lung tissue (the right middle lobe) from rats of each experimental and control group were measured. The tissue was weighed before and after oven-drying to constant weight at 80°C for 72 h.

### Data Analysis

Survival data were plotted on log-probability paper. The median lethal time ($LT_{50}$) in oxygen was calculated according to the method of Litchfield and Wilcoxon (13); $LT_{50}$'s were then compared by chi-squared analysis. Lung antioxidant enzyme activities and wet- to dry-weight ratios were analyzed as follows: each experimental group had an identically processed, matched control group. The control groups were tested by one-way analysis of variance (ANOVA) and found not to differ by the *F*-test. The controls were then pooled as a single mean, and each experimental group was compared to the control group by ANOVA followed by Dunnett's *t*-statistic for multiple comparisons (14).

## RESULTS

As previously reported by others, those rats pretreated with 500 μg/kg endotoxin had significantly longer survival in 1.0 ATA $O_2$ than did saline-pretreated controls (12 of 12 vs. 4 of 12 alive after 96 h, $P < 0.05$). When those endotoxin-pretreated rats which had survived 72 h of oxygen at 1.0 ATA were then exposed to 2.0 or 4.0 ATA $O_2$, there was no significant improvement of their survival compared to that of controls placed directly in hyperbaric hyperoxia. In 4.0 ATA $O_2$ such rats had a median lethal time that was significantly shorter than that of controls (1.8 h [1.2–2.6 h] vs. 5.9 h [5.2 h–6.7 h]; $LT_{50}$ [95% confidence limits], $P < 0.05$).

Endotoxin pretreatment immediately before exposure to 2.0 or 4.0 ATA $O_2$ (with no pre-exposure to 1.0 ATA $O_2$) did not significantly augment survival compared to controls. While in 4.0 ATA $O_2$, both endotoxin-pretreated 1.0 ATA $O_2$-preexposed and endotoxin-pretreated rats placed immediately in this high partial pressure of oxygen developed generalized convulsions sooner than did saline-pretreated controls (unpublished data). The survival data are summarized in Fig. 1.

### Lung Antioxidant Enzyme Activities

Our results confirmed that the endotoxin-pretreated 1.0 ATA $O_2$-preexposed rats (representative of those subsequently placed in 2.0 or 4.0 ATA $O_2$) had significantly increased activities of total SOD (+43%), GP (+27%), and CAT (+37%) in homogenates of whole lungs. Those groups sampled

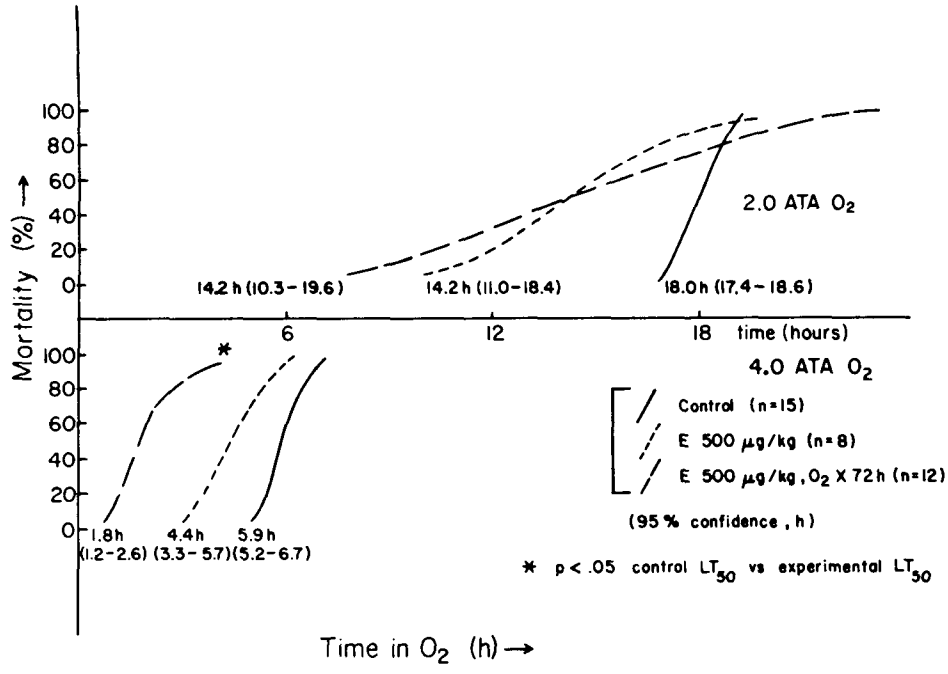



Fig. 1. Mortality rates of control, endotoxin-pretreated (E 500 µg/kg) and endotoxin-pretreated, normobaric oxygen-preexposed (E 500 µg/kg, $O_2$ × 72 h) rats in 2.0 ATA *(upper panel)* and 4.0 ATA *(lower panel)* oxygen. The median lethal times ($LT_{50}$) and 95% confidence limits *(in parentheses)* are displayed at the base of each curve. Although rats from both experimental groups began to die sooner than controls in 2.0 ATA $O_2$, there were no significant differences among their median lethal times. In 4.0 ATA $O_2$ the endotoxin-pretreated, $O_2$-preexposed rats had a significantly shorter survival time than did the other groups.

during the subsequent 2.0 or 4.0 ATA $O_2$ exposures retained the similar degrees of elevation of these enzyme activities. These data are summarized in Table I.

## Lung Water

Lung water content as estimated by the ratio of wet weight to dry weight of the right middle lobe was slightly increased over control in the endotoxin-pretreated rats after 72 h in 1.0 ATA $O_2$ (+17%), and was found to be similarly increased in rats sampled during subsequent exposure to 2.0 ATA $O_2$ (+17%) or 4.0 ATA $O_2$ (+12%)(Table I).

This accumulation of lung fluid was slightly greater than that previously reported for endotoxin-pretreated rats after 72 h in oxygen; however, the difference was small (17% greater than control in our study vs. 7% over control reported by Frank et al.) (15). This difference may reflect the higher

TABLE I

Lung Antioxidant Enzyme Activities and Wet- to Dry-Weight Ratios in Control and Endotoxin-Pretreated, $O_2$-Exposed Rats*

| | Air Controls† | $O_2$ Exposure | | |
|---|---|---|---|---|
| | | 72 h, 1.0 ATA | 72 h, 1.0 ATA<br>4 h, 2.0 ATA | 72 h, 1.0 ATA<br>1 h, 4.0 ATA |
| *N* | 12 | 6 | 6 | 6 |
| Wet/Dry Weight | 4.96 ± 0.04 | 5.79 ± 0.07‡ | 5.79 ± 0.08‡ | 5.57 ± 0.11‡ |
| SOD, units/lung | 220 ± 11 | 315 ± 9‡ | 337 ± 12‡ | 351 ± 32‡ |
| GP, μmol NADPH/min/lung | 34 ± 1 | 43 ± 2§ | 51 ± 4 | 56 ± 8‡ |
| CAT, I.U./lung | 5779 ± 245 | 7564 ± 507‡ | 9789 ± 510‡ | 10743 ± 629‡ |

* Data are mean ± SE and are taken from homogenates of both lungs. † Controls pooled after ANOVA demonstrated no intergroup differences. ‡ $P < 0.05$, $O_2$-exposed vs. controls; ANOVA and Dunnett's *t* statistic. § $P < 0.05$, $O_2$-exposed vs. respective control group ($n = 4$), unpaired *t* test. ANOVA revealed no difference when this experimental group was compared to the pooled controls.

mean [$O_2$] in our exposure chambers (>97% vs. >95%) and the lack of daily interruptions of our exposures for animal maintenance.

## DISCUSSION

When rats are pretreated with 500 μg/kg endotoxin and then placed in >95% $O_2$ at 1.0 ATA, almost all survive well beyond the median lethal time of non-pretreated controls (8,9). We found that this striking degree of resistance to hyperoxia did *not* occur when such endotoxin-pretreated rats were exposed to 2.0 or 4.0 ATA $O_2$ (after surviving 72 h in 1.0 ATA $O_2$), nor did it occur when rats were endotoxin-pretreated immediately befor exposure to 2.0 or 4.0 ATA $O_2$. Thus, in contrast to the finding in normobaric hyperoxia, there is *no* "protective" effect of endotoxin in these higher than atmospheric pressures of oxygen. This result was of interest, because the mechanisms resulting in pulmonary oxygen poisoning are presumed to be similar during normobaric and hyperbaric hyperoxia.

One interpretation of available data regarding the development of resistance to the toxic effects of oxygen (and other oxidants) is that enzymes capable of catalyzing the metabolism of superoxide anions and peroxides in effect protect lung tissue from cellular damage during exposure to oxidizing substrates (5,6,7,15). Although currently available data regarding resistance to normobaric hyperoxia are consistent with this postulate (16), we found no improvement in the survival rate of rats exposed to 2.0 or 4.0 ATA $O_2$ despite their having significant increases in lung SOD, GP, and CAT activities at the beginning of and during hyperbaric hyperoxia. However, cofactor depletion

(e.g., GSH, NADPH) may not be a limiting factor in 1.0 ATA $O_2$, but may become a determinant of cell function in higher partial pressures of oxygen where such depletion could impair the catalytic activity of otherwise functional enzyme systems (17). While the initial manifestations of oxygen poisoning at 2.0 ATA involve physiologic impairment and cellular damage of the lung, other organ systems, most notably the central nervous system, are eventually presumed to be affected by hyperbaric hyperoxia (18).

Kravetz and coworkers have previously shown that rats exposed to 0.8 ATA $O_2$ for 7 days are not subsequently tolerant to 2.0 ATA $O_2$, but do survive longer in 1.5 ATA $O_2$ than controls (19). Such rats were shown to survive for 1 week in 1.0 ATA $O_2$ and were thus considered *oxygen-adapted.* The authors did not report lung activities of SOD or other antioxidant enzymes. In a more analogous study of the effect of endotoxin on tolerance to hyperoxia, Akers and coworkers found no improvement of survival when endotoxin-pretreated rats or guinea pigs were exposed directly to 1.5 ATA $O_2$; they also estimated an increase in alveolar type II cell number in guinea pigs pretreated with 500 μg/kg endotoxin and exposed only to air (20). Thus our data are consistent with those of other investigators, a fact suggesting that tolerance to 1.0 ATA $O_2$ does not necessarily lead to improved survival in hyperbaric hyperoxia.

Models of adaptation to hyperoxia at 1.0 ATA (normobaric) commonly depend on either the administration of a chemical (e.g., alphanapthylthiourea) compound, which damages endothelial or alveolar lining cells (21), or pre-exposure to sublethal levels (0.80–0.85 ATA) of oxygen (22) or another oxidant (23). This form of sublethal lung injury typically leads to proliferation of alveolar type II cells (granular pneumocyte) and nonciliated terminal bronchiolar epithelial cells (22,24); such alveolar type II cells have been shown by Forman and othes to be rich in MnSOD and other antioxidant enzymes (25). Circulating endotoxin in the syndrome of septic shock is known to be injurious to the lung; although the dose of endotoxin we administered was small (1–2% of the $LD_{50}$ for rats), it could in association with hyperoxia for 72 h be sufficient to stimulate the adaptive-reparative cycle of cell changes thought to contribute to the lung's resistance to high oxygen tensions (26). In addition, the metabolic stimulatory effects of endotoxin (9) might result in an increase in rates of protein synthesis leading indirectly to accumulation of lung antioxidant enzymes in hyperoxic environments. The rate at which non-pretreated rats in hyperoxia develop increases in lung antioxidant enzyme activities is thought by Deneke and Fanburg (27) to be one determinant of survival.

Despite the overall improvement in survival of the endotoxin-pretreated rats after 72 h of 1.0 ATA $O_2$, we found some evidence of pulmonary edema. It is not known whether the small increase in lung fluid we observed correlated with ultrastructural evidence of endothelial cell damage. Furthermore, it is not known whether the number or distribution of alveolar type II cells changed during the 72 h exposure to oxygen in the lungs of the endotoxin-pretreated rats.

In conclusion, our studies demonstrate no protective effect of endotoxin when administered to rats immediately before exposure to 2.0 or 4.0 ATA $O_2$. Nor could we demonstrate an improvement in survival compared to controls when rats were pretreated with endotoxin immediately before a pre-exposure period in normobaric hyperoxia, despite their having significantly increased lung activities of the "protective" antioxidant enzymes SOD, GP, and CAT. Thus, while animal models of adaptation of oxygen at 1.0 ATA are useful in experiments designed to investigate mechanisms of pulmonary oxygen poisoning, such models may not be directly relevant to exposure to oxygen partial pressures of 2.0 ATA or greater. It is furthermore likely that mechanisms other than those described here are critical to survival in high oxygen tensions. While efforts to understand the mechanism by which oxygen injures the lung have been exhaustive, it is still not known with certainty which cellular functions are most specifically and critically altered by hyperoxia. In the absence of such knowledge, it has been impossible to date to develop a pharmacologic means to extend oxygen tolerance in humans. Thus, the operational extension of oxygen tolerance remains problematic.

## Acknowledgments

The author is indebted to Lee Frank, who inspired this study. Partial support came from Program Project Grant HL-08899-18, "Adaptations and Deteriorations Induced by Respiratory Environments."

## References

1. Hayatdavoudi G, O'Neil JJ, Barry BE, Freeman BA, Crapo JD. Pulmonary injury in rats following continuous exposure to 60% $O_2$ for 7 days. J Appl Physiol 1981;51:1220–1231.
2. Gerschman R, Gilbert DL, Nye SW, Dwyer P, Fenn WO. Oxygen poisoning and x-irradiation: a mechanism in common. Science 1954;119:623–626.
3. McCord JM, Fridovitch I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969;244:6049–6055.
4. Fridovitch I. The biology of oxygen radicals. Science 1978;201:875–880.
5. Chow CK, Tappel AL. An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone-exposed rats. Lipids 1972;7:518–524.
6. Kimball RE, Reddy K, Pierce TH, Schwartz LW, Mustafa MG, Cross CE. Oxygen toxicity: Augmentation of antioxidant defense mechanisms in rat lung. Am J Physiol 1976;230:1425–1431.
7. Tierney D, Ayers L, Herzog S, Yang J. Pentose pathway and production of reduced nicotinamide adenine dinucleotide phosphate. A mechanism that may protect lungs from oxidants. Am Rev. Resp Dis 1973;108:1348–1351.
8. Frank L, Roberts RJ. The role of endotoxin in protection of adult rats from oxygen-induced toxicity. J Clin Invest 1978;61:269–275.
9. Frank L, Chiang MJ, Massaro D. Protection from pulmonary oxygen toxicity by treatment with low doses of bacterial endotoxin. In Bachrach AJ, Matzen MM, eds., Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:65–74.
10. Crapo JD, McCord JM, Fridovitch I. Preparation and assay of superoxide dismutases. In Fletcher S, Packer L, eds., Methods in enzymology, Vol. LIII Biomembranes, Part D: Biological oxidations, mitochondrial and microbial systems. New York: Academic Press, 1978:382–393.

11. Puglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158–169.
12. Holmes RS, Masters CJ. Epigenetic interconversion of the multiple forms of mouse liver catalase. FEBS Letters 1970;11:45–48.
13. Litchfield JT, Wilcoxon F. A simplified method of evaluating dose effect experiments. J Pharmacol Exp Ther 1949;96:99–113.
14. Dunnett CW. New tables for comparison with a control. Biometrics 1964;20:482–491.
15. Frank L, Summerville JS, Massaro D. Protection from oxygen toxicity with endotoxin. Role of the endogenous antioxidant enzymes of the lung. J Clin Invest 1980;65:1104–1110.
16. Deneke SM, Fanburg BL. Normobaric oxygen toxicity of the lung. N Engl J Med 1980;303:76–86.
17. Haugaard N. Poisoning of cellular reactions by oxygen. Ann NY Acad Sci 1965;117:736–744.
18. Lambertsen CJ. Effects of hyperoxia on organs and their tissues. In Robin ED, ed., Extrapulmonary manifestations of respiratory disease. New York: Marcel Dekker, 1978:239–303.
19. Kravetz G, Fisher AB, Formon HJ. The oxygen-adapted rat model: Tolerance to oxygen at 1.5 and 2.0 ATA. Aviat Space Envir Med 1980;51:775–777.
20. Akers TK, Nichols G, Winsborough MM, Rowles PM. Synergism of endotoxin and oxygen exposure to produce lung damage. IRCS Medical Science 1982;10:15–16.
21. Tierney DF, Ayers L, Kasayuma RS. Altered sensitivity to oxygen toxicity. Am Rev Resp Dis (Suppl 2) 1977;115:59–65.
22. Crapo JD, Barry BE, Foscue HA, Shelburne J. Structural and biochemical changes in rat lungs occurring during exposures to lethal and adaptive doses of oxygen. Am Rev Resp Dis 1980;112:123–143.
23. Jackson RM, Frank L. Ozone-induced tolerance to hyperoxia in rats. Am Rev Resp Dis (in press).
24. Lum H, Schwartz LW, Dungworth DL, Tyler WS. A comparative study of cell renewal after exposure to ozone or oxygen. Am Rev Resp Dis 1978;118:335–345.
25. Forman HJ, Fisher AB, Antioxidant enzymes of rat granular pneumocytes. Constitutive levels and effect of hyperoxia. Lab Invest 1981;45:1–6.
26. Stephens RJ, Sloan MF, Evans MJ, et al. Early response of lung to low levels of ozone. Am J Pathol 1974;74:31–59.
27. Deneke SM, Fanburg BL. Involvement of glutathione enzymes in $O_2$ tolerance development by diethyldithiocarbamate. Biochem Pharmacol 1980;71:1367–1373.

# ALTERATIONS IN RAT LOCAL BRAIN AND SPINAL CORD GLUCOSE UTILIZATION DURING 30- TO 60-MINUTE EXPOSURE TO 2 AND 3 ATA $O_2$ AND NORMOXIC $N_2$-$O_2$ AT 3 ATA

*D. Torbati and C. J. Lambertsen*

The "asymptomatic" period of exposure at different oxygen pressures in rats is characterized by the absence of changes in electrocorticogram, electrocardiogram, respiratory rate, plasma glucose concentration and signs of nervous or respiratory distress (1–3). The regional cerebral metabolic rates for glucose (rCMRgl), have been autoradiographically measured in awake unrestrained rats during various periods of development of brain oxygen toxicity at 5 ATA $O_2$, as well as during asymptomatic periods of exposure at 1, 2, 3 and 5 ATA $O_2$ (4–6). Statistically significant increases in rCMRgl were observed in the majority of investigated brain structures during the asymptomatic first 30-min period of exposure to 5 ATA $O_2$ (4). The results during asymptomatic periods of 6-, 4-, and 2-h exposures at 1, 2, and 3 ATA $O_2$, respectively, showed that 51% of the investigated structures had an average of between 10 and 20% increase in rCMRgl without concomitant reduction in any other structure (5). Statistically significant increases during these exposures were also seen in lateral thalamus nuclei at 3 ATA $O_2$, in superior olivary nucleus and inferior colliculus at 2 ATA $O_2$, and in superior olivary nucleus at 1 ATA $O_2$. The elevated rCMRgl during those prolonged and asymptomatic periods of exposure could be either a physiological or pathological effect of hyperbaric oxygen (HBO). To investigate the development of the changes observed in prolonged oxygen breathing, we have measured rCMRgl during brief (30 to 60 min) exposures to 2 and 3 ATA $O_2$, as well as during an equivalent period of exposure to normoxic $N_2$-$O_2$ at 3 ATA.

## METHOD

The rCMRgl of male, Wistar albino rats weighing 220–330 g was autoradiographically measured in 26 brain structures and in gray and white matter of thoracic and lumbar spinal cord using $^{14}$C-2-Deoxyglucose (2-DG) as a tracer. For rCMRgl measurement, one femoral artery and vein were cannulated 3 to 4 days before the exposures. The rats were divided into four groups:

Group I: Air breathing at atmospheric pressure (14 rats).

Group II: During 30- to 60-min exposure to 2 ATA $O_2$ (12 rats).

Group III: During 30- to 60-min exposure to 3 ATA $O_2$ (12 rats).

Group IV: During 30- to 60-min exposure to normoxic $N_2$-$O_2$ at 3 ATA (14 rats).

The theoretical basis for the 2-DG autoradiographic technique has been extensively documented (7,8) and the experimental procedures used in this study during HBO were the same as previously described (4). On the day of the experiment, after the rat was placed in a pressure chamber, the respiratory rate was counted every 20 min and throughout the hyperbaric exposures. An arterial blood sample for determination of pre-exposure value of blood glucose concentration was taken from each rat during air breathing. During ongoing HBO or air exposures the 2-DG was intravenously injected (12 μc/100 g), while the rats were awake and moving freely inside the cage. Thirty minutes after the 2-DG injection, the rats were sacrificed by intravenous infusion of a lethal dose of pentobarbital. Following rapid decompression, the brain and a piece of thoracic and lumbar spinal cord were quickly removed and treated for autoradiography according to our previous protocol (4). The statistical significance of the results was evaluated by *t*-test (comparison between two means, control group vs. an experimental group).

## RESULTS

The regional brain and spinal cord glucose utilization rates in four investigated groups are presented in Table I. The relative changes as expressed by percentage difference in rCMRgl at three different exposures are illustrated in Figs. 1–3.

These data demonstrate that 23 out of 28 of the investigated structures at 2 ATA $O_2$ and 16 out of the 28 at 3 ATA $O_2$ had an average of between 10 and 34% increase in glucose utilization (Figs. 1 and 2). The increases were statistically significant in 14 out of 28 of the investigated structures at 2 ATA $O_2$ and only in 5 out of 28 at 3 ATA $O_2$, while no statistically significant changes were observed during 1 h at 3 ATA normoxic pressure (Table I). No difference was observed between pre-exposure value of plasma glucose concentration and exposure value. The respiratory rate remained stable throughout these short exposures.

TABLE I

Alterations in Regional Cerebral Metabolic Rate for Glucose during Exposure of 30–60 min to 2 and 3 ATA $O_2$ and 3 ATA Normoxic Pressure in Awake, Unrestrained Rats

| Structures | rCMRgl μm/100g/min ± SD | | | |
|---|---|---|---|---|
| | Air Breathing | 2 ATA $O_2$ (12 rats) | 3 ATA $O_2$ (12 rats) | 3 ATA Normoxia (14 rats) |
| Cortex | | | | |
| Sensory, motor, visual, olfactory | 115 ± 34 | 113 ± 17* | 126 ± 20 | 128 ± 23 |
| auditory | 113 ± 28 | 123 ± 18 | 115 ± 19 | 127 ± 20 |
| corpus callosum | 39 ± 12 | 42 ± 9 | 39 ± 6 | 46 ± 9 |
| Subcortex | | | | |
| hypothalamus | 73 ± 15 | 83 ± 11 | 75 ± 14 | 72 ± 17 |
| hippocampus | 93 ± 25 | 107 ± 12* | 97 ± 14 | 100 ± 19 |
| septum | 62 ± 21 | 73 ± 13 | 67 ± 12 | 68 ± 16 |
| amygdala | 96 ± 19 | 114 ± 21* | 105 ± 17 | 98 ± 19 |
| caudato-putamen | 113 ± 29 | 131 ± 19 | 121 ± 22 | 128 ± 27 |
| thalamus: | | | | |
| lateral nucleus | 122 ± 32 | 137 ± 14 | 140 ± 22 | 132 ± 25 |
| ventral nucleus | 117 ± 27 | 134 ± 18 | 128 ± 19 | 123 ± 21 |
| globus pallidus | 65 ± 18 | 78 ± 10* | 70 ± 13 | 67 ± 17 |
| mammillary body | 142 ± 31 | 164 ± 21* | 116 ± 23† | 144 ± 29 |
| geniculate body: | | | | |
| lateral | 100 ± 25 | 116 ± 16* | 111 ± 18 | 104 ± 18 |
| medial | 127 ± 25 | 134 ± 12 | 130 ± 24 | 134 ± 25 |
| substantia nigra | 93 ± 17 | 121 ± 17† | 110 ± 22* | 94 ± 19 |
| nucleus accumbens | 97 ± 28 | 129 ± 20† | 114 ± 24 | 111 ± 22 |
| olfactory nucleus | 88 ± 25 | 100 ± 14 | 93 ± 19 | 103 ± 25 |
| Midbrain and Pons | | | | |
| inferior colliculus | 239 ± 52 | 296 ± 38† | 283 ± 42† | 219 ± 48 |
| superior colliculus | 113 ± 27 | 125 ± 19 | 121 ± 15 | 122 ± 22 |
| superior olivary nucleus | 222 ± 59 | 284 ± 27† | 273 ± 43† | 196 ± 46 |
| reticular formation | 84 ± 17 | 98 ± 14* | 93 ± 10 | 84 ± 16 |
| pontine gray matter | 84 ± 19 | 94 ± 13 | 94 ± 9 | 86 ± 13 |
| Medulla and Cerebellum | | | | |
| vestibular nuclei | 115 ± 27 | 126 ± 20 | 128 ± 13 | 116 ± 22 |
| cochlear nuclei | 96 ± 22 | 114 ± 17* | 111 ± 13 | 98 ± 19 |
| cerebellar cortex | 73 ± 18 | 82 ± 13 | 80 ± 12 | 74 ± 13 |
| cerebellar white matter | 46 ± 12 | 55 ± 11* | 56 ± 11† | 48 ± 10 |
| Spinal Cord | | | | |
| gray matter | 78 ± 24 | 93 ± 9† | 86 ± 14 | 92 ± 30 |
| white matter | 32 ± 10 | 34 ± 7 | 33 ± 8 | 38 ± 17 |

Significantly different * $P < 0.05$; † $P < 0.01$.

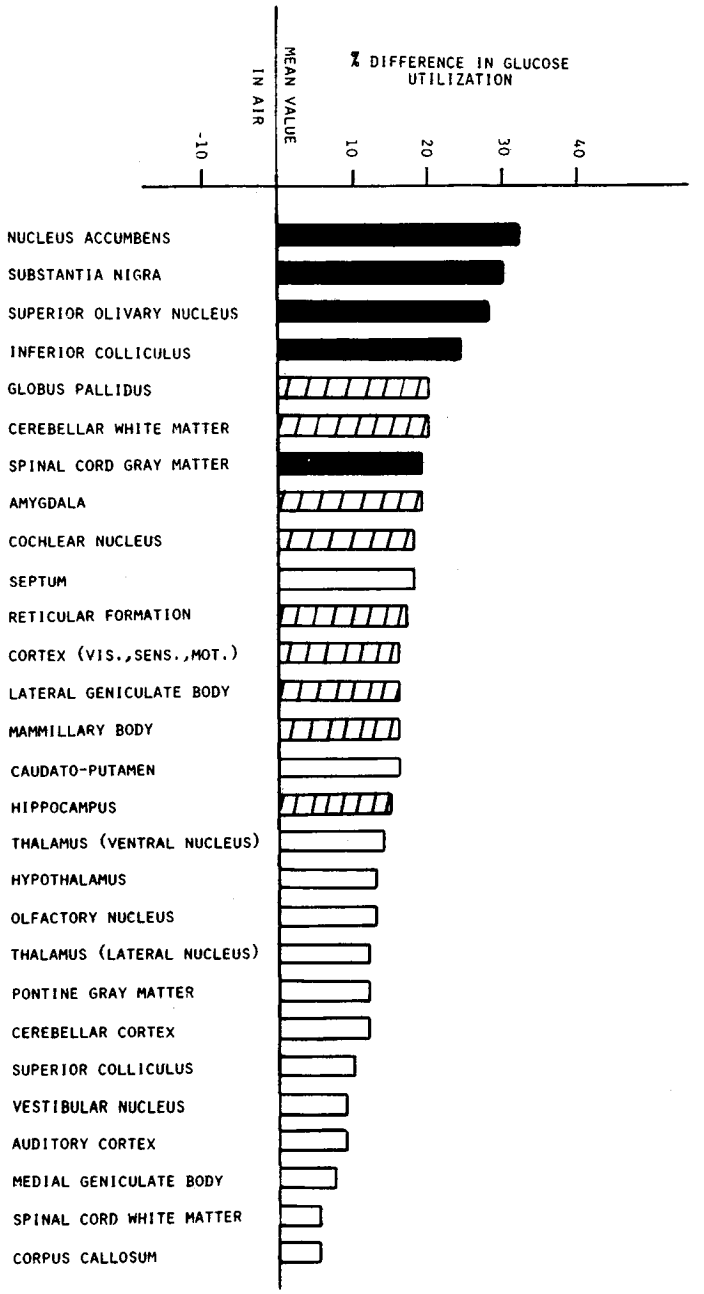



Fig. 1. The percentage difference in glucose utilization observed in each investigated neuronal structure during 30- to 60-min exposure to 2 ATA $O_2$. Significantly different: ▨ $P < 0.05$; ■ $P < 0.01$. The average values are arranged in descending order.

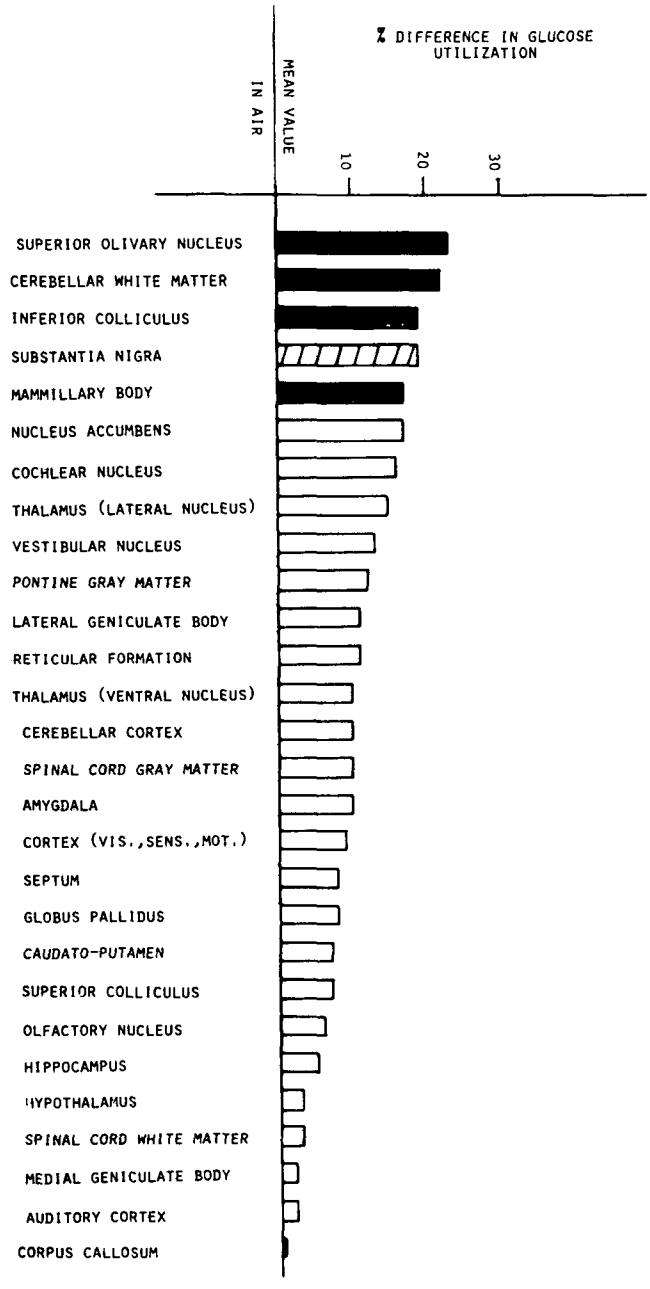



Fig. 2. The percentage difference in rCMRgl during 30- to 60-min exposure to 3 ATA $O_2$. Although changes in rCMRgl occurred in more structures at 2 ATA $O_2$ than in the group of rats exposed at 3 ATA $O_2$, the structures most affected are essentially the same at both pressures (e.g., inferior colliculus and superior olivary nucleus).

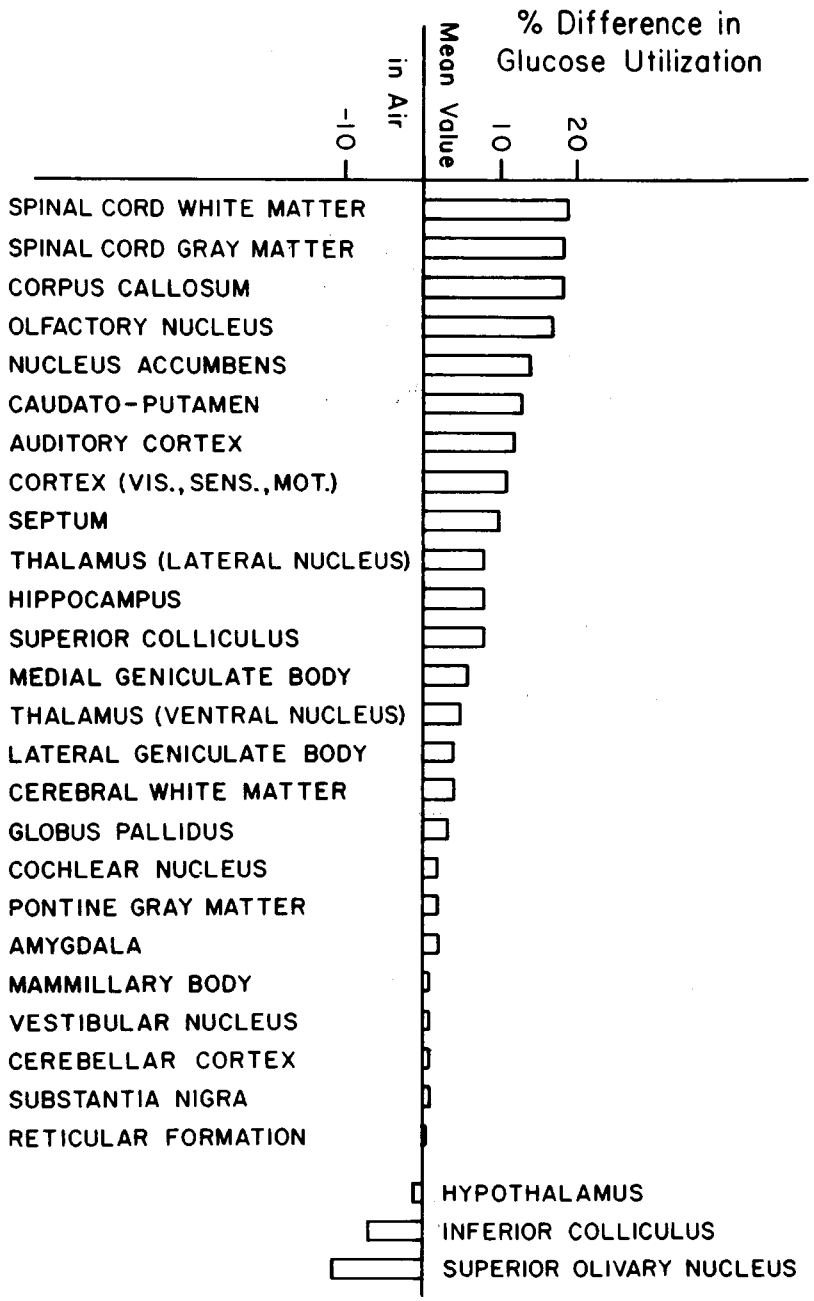



Fig. 3. The percentage difference in rCMRgl during 30- to 60-min exposure to normoxic $N_2$-$O_2$ at 3 ATA. Slight and insignificant average increases or decreases during this hyperbaric normoxic exposure are in opposite direction from those during HBO (as compared to Figs. 1 and 2). For example, inferior colliculus and superior olivary nucleus show an average negative value (as compared to control air breathing) while they showed the highest increases at 2 and 3 ATA $O_2$ (Figs. 1 and 2). On the other hand, spinal cord white matter and corpus callosum that had a normal value at 2 and 3 ATA $O_2$ (Figs. 1 and 2) achieved the highest average value at 3-ATA normoxia.

## DISCUSSION

The use of hyperbaric oxygen for treatment of central nervous system (CNS) decompression sickness and some cerebral disorders, such as ischemia, infarction, edema, injuries, and vascular diseases (9–11) requires a distinct differentiation between physiological and pathological CNS effects of HBO. This requires a continuous and correlated monitoring of various brain functions for different exposure durations at different oxygen pressures. Some physiological factors, such as cerebral electrical activity, cerebral tissue $P_{O_2}$, and cerebral blood flow have been extensively investigated in experimental animals (1,12–19). The brain energy metabolism in awake animals is among the important functions not adequately investigated at therapeutic oxygen pressures.

The variable effects upon rCMRgl in earlier studies during development of CNS oxygen toxicity and at prolonged asymptomatic exposures (4–6) led us to look for changes in shorter periods of exposure (5). The present data demonstrate an early increase in brain glucose utilization at pressures of 2 and 3 ATA $O_2$, pressures which are close to those used for therapeutic purposes in CNS decompression sickness. Comparison of these results with our previous data in exposures of 4 and 2 h to 2 and 3 ATA $O_2$, respectively, (5) indicates that early increase in glucose utilization during hyperoxia may be even greater than that observed in later stages of prolonged asymptomatic exposures. Furthermore, since the elevation in rCMRgl occurs during oxygen exposures which are known to be without visible or electroencephalographic effects in rats (2,3), these increases may be physiological responses to HBO, rather than toxic. Since in normal conditions glucose is the primary substrate of energy metabolism in neuronal elements (20), the elevations in glucose utilization, whether toxic or physiological, probably reflect an increase in brain energy metabolism. The physiological and clinical implications of the observed early and subsequent alterations of glucose utilization are beyond these experiments.

## Acknowledgments

The authors wish to thank Dr. Martin Reivich, Director of the Cerebrovascular Research Center, University of Pennsylvania School of Medicine, for providing the facilities required for autoradiographic determination of glucose utilization in this study. The technical assistance of Ms. Claire E. McCarthy and Mr. Peter Lo is gratefully acknowledged. This study was supported in part by NIH Grant HL-08899 and ONR Contract N00014-81-K-0404.

## References

1. Torbati D, Simon AJ, Ranade A. Frequency analysis of EEG in rats during the preconvulsive period of $O_2$ poisoning. Avait Space Environ Med 1981;52:598–603.

2. Simon AJ. Torbati D. Effects of hyperbaric oxygen on heart, brain, and lung functions in rat. Undersea Biomed Res 1982;9:263–245.
3. Torbati D, Torbati A. Alterations in arterial blood glucose concentration during asymptomatic exposure to hyperbaric oxygenation and during development of brain oxygen toxicity in awake unrestrained rats. Fed Proc 1983;42:1254.
4. Torbati D, Greenberg J. Lambertsen CJ. Correlation of brain glucose utilization and cortical electrical activity during development of brain oxygen toxicity. Brain Res 1983;262:267–273.
5. Torbati D, Lambertsen CJ, Greenberg J. Regional cerebral glucose utilization rates in rats during asymptomatic period of exposure to 1, 2, and 3 ATA $O_2$. Brain Res 1983;262:267–273.
6. Torbati D, Lambertsen CJ. Regional cerebral metabolic rate for glucose during oxygen-induced convulsions. Physiologist 1982;25(4):272 (Abstr.)
7. Sokoloff L, Reivich M, Kennedy C, et al. The $^{14}C$-deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure and normal values in the conscious and anesthetized albino rat. J Neurochem 1977;28:897–916.
8. Sokoloff L. The deoxyglucose method for the measurement of local glucose utilization and the mapping of local functional activity in the CNS. In: Smythies JR, Bradley RJ, eds. International review of neurobiology. Vol. 22. San Francisco: Academic Press, 1981:287–333.
9. Hayakawa T. Hyperbaric oxygen treatment in neurology and neurosurgery. TITJ Life Sci 1974;4:1–25.
10. Holbach KH, Wassman H. Neurological and EEG analytical findings in the treatment of cerebral infarction with repetitive hyperbaric oxygenation. In: Smith G, ed. Proceedings of the sixth international congress on hyperbaric medicine. Aberdeen: University Press, 1979:205–210.
11. Heyman A, Saltzman HA, Whalen RE. The use of hyperbaric oxygenation in the treatment of cerebral ischemia and infarction. Circulation 1966;33:20.
12. Rucci FS, Giretti ML, Laroca M. Changes in electrical activity of the cerebral cortex and of some subcortical centers in hyperbaric oxygen. EEG Clin Neurophysiol 1967;22:231–238.
13. Harel D, Kerem D, Lavy S. The influence of high oxygen pressure on the electrical activity of the brain. EEG Clin Neurophysiol 1969;26:310–317.
14. Torbati D, Parolla D, Lavy S. Changes in the electrical activity and $Po_2$ of the rats brain under high oxygen pressure. Exp Neurol 1976;50:439–447.
15. Torbati D, Parolla D, Lavy S. Changes in local brain tissue $Po_2$ and electrocortical activity of unanesthetized rabbit under high oxygen pressure. Aviat Space Environ Med 1977;48(4):347–350.
16. Bean JW. Cerebral $O_2$ in exposures to $O_2$ at atmospheric and higher pressure and influence of $CO_2$. Am J Physiol 1961;201:1192–1198.
17. Hunt RC, Blackburn JG, Ogilvie RW, Balentine JD. Oxygen tension in the globus pallidus and Neastriatum of unanesthetized rats during exposure to hyperbaric oxygen. Exp Neurol 1978;62:698–707.
18. Bean JR, Lignell J. Burgess W. Cerebral $O_2$, $CO_2$ regional cerebral vascular control, and hyperbaric oxygenation. J Appl Physiol 1972;32(5):650–657.
19. Torbati D, Parolla D, Lavy S. Blood flow in rat brain during exposure to high oxygen pressure. Aviat Space Environ Med 1978;49:963–967.
20. Sokoloff L. Circulation and energy metabolism of the brain. In: Siegel GJ, Albers RW, Katzman R, Agranat BW, eds. Basic neurochemistry. Boston: Little Brown, 1976:388–413.

# INVITED REVIEW: BRAIN OXYGEN TOXICITY

*A. Mayevsky*

The universality of oxygen toxicity is a well known and accepted phenomenon since the pioneering work done by Paul Bert and published in detail in 1878 (1). During the years, many review articles on "oxygen toxicity" have been published dealing with various aspects of the phenomenon (2–11). In 1982 Balentine published a book on the pathology of oxygen toxicity (12).

To avoid repetition, I will concentrate the current review mainly on the inter-relationship between brain energy metabolism, electrical activity, and ionic distribution as measured in situ in the awake animal exposed to hyperbaric oxygenation (HBO).

As an introduction to my review, I will describe the basic principles of the processes connected with energy metabolism taking place in a typical neuronal tissue. Figure 1 presents in a schematic way the main biochemical events occurring in the cerebral cortex. Production of adenosine triphosphate (ATP) is fully dependent upon normal blood flow leading to continuous supply of oxygen and availability of glucose. Glycolysis in the brain contributes only a very small fraction to the overall ATP production taking place mainly in the mitochondria. The major part of the ATP produced is utilized by the sodium potassium ATPase system keeping the normal distribution of ions around the cell membrane. Activation of energy utilization will cause oxidation of the mitochondrial NADH (13,14) to facilitate ATP production. Changes in oxygen delivery to the neurons induced by hypoxia or ischemia may affect energy availability so that pumping activity will be inhibited and as a result accumulation of $K^+$ in the extracellular space will occur (15). A high level of intracellular $P_{O_2}$ may affect various intracellular events such as glycolysis, mitochondrial activity, free radicals production, various cytosolic enzyme activity, or membrane integrity reflected by the ATPase activity (for details *see* Ref. 11). In my review, I will not discuss other neuronal activities such as

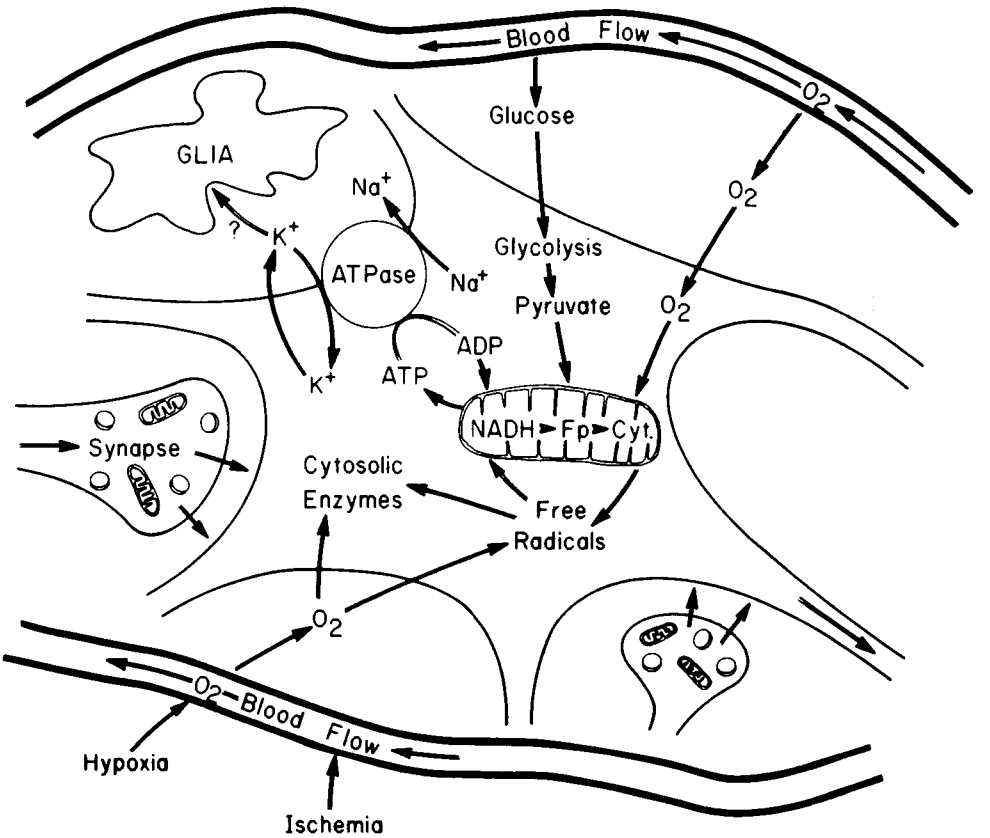



Fig. 1. Basic principles of energy-producing and -consuming systems taking place in a neuronal tissue under normoxic or hyperoxic conditions.

chemical transmission (synthesis, release, or activity), which are also correlated to HBO toxicity (10).

## RESPONSES OF BRAIN ENERGY METABOLISM TO HBO

Various aspects of brain energy metabolism were investigated under hyperbaric oxygenation conditions such as cerebral blood flow, tissue $P_{O_2}$ level and respiration, mitochondrial activity, concentration of high energy phosphate compounds, and glucose metabolism. In vitro systems were used in many of the studies; in others in vivo preparations were used.

The effects of HBO on cerebral blood flow depend upon the duration of exposure as well as the pressure level. Various investigators (16–19) described the responses and one can summarize them as follows: Under pressure of 3–6

ATA $O_2$ an initial decrease in blood flow was found due to the well-known vasoconstrictive autoregulation mechanism. As compression continued, this mechanism failed and an increase in blood flow occurred (vasodilation) before convulsions appeared. The vasoconstrictive response, which is probably a protective mechanism against HBO, may deteriorate as the toxicity processes are taking place. Tissue $Po_2$ measurements were performed by various investigators and all of them found increased levels due to HBO. The differences between the various experiments are in relation to the magnitude of the increased $Po_2$ (20–25).

Glucose metabolism under HBO was studied both in in vitro (26) and in vivo (27) conditions and some discrepancies between the results were documented (for details *see* Ref. 27). Inhibition of cellular respiration was suggested as a possible mechanism taking place under HBO conditions (for details *see* Refs. 11,12). Here, also, different results were described by various investigators. Sanders et al. (28) found a decrease in brain ATP before the onset of convulsion while Nolan and Faiman (29) were unable to confirm it. Recently Faiman et al. (30) re-evaluated the effects of HBO and were unable to change their original findings, namely, high energy phosphate levels did not fall before convulsions.

It is well accepted that the oxidation of pyridine nucleotides is the first biochemical change that takes places under HBO conditions. The pioneering work of Chance and collaborators (8) showed that various tissues or organs exposed to HBO in vitro as well as in vivo react in the same way, namely, NADH becomes more oxidized. The main questions are, What are the reasons for this oxidation, and How is this oxidation correlated to the toxicity processes developed under HBO? Using the fiber optic surface fluorometer, we exposed an awake brain to HBO and found also a clear oxidation of NADH (31). Later on, Hempel et al. were able to monitor the oxidation of cerebral cytochrome $aa_3$ under HBO (32). In 1979 Hempel (33) indicated an increase in intracellular $Po_2$ under HBO using pyrenbutynic acid (PBA).

## THE FUNCTIONING AWAKE BRAIN UNDER HBO

As stated before, my review will emphasize only a few aspects of brain activity, and they are shown in Fig. 2. Hyperbaric oxygenation may have a direct effect and may lead to either decrease or increase in energy availability. The data to be presented will not support a decrease, but rather will prove that energy metabolism is stimulated at least during the initial stage under HBO. Electrical activity could be affected directly or indirectly through the changes in extracellular $K^+$ levels. It will be shown that energy metabolism reflects secondary responses to primary change in either electrical activity or extracellular $K^+$ levels, or both.

For the convenience of my discussion, the various responses to hyperbaric oxygenation will be divided into three phases as shown in Fig. 3. During the first phase, intracellular and extracellular events may lead to the development

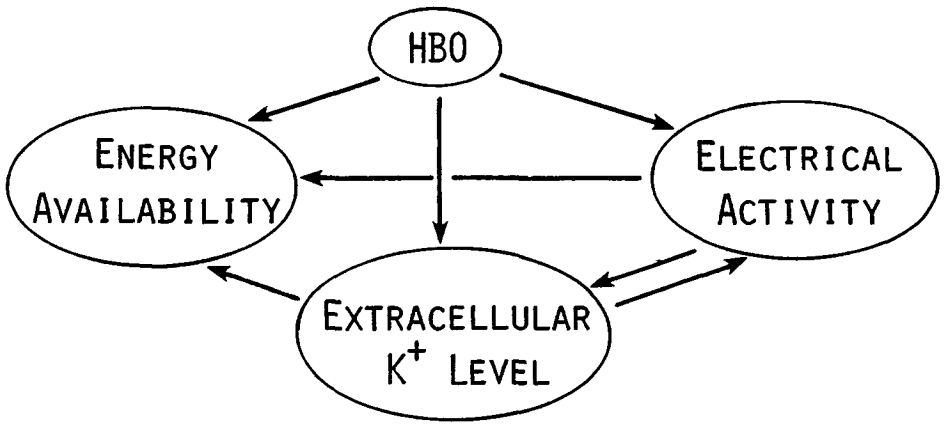



Fig. 2. Three possible brain activities suspected to be involved in the development of oxygen toxicity under hyperbaric oxygenation (HBO). The *arrows* represent the reviewer evaluation regarding the sequence of events.

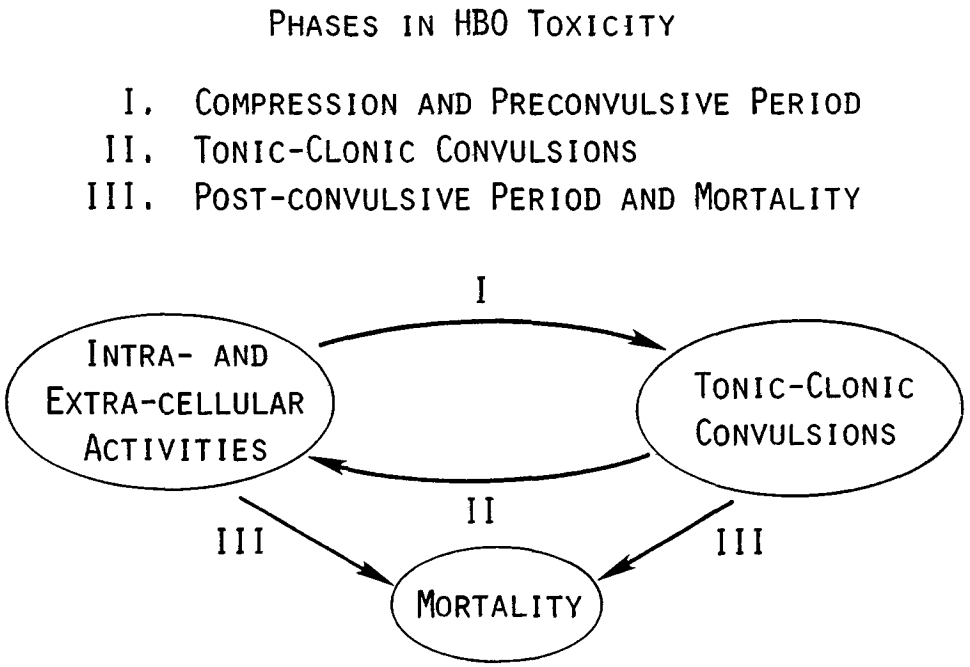



Fig. 3. Three major stages of oxygen toxicity developed under conditions in which brain toxicity is the dominant one (above 3 ATA $O_2$).

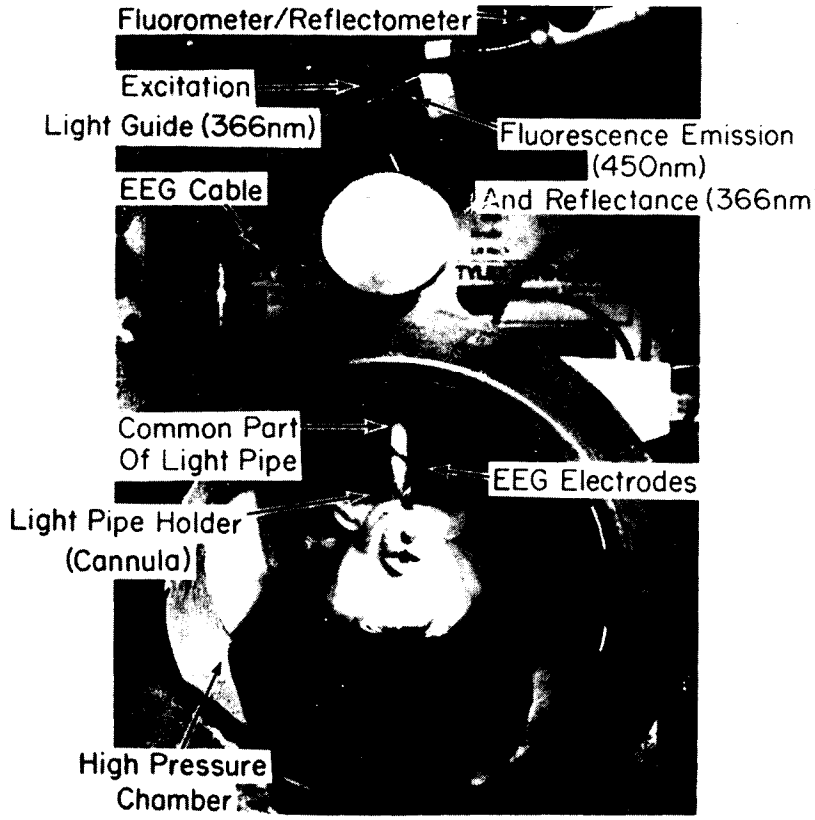



Fig. 4. Fiber optic fluorometer/reflectometer used for monitoring the redox state of NADH and 366 nm reflectance from the surface of the brain in the awake animal. Electrocorticogram (ECoG) obtained by bipolar electrodes.

taneously with changes in the reflectance. These cycles are probably of the same nature as cortical spreading depression (SD), which is also identified by the changes in extracellular $K^+$ levels and oxidation cycles of NADH (14,15). During the last phase, the postconvulsive period, deterioration of the brain was recorded and, finally, ECoG disappeared and the spontaneous breathing stopped. As a result the $Po_2$ decreased and NADH increased to its maximal level. A few minutes later a complete depolarization of the neuronal elements occurred as recorded by the accumulation of $K^+$ in the extracellular space and a dramatic increase in the reflectance (blood vessels constricted).

## Multiparametric Monitoring

The second approach, the multiparametric monitoring system, was described in detail recently (therefore only a brief description is given here).

of tonic-clonic convulsions, which later on, in the second phase, will affect various activities in the organism, and, finally, will affect directly or indirectly the mortality of the animal.

To study the functioning awake brain under HBO conditions, we used two main approaches. In 1973 we began to use the fiber optic surface fluorometer and to monitor the NADH oxidation-reduction state in correlation with the electrocorticograph (ECoG). In the last year [1982–83] we applied the two-channel fluorometer reflectometer (Mayevsky et al., unpublished results) to HBO studies in which the two hemispheres of the brain are monitored simultaneously and the effect of local ischemia on the responses to HBO were under investigation. In 1982 we applied the new multiparametric monitoring system (34) to study the effects of HBO on the metabolic, ionic, and electrical responses of the awake brain. To introduce the reader to these two approaches, I will briefly describe the methodology involved.

## Surface Fluorometry/Reflectometry

The technique is based upon the fact that NADH when illuminated by 366-nm light may fluoresce and a blue light having a peak at 450 nm is emitted. In the original studies by Chance et al. (35) the investigators exposed anesthetized animals to the Ultrapact optical system and the light was transmitted through the window of the hyperbaric chamber. In the new system developed (31), the light was guided by way of a bundle of optic fibers penetrating the wall of the hyperbaric chamber and connected chronically to the surface of the brain of an *awake* animal. Figure 4 shows a rat placed in the hyperbaric chamber and connected to the fluorometer.

Since 1973 another improvement has been introduced to the NADH measurement system under HBO. As compensation for hemodynamic changes in the tissue monitored, the intensity of the reflected light at the excitation wavelength (366 nm) was measured simultaneously with the NADH fluorescence. Electronic subtraction of the change in the reflectance signal (*R* in Fig. 5) from that of the fluoresence (*F* in Fig. 5) provided the corrected fluorescence (*CF*), which represented the net change of the intramitochondrial NADH (36,37). Typical results obtained by using the fluorometer reflectometer are shown in Fig. 5. As one can see in this figure, at the end of the compression few changes were recorded in various parameters. As shown by others and recently by us, too, brain $P_{O_2}$ increased simultaneously with the oxidation of NADH. At the same time the reflectance signal (having inverse correlation to blood flow or volume) showed an increase, which means a vasoconstriction response. In 1974 we speculated that during the activation of ECoG a possible change in extracellular $K^+$ might happen. As I will show later, this possibility may exist and we were able to monitor it in the awake rat (34). During the late preconvulsive stage, the reflectance signal showed a decrease, which means increased blood flow, and changes in electrical activity were noted. During the convulsive period, NADH showed an oxidation, and during the post ictal depression period, oxidation cycles of NADH were recorded simul-

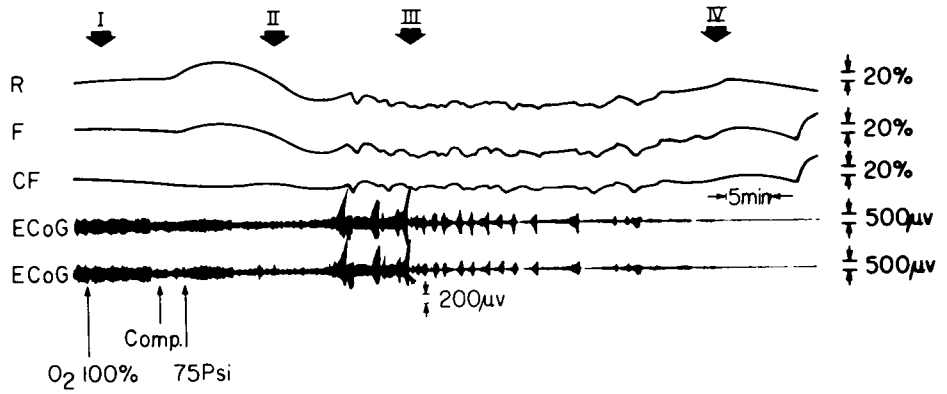



Fig. 5. Results from use of fluorometer/reflectometer. Discussion *in text*.

Figure 6 shows in a schematic way the connection between the animal and the monitoring system located outside the hyperbaric chamber. Metabolic activity was measured by monitoring the tissue $Po_2$ as well as the redox state of NADH. The ionic activity was evaluated by the changes in extracellular $K^+$ levels as determined by a Valinomycine surface electrode. The ECoG and DC steady potentials represent the electrical activity of the cortex. Brain, core, and chamber temperature were measured also. All signals were recorded on a multichannel paper recorder and a typical response of the rat brain to anoxia is shown in Fig. 7.

Soon after breathing 100% $N_2$ a sharp decrease in $Po_2$ was recorded simultaneously with the increase in NADH corrected fluorescence signal. Due to autoregulation mechanism blood flow was increased and recorded as a decrease in reflectance (*R*). Under such conditions (decrease in available energy) the ECoG disappeared and extracellular $K^+$ showed a slight increase. During the recovery from anoxia an overshoot in $Po_2$ was recorded due to high blood flow at this stage (decrease in *R* below baseline level). All parameters did recover within 10 min to the preanoxic level.

## RESULTS AND DISCUSSION OF EVENTS OCCURRING DURING PHASES OF OXYGEN TOXICITY

At this stage I would like to discuss in detail a few of the events occurring during the various phases of oxygen toxicity (Fig. 3).

In regard to the first phase, the following three questions have to be answered:

1) Does available energy become a limiting factor at the preconvulsive phase, and thus lead to the development of convulsions?

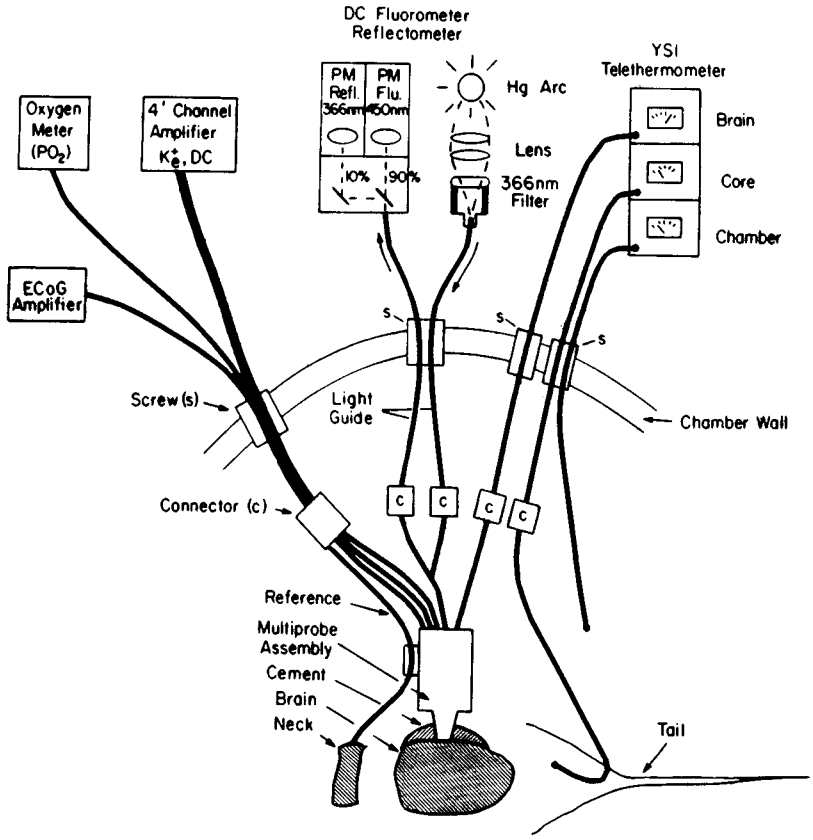



Fig. 6. Schematic presentation of the system used in monitoring the metabolic, ionic, and electrical activities of the awake brain under hyperbaric oxygenation (for details, *see* Ref. 34).

2) What is the meaning of the oxidation of pyridine nucleotides under these conditions?

3) What happens to extracellular $K^+$ level during the first phase?

In the second phase I will discuss the ionic and metabolic concomitant of the electrical changes. I will suggest a mechanism by which the termination of seizure may occur. Finally, the correlation between various parameters during the last terminal phase will be discussed.

The effects of oxygen at HBO conditions upon cellular energy metabolism have been noted already by Bert, and he described a slow-down in oxygen consumption, glucose breakdown, and the like. Later on, other investigators showed inhibition of respiration in vitro. Haugaard (38,39) reviewed the subject of enzyme inhibition under HBO conditions, but the time scale for those effects is much longer as compared to the appearance of convulsions in vivo. As concluded by Balentine, "Most of the available critical data on the effects of hyperoxia on glycolysis at least in the brain, do not support the idea that inhibition of glycolysis is fundamental to oxygen toxicity" (12, p. 41).

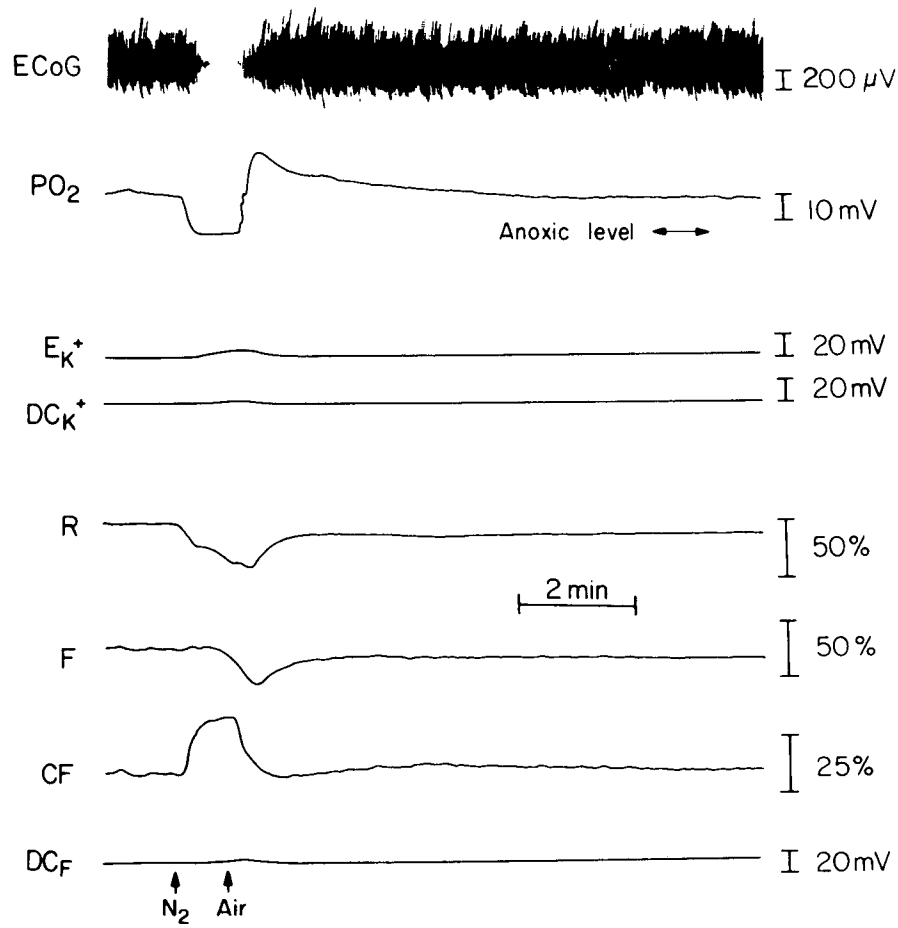



Fig. 7. The effects of a short anoxic episode on the metabolic, ionic, and electrical activities in the anesthetized rat.

Using the 2-deoxy-glucose method, Torbati et al. in 1983 (27) showed an increase in glycolysis during the preconvulsive period in most of the rat's brain structures. There is a general agreement about the increased level of intracellular $P_{O_2}$ in the brain exposed to HBO, although a compensatory decrease of blood flow was recorded in various organs.

Using surface fluorometry in the awake brain (Table I), we found the same level of oxidation of NADH before the seizures as well as during the oxidation cycles. The addition of $CO_2$ to the compression mixture accelerated the toxicity process as evaluated by the onset of convulsions, onset of oxidation cycles, and survival time, which were shorter. Pretreating the rats with succinate delayed the appearance of the seizures and postponed the death of

TABLE I

Effects of 6 ATA Hyperbaric Oxygenation on Various Responses of the Awake Rat

| Breathing Mixture | NADH Oxidation before Seizures (%) | NADH Decrease during Oxidation Cycle (%) | Onset of Seizures (min) | Onset of Oxidation Cycles (min) | Survival Time (min) |
|---|---|---|---|---|---|
| 100% $O_2$ | 14 | 10 | 20 | 27 | 78 |
| 98.5% $O_2$ + 1.5% $CO_2$ | 19 | 9 | 10* | 15* | 38† |
| 100% $O_2$ + succinate | 13 | 14 | 32* | 43* | 110 |

* $P < 0.01$. † $P < 0.001$. Modified data from Mayevsky, et al. (31).

the animal as reported previously by Sanders et al. (40). No correlation was found between the level of toxicity and the percentage change of NADH oxidation. Exposing the rat to various levels of $CO_2$ exhibited different patterns of reflectance changes (41), although the amount of oxidation of NADH was very similar. Figure 8 shows the effects of 1% $CO_2$ in the mixture on the responses to 6 ATA $O_2$. The vasoconstrictive response of the blood vessels was smaller (as measured by the increase in the *R* signal) as compared to 100% $O_2$ exposure (Fig. 5). Under those conditions, tonic-clonic seizures were recorded. Figure 9 shows that under 4% $CO_2$ in 6 ATA the reflectance signal decreased rather than increased due to the vasodilation effects of high $CO_2$

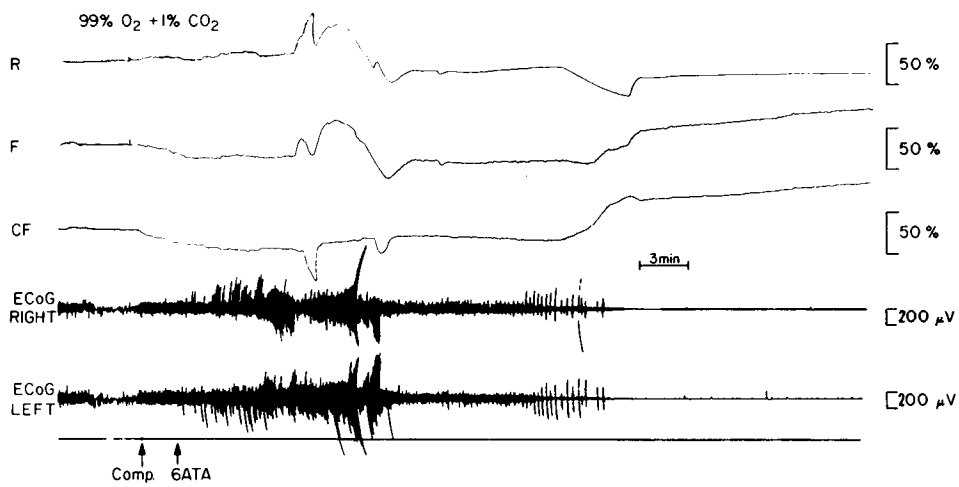



Fig. 8. Metabolic and electrical responses to 6 ATA $O_2$ containing 1% $CO_2$ measured in the awake rat.

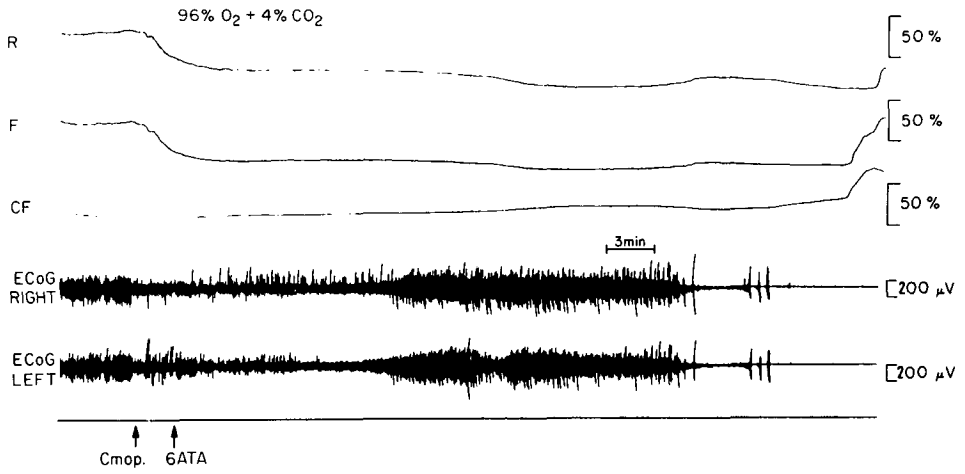



Fig. 9. The effects of hypercapnia (4% $CO_2$) on NADH fluorescence, light reflectance, and electrical activity measured in the brain of the awake rat.

level. The tonic-clonic seizures were blocked in this animal and Table II summarizes the results of this set of experiments. The level of oxidation of NADH was about the same under all levels of $CO_2$ but the number of convulsions decreased as well as the number of oxidation cycles, which were in good correlation with the seizures. The survival time in all five groups of animals was about the same.

The conclusions from the $CO_2$ experiments are the following: Intracellular $P_{O_2}$ at the intramitochondrial space in the first phase was not dependent upon the amount of blood flowing to the brain. No correlation was found between the level of $CO_2$ and the survival time although the number of tonic-clonic seizures correlated inversely with the $CO_2$ level. Carbon dioxide cannot be

TABLE II

Effects of $CO_2$ Level on the Responses to Hyperbaric Oxygenation in the Awake Rat

| % $CO_2$ in Mixture | No. of Convulsions | No. of Oxidation Cycles | Survival Time (min) |
|---|---|---|---|
| 1 | 3.8 ± 1.6 | 2.6 ± 0.9 | 42 ± 12 |
| 2 | 1.4 ± 1.1 | 1.4 ± 0.5 | 40 ± 14 |
| 3.1 | 0 | 0.4 ± 0.5 | 48 ± 20 |
| 4 | 0 | 0.2 ± 0.4 | 51 ± 25 |
| 5 | 0 | 0 | 42 ± 11 |

Modified data from Mayevsky (41).

considered as a protective agent although convulsions were blocked (as shown in Table II).

Under conditions where the blood flow to the brain was limited by partial ischemia, the following results obtained (Table III). The onset time for convulsions and survival time was not different in the two groups, but the number of convulsions was smaller and the convulsive period was shorter (42). The same was true for the oxidation cycles. Again, we can conclude that induced partial ischemia blocked the number of convulsions but the onset time for convulsions, oxidation cycles, or survival times was not affected, a finding which shows that intracellular $P_{O_2}$ levels are not the main key factor in oxygen toxicity as evaluated by the survival time.

According to the results accumulated, it is very difficult to assume that the energy availability during the preconvulsive phase is the limiting factor. Indirect evidence also was found. In a few of our multiparametric monitoring experiments, we found that spreading depression occurred during the postcompression phase and under those conditions the rate of pumping of $K^+$ from the extracellular space, as well as the NADH response, were as found in the normal brain (15). Therefore we can conclude that energy availability is not the rate-limiting step during phase one.

Now, I would like to discuss the effects of HBO on the extracellular level of $K^+$ in vivo. In 1957, Kaplan and Stein (7) showed a shift of $K^+$ into the extracellular space of brain slices under HBO. The level of $Na^+K^+$ activity in vivo is a key factor for the understanding of neuronal integrity as well as the functional state of the brain. The activity of the ATPase could be either a cause to some changes or a response to other processes going on in the brain. Because one cannot measure the ATPase activity in situ, we use the level of extracellular $K^+$ as an indicator, as compared to other investigators (43–46). Using the multiprobe approach, we exposed rats to 5 ATA $O_2$; preliminary results were described recently (34).

Figure 10 shows the responses to 5 ATA $O_2$ and the development of the first tonic-clonic seizures in the awake rat. One can see the increased $P_{O_2}$ level

TABLE III

Effects of Partial Brain Ischemia on Metabolic and Electrical Responses to 7 ATA $O_2$

| | Convulsions | | Convulsive Period (min) | Oxidation Cycles | | Survival Time (min) |
|---|---|---|---|---|---|---|
| | Onset Time (min) | Number | | Onset Time (min) | Number | |
| Control | 29 ± 2 | 14 ± 1 | 28 ± 4 | 25 ± 2 | 5 ± 0.3 | 75 ± 7 |
| Carotids occluded | 32 ± 4 | 3 ± 1† | 15 ± 4* | 31 ± 5 | 1.5 ± 0.4† | 79 ± 7 |

* $P < 0.05$; † $P < 0.01$. Modified data from Mayevsky et al. (42).

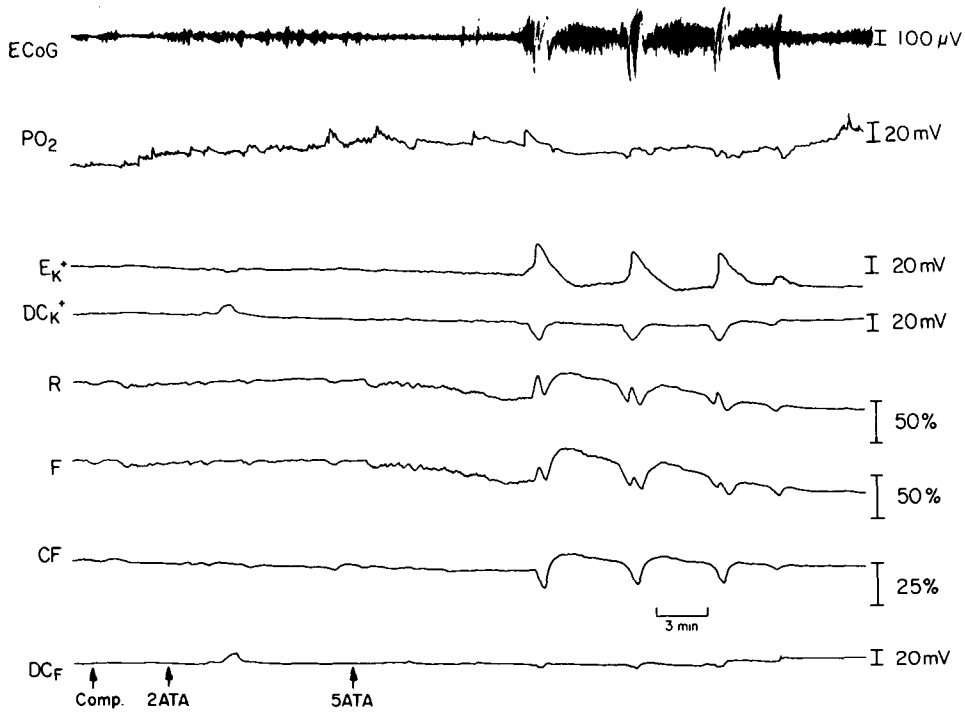



Fig. 11. Metabolic, ionic, and electrical responses to hyperbaric oxygenation (5 ATA $O_2$) of the awake rat. ECoG: electrocorticogram. $Po_2$: partial pressure of $O_2$. $E_K{}^+$: level of extracellular $K^+$. R: reflectance. F: NADH fluorescence. CF: corrected fluorescence. $DC_{K+}$, $DC_F$: steady potential measured near the $K^+$ electrode or the light guide.

One of the problematic points in HBO toxicity is the best definition for this process. Does the number of convulsions or the onset time for convulsions represent the main toxic event, or, perhaps, is survival time a better parameter? In this review I am not pretending to define it but rather discuss the various parameters used by others as they are reflected in our animal models and results.

In a detailed study in 1980 (48) we were able to show a differentiation between the convulsive activity and mortality of the animal exposed to HBO. Figure 13 shows the effects of various pressures of oxygen on the responses of two types of parameters, namely, the time parameters and number of events connected to the convulsive activity. The three parameters shown on the *left side* of the figure are probably correlated to each other and in most animals occurred in the same order (i.e., the change in reflectance is the first event, followed by the convulsive activity, and the oxidation cycles, which appeared later). Between 30 and 60 psi the slopes of changes of all three parameters are very sharp, whereas between 60 and 150 psi they are more moderate. Thus,

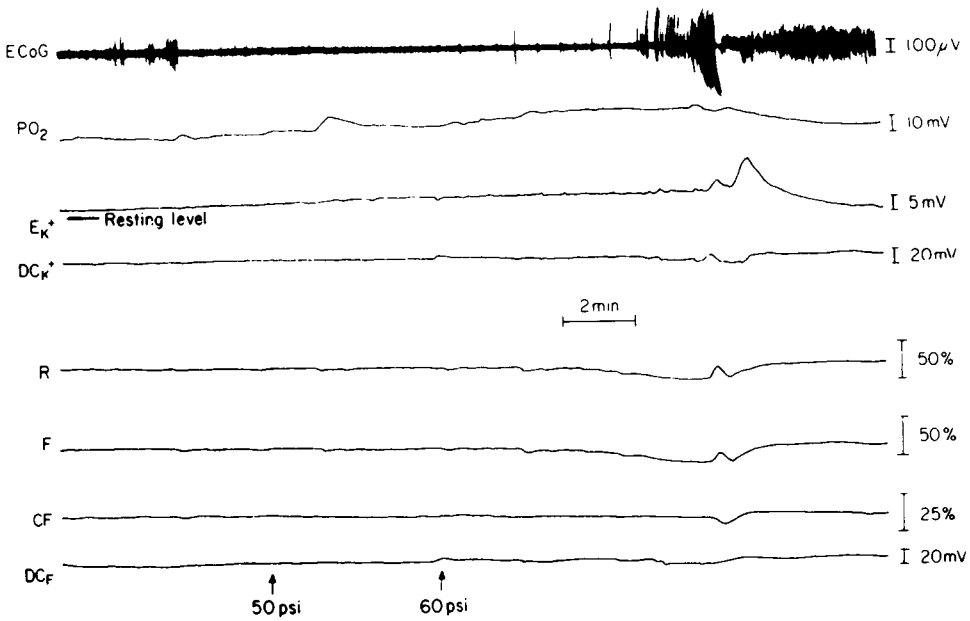



Fig. 10. The first tonic-clonic convulsion developed under hyperbaric oxygenation of the awake rat (5 ATA $O_2$). ECoG: electrocorticogram. $Po_2$: partial pressure of $O_2$. $E_K{}^+$: level of extracellular $K^+$. R: reflectance. F: NADH fluorescence. CF: corrected fluorescence. $DC_{K+}$, $DC_F$: steady potential measured near the $K^+$ electrode or the light guide (Modified from Mayevsky, 34).

simultaneously with the oxidation of NADH. The $K^+$ accumulated gradually (in this animal) in the extracellular space. During the seizure itself, $K^+$ showed a transient increase and then a secondary increase was recorded together with a large negative shift in DC steady potential; these changes are characteristic of the spreading depression phenomenon (15). Energy metabolism was stimulated as seen by the wave of NADH oxidation. After the SD wave, $K^+$ showed an undershoot, which may suggest an activation of the $Na^+K^+$ATPase system. Figure 11 shows another rat exposed to 5 ATA $O_2$. In this animal the resting level of $K^+$ remained normal during the preconvulsive stage and an undershoot was also recorded after the SD wave. These results suggest that the SD wave following the epileptic activity may serve as a mechanism for termination of the high electrical activity occurring during the seizure. During the seizure $K^+$ accumulated and reached a level that could initiate a wave of SD. Similar results were obtained when epilepsy was induced by other means (Metrazol, Penicillin) and preliminary results were published recently (47).

Figure 12 shows the terminal phase and death of a rat under 5 ATA $O_2$. One can see the decrease in $Po_2$ followed by an increase in NADH and in ECoG disappearance. Finally, $K^+$ accumulated, and a complete depolarization occurred as shown by the negative DC steady potential shift.

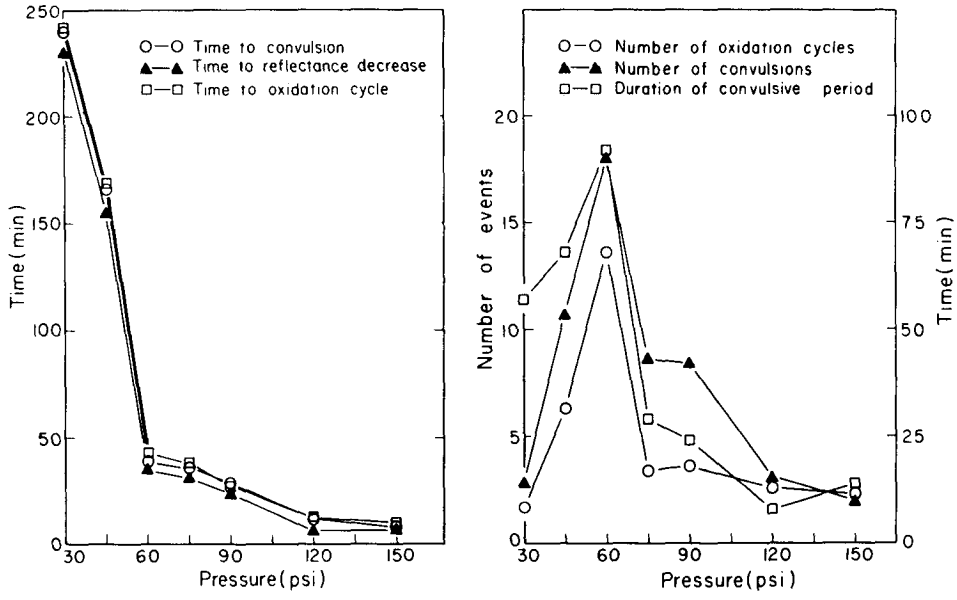



Fig. 13. The effects of various pressures on the responses of the awake rat to hyperbaric oxygenation (Modified from Mayevsky and Shaya, 49).

TABLE IV

Effects of Diazepam and Pentobarbital on Responses to 7 ATA and 5 ATA $O_2$

| | Time to Reflectance Decrease (min) | Convulsions | | Oxidation Cycles | | Survival |
|---|---|---|---|---|---|---|
| | | Onset Time (min) | Number | Onset Time (min) | Number | Time (min) |
| *7 ATA $O_2$* | | | | | | |
| Control | 24 | 28 | 8.4 | 28 | 4 | 65 |
| Diazepam | 65* | 53* | 0.3† | 55* | 0.7* | 118* |
| Pentobarbital | 97* | * | 0† | 91† | 0.4* | 121* |
| *5 ATA $O_2$* | | | | | | |
| Control | 34 | 38 | 18 | 43 | 14 | 243 |
| Diazepam | 161* | 164* | 3 | 109* | 1* | 283 |
| Pentobarbital | 204* | * | 0† | 218* | 0.3* | 297 |

* $P < 0.05$; † $P < 0.001$. Modified data from Mayevsky and Shaya (48).

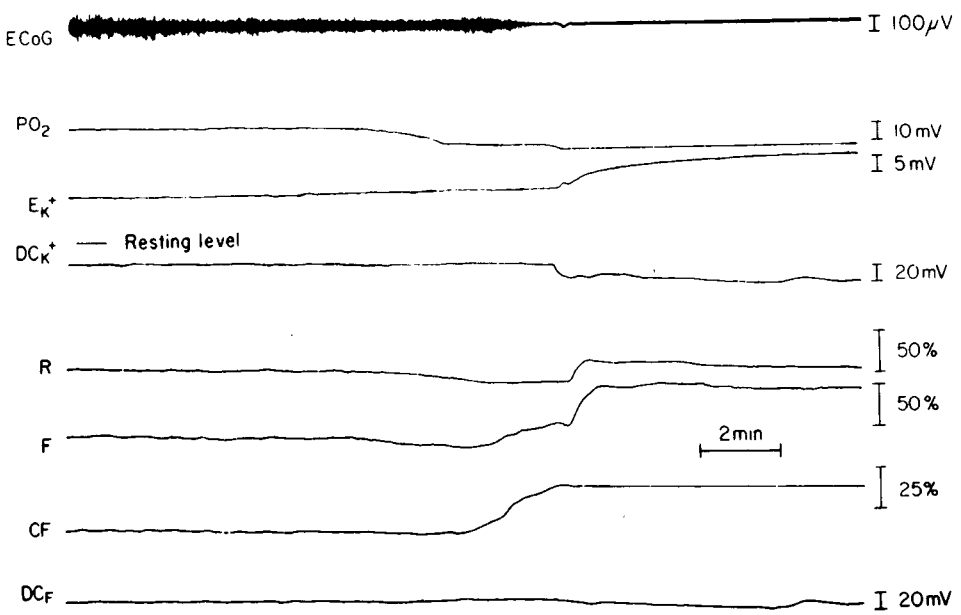



Fig. 12. The terminal phase of the responses of the awake rat to 5 ATA $O_2$. ECoG: electocorticogram. $P_{O_2}$: partial pressure of $O_2$. $E_{K^+}$: level of extracellular $K^+$. R: reflectance. F: NADH fluorescence. CF: corrected fluorescence. $DC_{K+}$, $DC_F$: steady potential measured near the $K^+$ electrode or the light guide (Modified from Mayevsky, 34).

the 60-psi pressure is a breaking point of the line. On the other hand, the other three parameters shown on the *right side* of the figure are affected differently by the pressure. The maximum effect was observed at 60 psi, and the curves had a bell shape. The differences between the 60-psi point and the 30- or 150-psi points are statistically significant ($P < 0.005$), as calculated by the Student's *t*-test.

The same type of discrepancy between epileptic activity and survival time was found when rats were pretreated by pentobarbital or diazepam, which served as protective agents against HBO toxicity (49–52). The results in Table IV show the significant effects of pentobarbital as well as diazepam on the various parameters measured. Pentobarbital completely inhibited the tonic convulsions under 60 and 90 psi. As a result, the oxidation cycles did not occur in most of the animals treated by pentobarbital. Diazepam also inhibited the convulsions and the oxidation cycles under 60 and 90 psi, but was not so efficient as pentobarbital. The main difference between the 60 and 90 psi can be seen in the survival time values. At 90 psi the pentobarbital and diazepam significantly increased the survival time, whereas at 60 psi no significant difference between the three groups of animals was observed although the convulsions were almost completely inhibited. Using trimethadione (TMO) as

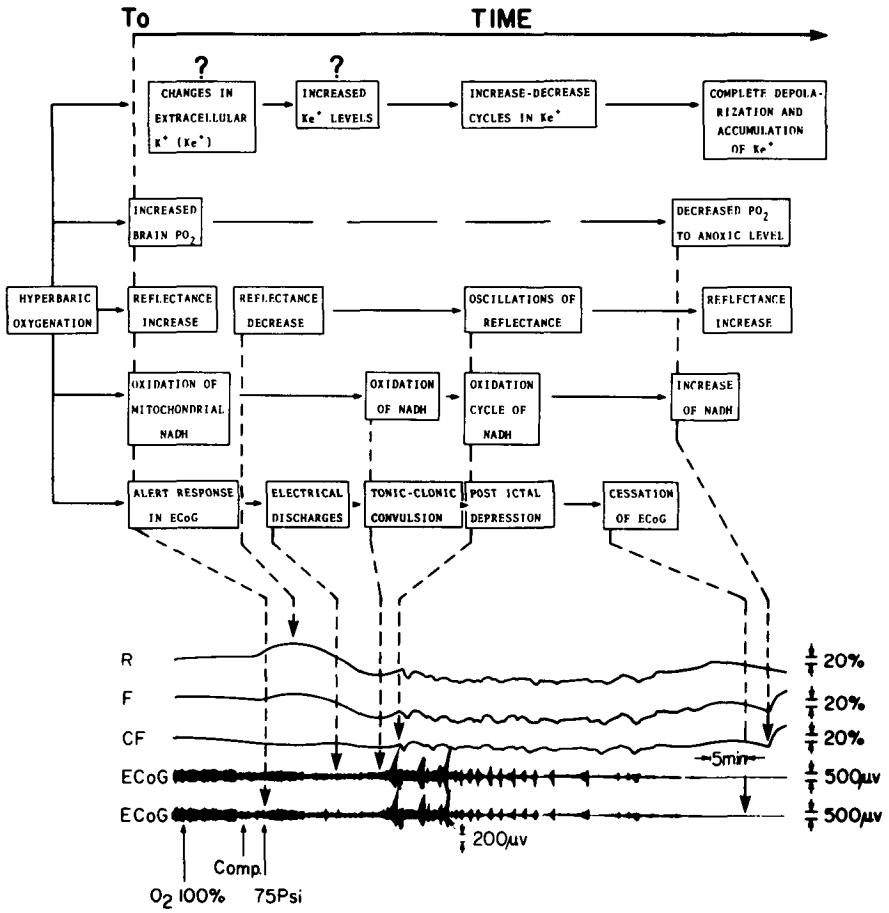



Fig. 14. Summary of the various events recorded from the surface of the brain under hyperbaric oxygenation.

vivo as was suggested also by Hempel et al. (33) for cytochrome $aa_3$ and by Hempel (33) using the pyrenbutynic acid as an intracellular $PO_2$ indicator. This idea is supported by the increase of glycolysis that occurs in vivo and supplies more substrate to the mitochondria (27). One possible explanation for the activation of energy metabolism is the availability of more oxygen intracellularly, which in normal air-breathing conditions is not the optimal one (32). We believe that under the preconvulsive stage of hyperbaric oxygen, energy production and availability are not impaired and even may be stimulated because more oxygen is available intracellularly.

We still do not have clear-cut statistical evidence about the level of extracellular $K^+$ at the first phase. Extracellular $K^+$ may increase because of a membrane permeability change and thus may stimulate the activity of the $Na^+K^+$ATPase, as shown by Hemrick and Gottlieb, and Gottlieb et al. (44).

TABLE V

Effects of Trimethadione (TMO) on Responses to 6 ATA $O_2$

| | Convulsions | | Oxidation Cycles | | Survival |
|---|---|---|---|---|---|
| | Onset Time (min) | Number | Onset Time (min) | Number | Time (min) |
| Control | 9 ± 3 | 11 ± 4 | 13 ± 4 | 5 ± 1 | 70 ± 32 |
| TMO | 24 ± 7† | 0.5 ± 0.8† | 31 ± 9† | 3 ± 1 | 102 ± 23* |

* $P < 0.05$; † $P < 0.01$. Modified data from Mayevsky (14).

a protective agent (53) against HBO toxicity (Table V), we found a good correlation between seizure activity and survival time, although NADH oxidation was the same in the two groups (54).

The results presented to this point suggest that limitation in available energy is not the key factor in the development of oxygen toxicity as evaluated by convulsive parameters. This conclusion was supported also by experiments exposing rats in vivo to HBO: mitochondria were isolated and state 3 and 4 respiration was measured in vitro after various in vivo behavioral stages (55,56). Table VI shows a very small decrease in state 3 respiration as well as the small change in the *Respiratory Control Ratio* at the various stages of HBO toxicity correspond to *Stages I, II, III,* and *IV* in Fig. 5. These results suggest that the mitochondria were in a good biochemical condition after in vivo exposure to HBO, and one may assume that under in vivo condition they may act even better.

Figure 14 summarizes our findings as regards the HBO toxicity processes. According to the results accumulated in our laboratory, we are interpreting the oxidation of NADH as an activation of the energy metabolism of the brain in

TABLE VI

Brain Mitochondrial Respiratory Activity Measured In Vitro after In Vivo Exposure to Hyperbaric Oxygenation

| Time at 6 ATA $O_2$ (min) | Behavioral State | State 3 $O_2$ Uptake (nmoles $O_2$/min) | Respiratory Control Ratio |
|---|---|---|---|
| 0 | Awake | 61 ± 2 | 11 ± 0.6 |
| 10 | Preconvulsive | 51 ± 5* | 15 ± 0.9† |
| 20 | Convulsive | 56 ± 3 | 11 ± 1.3 |
| 40 | Postconvulsive | 53 ± 4* | 12 ± 1.0 |

* $P < 0.05$. † $P < 0.01$. Modified data from Mayevsky et al. (56).

14. Mayevsky A, Chance B. Metabolic responses of the awake cerebral cortex to anoxia, hypoxia, spreading depression, and epileptiform activity. Brain Res 1975;98:149–164.
15. Friedli CM, Sclarsky DS, Mayevsky A. A new multiprobe assembly for surface monitoring of ionic metabolic and electrical activities in the awake brain. Am J Physiol 1982;243:R462–R469.
16. Leidingham I McA, McDowall DG, Harper AM. Cerebral cortical blood flow under hyperbaric conditions. In: Brown IW, Cox BG eds. Proceedings of the third international conference on hyperbaric medicine. Washington DC: National Academy of Sciences 1966:243–249. (NAS-NRC publ. 1404)
17. Bean JW, Leatherman NE. Cerebral blood flow during convulsions. Alterations induced in animals by pressure oxygen. Arch Neurol 1969;20:396–405.
18. Bean JW, Lignell J, Burgess DW. Cerebral $O_2$, $CO_2$, regional cerebral vascular control, and hyperbaric oxygenation. J Appl Physiol 1972;32:650–657.
19. Torbati D, Parolla D, Lavy S. Blood flow in rat brain during exposure to high oxygen pressure. Avait Space Environ Med 1978;49:963–967.
20. Stein SN, Sonnenschein RR. Electrical activity and oxygen tension of brain during hyperoxic convulsions; experimental method and results. J Aviation Med 1950;21:401–404.
21. Jamieson D, van den Brenk, HAS. Measurement of oxygen tensions in cerebral tissues of rats exposed to high pressures of oxygen. J Appl Physiol 1963;18:869–876.
22. Torbati D, Parolla D, Lavy S. Changes in electrical activity and $Po_2$ of the rat's brain under high oxygen pressure. Experimental Neurol 1976;50:439–447.
23. Blackburn JG, Ogilvie RW, Balentine JD. Effects of hyperbaric oxygenation on electrical activity of Globus Pallidus and Neostriation. Exp Neurol 1977;56:158–167.
24. Torbati D, Parolla D, Lavy S. Changes in local brain tissue $Po_2$ and electrocortical activity of unanesthetized rabbits under high oxygen pressure. Aviat Space Environ Med 1977;48:347–350.
25. Lehmenkuhler A, Bingmann D, Lange-Asschenfeldt H, Berges D. Oxygen pressure and ictal activity in the cerebral cortex of artificially ventilated rats during exposure to oxygen at high pressure. In: Silver IA, Erecinska M, Bicher HT, eds. Oxygen transport to tissue, III. New York: Plenum Press, 1978:679–685.
26. Kovachich GB, Haugaard N. Biochemical aspects of oxygen toxicity in the Metazoa. In: Gilbert DL, ed. Oxygen and living processes. An Interdisciplining approach. New York: Springer, 1981:210–234.
27. Torbati D, Greenberg J, Lambertsen CJ. Correlation of brain glucose utilization and cortical electrical activity during development of brain oxygen toxicity. Brain Res 1983;262:267–273.
28. Sanders AP, Hall HI, Cavanough PJ, Woodhall B. Effects of HBO on metabolism. I. ATP concentration in rat brain, liver and kidney. Proc Soc Exp Biol 1966;121:32–34.
29. Nolan RJ, Faiman MD. Brain energetics in oxygen-induced convulsions. J Neurochem 1974;22:645–650.
30. Faiman MD, Nolan RJ, Dodd DE, Waechter JM, Driks RC, Haya K, Zempel HA. In Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:25–36.
31. Mayevsky A, Jamieson D, Chance B. Oxygen poisoning in the unanesthetized brain: Correlation of the oxidation reduction state of pyridine nucleatide with electrical activity. Brain Res 1974;76:481–491.
32. Hempel FG, Jobsis FF, LaManna JL, Rosenthal MR, Saltzman HA. Oxidation of cerebral cytochrome $aa_3$ by oxygen plus carbon dioxide at hyperbaric pressures. J Appl Physiol: Respirat Environ Exercise Physiol 1977;43:873–879.
33. Hempel FG. Oxygen tensions measured in cat cerebral cortex under hyperbaric conditions. J Appl Physiol 1979;46:53–60.
34. Mayevsky A. Multiparameter monitoring of the awake brain under hyperbaric oxygenation. J Appl Physiol: Respirat Environ Exercise Physiol 1983;54:740–748.
35. Chance B, Jamieson D, Coles H. Energy-linked pyridine nucleotide reduction: Inhibitory effects of hyperbaric oxygen in vitro and in vivo. London: Nature 1955;206:257–263.
36. Jobsis FF, O'Connor M, Vitale A, Verman H. Intracellular redox changes in functioning cerebral cortex. Metabolic effects of epilepiform activity. J Neurophysiol 1971;34:735–749.
37. Harbig K, Chance B, Kovach AGB, Reivich M. In vivo measurement of pyridine nucleotide fluorescence from cat brain cortex. J Appl Physiol 1976;41:480–488.
38. Haugaard N. Cellular mechanisms of oxygen toxicity. Physiol Rev 1968;48:311–373.

The other possibility is that $Na^+K^+$ATPase is inhibited initially as shown by Kovachich et al. (45,46), and this induces $K^+$ to be accumulated in the extracellular space. During the beginning of the convulsive period, our results suggested that the epileptic activity followed by the spreading depression mechanism are energy-consuming processes and thus stimulate the mitochondrial activity to produce more needed ATP. We do not see any results that support inhibition of the $Na^+K^+$ATPase system at the second stage. Only at the final stage of the seizures such an event may occur, and as a result $K^+$ will accumulate in the extracellular space. It seems to us that the mechanism of initiation of the epileptic activity cannnot be drawn from our results; we assume that other physiological or biochemical events are in charge of this mechanism, such as impaired neutrotransmission or an event occurring in subcortical structures. As shown by Balentine (12) the cortical areas are not connected to the neuronal necrosis found in other areas of the brain.

## Acknowledgments

This work was supported by NIH Program Project HL 08899-18 "Responses Induced by Respiratory Environments," U.S. Navy Office of Naval Research "Pressure Physiology," and the Program Project Grant NINCDS-10939.

## References

1. Bert P. Barometric pressure: Researches in experimental physiology. Paris, 1878. Translated by Hitchcock MA, Hitchcock FA, Columbus, OH: College Book Co., 1943.
2. Bean JW. Effects of oxygen at increased pressure. Physiol Rev 1945;25:1–147.
3. Stadie WC, Riggs BC, Haugaard N. Oxygen poisoning. Am J Med Sci 1944;207:84–114.
4. Dickens F. The toxic effect of oxygen on brain metabolism on tissue enzymes. 1. Brain metabolism. Biochem J 1946;40:145–171.
5. Dickens F. The toxic effects of oxygen on brain metabolism and on tissue enzymes. 2. Tissue enzymes. Biochem J 1946;40:171–187.
6. Dickens F. The toxic effect of oxygen on nervous tissue. Neurochemistry. Elliott KAC, Page IH, Quaster JH, eds. Springfield IL: Charles C Thomas 1962:851–869.
7. Kaplan SA, Stein SN. Effects of oxygen at high pressure on the transport of potassium sodium and glutamate in guinea pig brain cortex. Am J Physiol 1957;190:157–162.
8. Chance B, Jamieson D, Williamson JR. Control of the oxidation-reduction state of reduced pyridine neuclotides in vivo and in vitro by hyperbaric oxygen. In: Brown IW, Cox BG, eds. Proceedings third international conference on hyperbaric medicine. Washington, DC: National Academy of Sciences, 1966:15–41.
9. Lambertsen CJ. Effects of hyperoxia on organs and their tissues: In: Robin ED, ed. Extrapulmonary manifestations of respiratory disease. New York: Marcel Dekker 1978:239–303.
10. Wood JD. Oxygen toxicity. In Bennett PB, Elliott DH, eds. The physiology and medicine of diving and compressed air work, London: Baillière, Tindall and Cassell 1969:113–143.
11. Clark JM. Current concepts of oxygen toxicity. In: Bachrach AJ, Matzen MM, eds. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society Inc., 1981:3–24.
12. Balentine JD. Pathology of oxygen toxicity. New York: Academic Press, Inc., 1982.
13. Chance B, Williams GR. Respiratory enzymes in oxidative phosphorylation. I. Kinetics of oxygen utilization. J Biol Chem 1955;217:383–393.

39. Haugaard N. The scope of chemical oxygen poisoning. In: Lambertsen CJ, ed. Underwater physiology. Proceedings of the fourth symposium on underwater physiology. New York and London: Academic Press, 1971:1–18.
40. Sanders AP, Hall IH, Cavanaugh PJ, Woodhall B. In vivo effects of hyperbaric oxygen toxicity and succinate as a preventative. In: Brown IW, Cox BG, eds. Third international conference on hyperbaric medicine. Washington DC: National Academy of Sciences, 1966:73–78.
41. Mayevsky A. The responses of an awake brain to HBO under increased $CO_2$ concentrations. In: Silver IA, Erecinska M, Bicher HI, eds., Oxygen transport to tissue, III. New York: Plenum Press 1978:735–739.
42. Mayevsky A, Gugenhaimer E, Zarchin N. The effects of brain partial ischemia on the metabolic responses to hyperbaric oxygen. Undersea Biomed Res 1981;8(Suppl 1):A45.
43. Hemrick SK, Gottlieb SF. Effect of increased pressure of oxygen, nitrogen, and helium on activity of Na-K-ATPase of beef brain. Aviat Space Environ Med 1977;48:40–43.
44. Gottlieb SF, Schmit PL, Stewart JR, Bendixen G. The in vivo effect of oxygen on Na-K-ATPase: The beginning of a new cellular theory of oxygen toxicity. In: Smith G, ed. Proceedings of the sixth international congress on hyperbaric medicine. Aberdeen, Scotland: Aberdeen University Press, 1979:8–12.45.
45. Kovachich GB, Mishra OP, Clark JM. Depression of cortical Na-K-ATPase activity in rats exposed to oxygen at 4 ATA $O_2$. Brain Res 1981;206:229–231.
46. Kovachich GB, Mishra OP. Partial inactivation of Na-K-ATPase in cortical brain slices incubated in normal Krebs-Ringer phosphate medium at 1 and 10 ATA $O_2$ pressure. J Neurochem 1981;36:333–335.
47. Mayevsky A. Metabolic ionic and electrical responses to experimental epilepsy in the awake rat. First International Congress CBF, metabolism and epilepsy, France, 1983 (in press).
48. Mayevsky A, Shaya B. Factors affecting the development of hyperbaric oxygen toxicity in the awake rat brain. J Appl Physiol 1980;49:700–707.
49. Gutsche BB, Harp JR, Stephen CR. Physiologic responses of the anesthetized dogs to oxygen at five atmospheres absolute. Anesthesiology 1966;27:615–623.
50. Harp JR, Gutsche BB, Stephen CR. Effect of anesthetics on central nervous system toxicity of hyperbaric oxygen. Anesthesiology 1966;27:608–614.
51. Beubler E, Lambeck F. Seizures induced by hyperbaric oxygen (OHP) and their inhibition by diazepam. Naunyn Schemiedebergs Arch Pharmakol 1976;293(Suppl):R14. (Abstr)
52. Ruszczewski P. The protective action of certain anesthetics and tranquilizers against the effects of hyperbaric oxygen. Acta Physiol Pol 1976;27:435–447.
53. Paton WDM. Experiments on the convulsant and anesthetic effects of oxygen. Br J Pharmocol Chemother 1967;29:350–366.
54. Mayevsky A. The effect of trimethadione on brain energy metabolism and EEG activity of the conscious rat exposed to HBO. J Neurosci Res 1975;1:131–142.
55. Mayevsky A, Mela L, Chance B. In vivo effects of hyperbaric oxygenation on brain pyridine nucleotide redox state, EEG, and mitochondrial respiration. Fed Proc 1974;33:1399. (Abstr)
56. Mayevsky A, Korbe-Kuhl K, Mela L. Oxygen toxicity in the awake rat brain: Correlation between in vitro mitochondrial activity and metabolic activity in vivo. Neurol Res 1980;1:305–311.

# Part II

## INERT GAS EXCHANGE, COUNTERDIFFUSION, AND BUBBLE FORMATION

# ACCLIMATIZATION TO DECOMPRESSION STRESS

*R. G. Eckenhoff and J. S. Hughes*

That humans can adapt to decompression stress has long been suspected, due to evidence which is largely anecdotal, incidental, and retrospective. Few prospective studies exist which have examined acclimatization in a systematic fashion. Also, evidence concerning the optimal profiles for the induction of acclimatization is unavailable. Nevertheless, several military and commercial diving units have incorporated *work up* dives (often performed in a hyperbaric chamber) into their preparation for deep diving tasks, and even for research hyperbaric exposures. Therefore, it is important to know whether acclimatization actually occurs in humans repetitively exposed to decompression stress, so that the concept can be maximally exploited to reduce the risk of decompression sickness (DCS) in subsaturation diving. This report describes the effect of 12 consecutive daily exposures for 30 min to a pressure equivalent to 150 feet sea water gauge (fswg) on the quantity of intravascular bubbles and symptomatology in 15 human subjects.

## LITERATURE REVIEW

In a large retrospective study, the incidence of reported decompression symptoms was found to decline with a half time of 7 ± 4 days to a maximum effect in about 15 days in a large population of caisson workers exposed daily to hyperbaric air (1,2). These authors suggest that acclimatization at one pressure does not infer protection at another and that deacclimatization is essentially complete after 10 days of nonexposure. Furthermore, they rule out the elimination of susceptible individuals as an explanation for the decline and do not consider under-reporting or becoming accustomed to symptoms as a possibility. In experiments at the AMTE Physiologic Laboratory, investigators

found that decompression symptoms occurred more frequently in subjects who had not had prior hyperbaric exposure than in those who had (3–5). The concept of the work-up dive probably grew out of these studies and is presently used on a routine basis in the Royal Navy. In a similar but not entirely analogous decompression stress, the incidence of bends symptoms was shown to decline in 35 subjects exposed to twice-daily hypobaric runs (20 000 ft) for each of 34 days, but only after the 28th day (6). In this study, day-to-day variability in symptoms was noteworthy.

All of these studies have relied on the reporting of symptoms and therefore are susceptible to the limitations of subjective data. It seems that objective measures of decompression stress are either unavailable, impractical, or insufficiently refined. However, the quantification of venous gas embolii (VGE) by Doppler ultrasound detection, an easily performed technique, may provide a means of estimating decompression stress. In at least one study using the Doppler technique for determining the stressfulness of helium-oxygen decompression schedules, the mean VGE score obtained in seven human subjects declined significantly after only three hyperbaric exposures (120 fswg for 20 min), even with 5 days between each dive (Vann, personal communication). No other Doppler data from human repetitive exposure experiments is available.

The goal of much of the animal work in this area has been the crushing of bubble *micronuclei* by very large and brief pressure increases before the test exposure. Although this has been shown to decrease the incidence of DCS (7), it is probably via a different mechanism than repetitive, shallow exposures, during which micronuclei crushing would not occur. Few animal experiments with repetitive similar exposures have been reported. However, as an incidental finding in one study, the *minimum bends depth* was shown to increase in a small number of goats after repetitive hyperbaric exposure (8). Additionally, a reduction in sensitivity of cats to decompression sickness was noted with repetitive exposure to 8 ATA of air (9). However, in a study of minipigs implanted with pulmonary artery Doppler cuffs, no significant changes were reported in the Doppler score on direct decompression from exposures of 20 m for 340 min every other day for an unspecified period of time (10). In this study, substantial day-to-day variation in the Doppler score was noted. In another animal study (rat and dog) utilizing the Doppler technique, the pressure reduction required to produce VGE increased on repeat exposures (11). Thus, it seems that much of the available evidence for both man and animals is contradictory. However, this may be a result of differences in the exposure itself, the intervals between the exposures, or the total number of repeat exposures.

Several mechanisms have been suggested for acclimatization, but they can be simplified into two broad categories. First, because the bubble is still considered the initiating event in DCS, the rate of bubble production, or the total body evolved gas load, may be reduced by repeated compression-decompression cycles. The best example in this category would be the concept of bubble micronuclei depletion (12,13). The second category would not

require a reduction in the evolved gas phase. A change in the body's handling of bubbles, either anatomically or biochemically, could produce acclimatization to decompression stress. Possibilities in this category include passive tissue relaxation (14), increased efficiency of handling or tolerating blood-bubble interactions, or anatomical changes resulting in a decreased appreciation of, or an increased clearance of bubbles (2). Mechanisms that have characteristics of both categories are possible as well. Nevertheless, evidence in support of either is lacking at present.

## MATERIALS AND METHODS

### Subjects

Fifteen healthy male volunteers, ages 20 to 41 years, served as subjects. Vital statistics for the subjects are shown in Table I. The subjects were instructed not to undergo any exposure to pressure for at least 2 weeks prior to the test exposures. Furthermore, they were instructed to avoid any diving, aviation, unusually strenuous or contact sports, or medications the week before and during the course of the study. Informed consent was obtained from all participating subjects.

### Exposures

Four groups of 4, 4, 4, and 3 subjects each were compressed with air to 148 fswg at the Environmental Simulation Facility of the Naval Submarine Medical Research Laboratory located in Groton, Connecticut. The bottom time was 28 min and standard air decompression was performed according to the profile in the *U.S. Navy Diving Manual* for 150 fsw for 30 min, which resulted in 32 min of decompression, 8 min at 20 fsw and 24 min at 20 fsw. This procedure was followed at the same time of the day for each of 12 consecutive days. No exercise was performed during either the exposure or decompression.

TABLE I

Subject Vital Statistics
(n = 14)

| | Age (yr) | Weight (g) | Height (cm) | Body Fat (%) |
|---|---|---|---|---|
| Mean | 29.2 | 74.4 | 175.1 | 15.7 |
| SD | 6.6 | 13.7 | 7.3 | 4.6 |
| Range | 20–41 | 52.3–95.3 | 163–186 | 9.7–23.6 |

### Monitoring

After surfacing, subjects were monitored at 15-min intervals for a total of 2 h with a Sodelec D.U.G. precordial Doppler ultrasound sensor. Each session consisted of monitoring subjects in the standing position for 40 cardiac cycles, then for 20 cardiac cycles after each of 3 deep knee bends. The signals were recorded on cassette tapes. The recordings from the 12 dives were randomized, coded, and then interpreted by the Doppler group at the Defense and Civil Institute for Environmental Medicine (DCIEM), Toronto, Canada, in a single-blind fashion. The recordings were scored in accordance with the KM grading system (15).

For each of the 12 exposures, the subjects filled out questionnaires describing diet, medications, and exercise, as well as any symptoms resulting from the exposures. Because pruritus is a common symptom during dry chamber exposures of this type, the subjects were asked to score this condition on a 1–10 scale based on severity and distribution.

Subsequent to the analysis by DCIEM, the daily pruritus and both peak and mean Doppler scores were recorded for each subject. Trends were identified by obtaining the slope and confidence limits of a line described by a least-squares regression of the data. The data were also subjected to a repeated-measures one-way analysis of variance. Additionally, the time course of VGE appearance over the 2-h postdive was compared between the 12 days.

## RESULTS AND DISCUSSION

The entire 12-day protocol was completed by 14 of the 15 subjects. On the first dive day, one subject suffered DCS (spinal cord) and was therefore excluded from the remainder of the experiment. The Doppler and pruritus data are shown in Figs. 1 and 2, respectively. No detectable VGE was found in 4 of the 14 subjects for the entire 12-day period, whereas only 4 subjects had detectable VGE on each of the 12 days. The remaining six subjects had intermittent VGE, in no predicable pattern. No significant trend was noted in either the peak or mean Doppler score, during rest or after movement, over the 12-day period. The slope of the line obtained using least-squares linear-regression analysis was $+0.023$ (about 2.5% of the $y$ intercept), with a 95% confidence interval of $\pm$ 0.065 (*see* Fig. 3), which is not significantly different from zero. The maximum downward or upward trend described by these limits is about 4.5 or 9.5% per day, respectively. Forcing the data to an exponential curve did not improve the fit and gave similar results: the *minimum* half life described by the slope confidence limits was 14.5 days. The maximum slope according to this exponential fit gave a *doubling* time of about 6 days. No adaptive trends were observed in any single subject. Doppler scores varied widely—not only between individuals, but also between days in the same individual. No change in the time course of VGE appearance in the 2-h

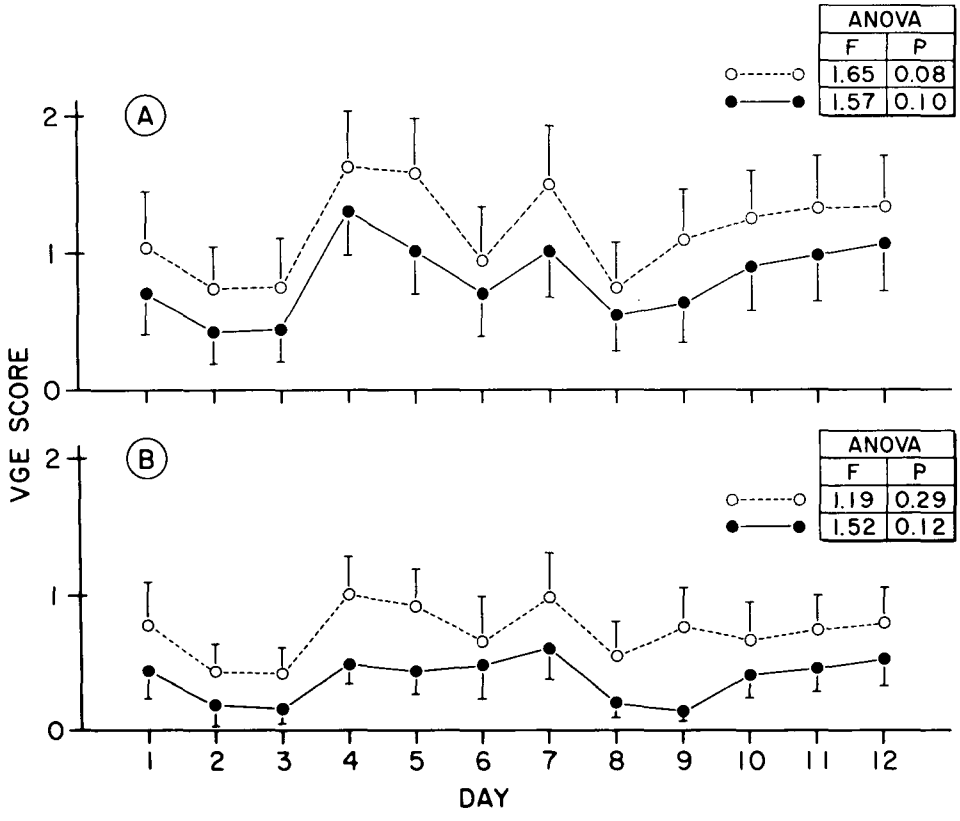



Fig. 1. Average VGE scores (Kisman-Masurel grading scheme) for all 14 subjects on each of the 12 days. *Open circles* represent the score after movement (deep knee bends); *closed circles* represent the score at rest. (*A*) is the average *peak* VGE score during the 2-h monitoring session; (*B*) is the average *mean* VGE score over the same period. *F* and *P* values for a repeated-measures analysis of variance are indicated for each set of data. SEM is indicated.

monitoring period was noted over the 12 days. The mean time to the peak VGE score was approximately 60–70 min after surfacing.

On the other hand, subjective data were consistent with some sort of adaptive trend. A significant decrement in pruritus was observed, ($P<0.001$); the subjective score was approximately halved in the 12-day period. Here, regression analysis revealed a line with a slope of $-0.21$ and confidence limits of $\pm$ 0.12. It is difficult to determine if this represents a physiologic reduction, or a "getting-used-to-symptoms" reduction. Also, out of a total of 169 man dives, the only case of DCS occurred on the first day. This should not, however, be construed as having any statistical meaning, as the numbers are too small, and this subject was necessarily eliminated from further exposures. It also merits mention here that the overall incidence of DCS seen in this study

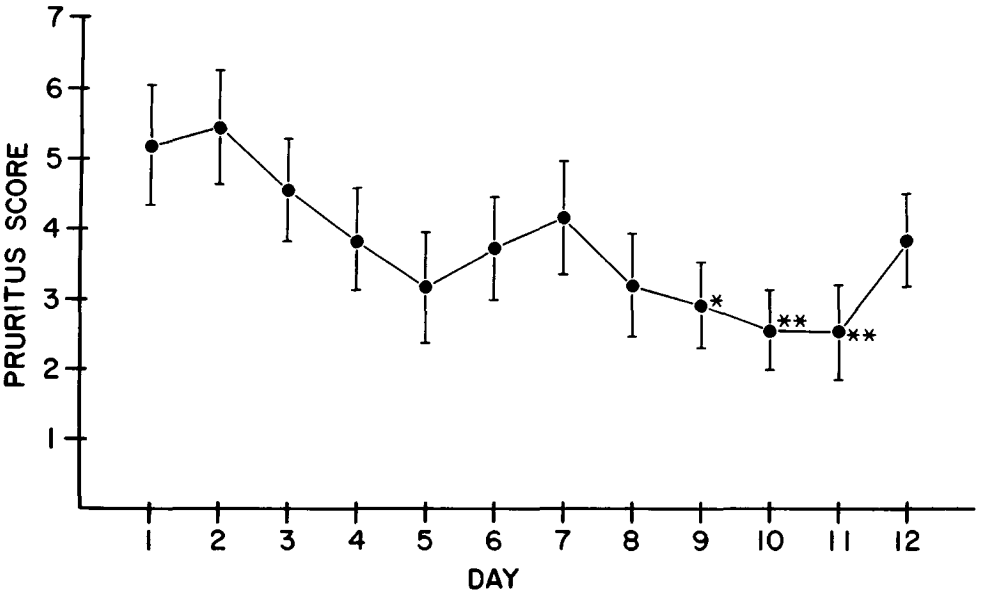



Fig. 2. Average pruritus (itching) score for all subjects on each of the 12 days. The score is a subjective determination of the severity and distribution of itching on a scale of 1–10. SE is indicated. $F$ and $P$ values for a repeated-measures analysis of variance are 3.23 and $<0.001$, respectively. * = $P<0.05$; ** = $P<0.01$.

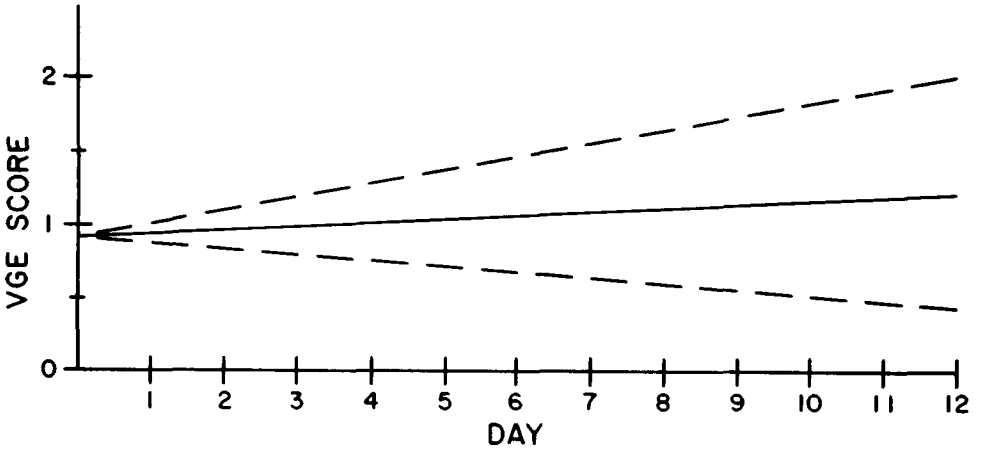



Fig. 3. Least-squares regression of the combined data results in the above line (*solid line*) with a $y$-intercept of 0.93 and slope of + 0.023. The 95% confidence interval for the slope of this line is − 0.042 to + 0.088, and these limits are also shown by the *dashed lines*. Therefore, the actual trend for VGE quantity lies within these *dashed lines*.

(about 0.6%) agrees with that reported for this exposure in the past: about 1.1% (16). However, if only the first day is considered, the incidence would increase to about 7%.

Any conclusions drawn depend in large part upon how much faith one puts in the detection of VGE as a reliable indicator of decompression stress. Insofar as it is a reliable index, this study suggests that acclimatization to decompression stress does not occur. On the other hand, if the quantification of VGE is not a reliable index of decompression stress, it can at least be considered a rough measure of the evolved gas load in the body. And the results of this study suggest that there is no change in the quantity of gas phase produced by repeated, daily, compression-decompression cycles. Although the confidence limits for this set of data could also describe a decrease in the VGE score, it is not of sufficient magnitude to explain the larger and more rapid effect observed in previous studies—although it could conceivably explain the decrement in pruritus observed in this study. Nevertheless, a change in the quantity of VGE produced is unlikely to account for acclimatization. Therefore, we hypothesize that acclimatization, if it indeed exists, occurs not because of a reduction in evolved gas phase, but more likely as a result of some change in the body's handling of the gas phase, whether anatomical, biochemical, or a combination of both.

In summary, acclimatization to decompression stress may indeed exist, but objective evidence remains elusive. The change in the quantity of VGE with repetitive daily exposures is not significantly different from zero, but may range from $-4.5$ to $+9.5\%$ per day. If acclimatization does occur, the mechanism probably does not reside in any reduction in the gas phase produced by a given series of decompressions, but rather in the organism's handling of the gas phase.

## Acknowledgments

Supported by Naval Medical Research and Development Command Work Unit No. MR000.01.01–5094. The opinions and 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.

The authors gratefully acknowledge the assistance of: the subjects, for participating in this somewhat tedious and not altogether stimulating experiment; B. Eatock, S. MacDonald, D. Eastman, and G. Carrod of DCIEM, Toronto, Canada, for analysis of the Doppler tapes; G. Masurel of CERTSM, Toulon, France, for providing Doppler equipment; J. Jordan, J. Leonard, and K. Smith for chamber operations; J. Parker for assistance with data analysis, and H. Fiske for graphic arts.

## References

1. Paton WDM, Walder DN. Compressed air illness. An investigation during the construction of the Tyne Tunnel, 1948–50. London: Her Majesty's Stationery Office, 1954. (Med Research Council special report no. 281)
2. Walder DN. Adaption to decompression sickness in caisson work. Biometeorology 1966;2:350–359.

3. Hempleman HV. Decompression procedures for deep, open sea operations. In: Lambertsen CJ, ed. Underwater physiology. Proceedings of the third symposium on underwater physiology. Baltimore: Williams & Wilkins, 1967:255–266.
4. Elliott DH. Some factors in the evaluation of oxy-helium decompression schedules. Aerospace Med 1969;40:129–132.
5. Rubenstein CJ. Role of decompression conditioning in the incidence of decompression sickness in deep diving. Washington, DC: Navy Experimental Diving Unit, 1968. (NEDU report No. 12–68)
6. Smedal HA, Graybiel A. Effects of decompression on thirty five subjects repeatedly exposed to a simulated altitude of 20,000 feet during approximately one month. Pensacola: Naval School of Aviation Medicine and Research, 1947. (Reports no. 1 and 2, project no. X–762 Av–390–k)
7. Vann RD, Grimstad J, Nielsen CH. Evidence for gas nuclei in decompressed rats. Undersea Biomed Res 1980;7:107–112.
8. Hills BA. Limited supersaturation versus phase equilibration in predicting the occurrence of decompression sickness. Clin Sci 1970;38:251–267.
9. Nazarkin VYa. A change of animal sensitivity to caisson disease in chronic experiments. Patol Fiziol Eksp Ter 1972;16:88–89.
10. Masurel G, Guillerm R. Kinetics of circulating bubble flow in mini-pigs following hyperbaric exposures to air. Intraindividual variations and $Pi_{CO_2}$ effect. Undersea Biomed Res 1982;9:A25.
11. Lin YC. Species independent maximal no-bubble pressure reduction from saturation dive. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981;699–706.
12. Harvey EN. Bubble formation in animals. J Cell Comp Physiol 1944;24:1–22.
13. Yount DE, Strauss RH. Bubble formation in gelatin: a model for decompression sickness. J Appl Phys 1976;47:5081–5089.
14. Hills BA. Acclimatization to decompression sickness: A study of passive relaxation in several tissues. Clin Sci 1969;37:109–124.
15. Kisman KE, Masurel G, Guillerm R. Bubble evolution code for Doppler ultrasonic decompression data. Undersea Biomed Res 1978;5:A28.
16. Berghage TE, Durman D. U.S. Navy air decompression schedule risk analysis. Bethesda: Naval Medical Research Institute, 1980. (NMRI report no. 80–1)

# SUPERFICIAL ISOBARIC COUNTERDIFFUSION GAS LESION DISEASE: EFFECTS LEADING TO MORTALITY

*J. Pisarello, M. Fried, D. G. Fisher, and C. J. Lambertsen*

The new gas lesion disease, identified and named *superficial isobaric inert gas counterdiffusion syndrome* by its discoverers (1), has proved by detailed investigation in animals to produce continuous venous gas embolism, which is lethal but preventable (2,3). The prior occurrence of the isobaric gas lesion disease in laboratory, and presumably in open-sea diving, went unrecognized partly because it was considered impossible and partly due to confusion with either effects of gas osmosis (4) or decompression (C. J. Lambertsen, personal contact with offshore diving operations). Theoretical bases and kinetics of the superficial isobaric supersaturation have been described (5–8), leading to the distinct impression of the discoverers that more than one mechanism, at an infinite number of sites, is involved (3). The extensive animal experimentation, reinforcing the theoretical bases for gas supersaturation development, has allowed prediction of occurrence, and hence prevention of gas lesion development.

Recognition that a transient *deep tissue* isobaric inert gas counterdiffusion process must lead to potentially prolonged inert gas supersaturation of deep tissue in man when specific inert gases were breathed sequentially (1,9), stimulated search and detection in animals of transient venous gas embolism during sequential isobaric inert gas administration (10). It is not yet known whether this phenomenon produces pathological effects in man; hence it is not yet appropriate to designate it a human disease.

A special feature of the superficial isobaric inert gas counterdiffusion process, occurring in the *stable condition* of respiring one inert gas-oxygen mixture while surrounded by a specific different inert gas, is that continuous venous gas embolism occurs, which may lead to death (2,3). Concurrently,

there develops a pattern of dermal subcutaneous gas lesions, which proceed to severe damage to subcutaneous structures (3), as well as incapacitating vestibular derangement (1).

Of additional pertinence to diving, decompression, and therapy of decompression sickness is the clear warning that both superficial and deep tissue forms of isobaric counterdiffusion necessarily interact with and can aggravate tissue supersaturations which occur in decompression and the gas lesions which occur in decompression sickness (11). This interaction has been demonstrated for deep tissue counterdiffusion and decompression in animals (unpublished study, Ashkar, De Long, Lambertsen, Institute for Environmental Medicine).

Against this background the present program of investigation is designed to determine effects of the superficial isobaric gas lesion disease, which lead to fatality. In view of the complex, massive, and lethal consequences of the overall process, the program necessarily has several facets. This preliminary report concerns only aspects of circulatory function in a series of experiments as described.

## CIRCULATORY EFFECTS OF SUPERFICIAL INERT GAS COUNTERDIFFUSION

Anesthetized pigs artificially ventilated with $N_2O$-$O_2$ mixture at 1 ATA and exposed to helium over the trunk and extremities were studied until death or during multihour periods of venous gas embolism. Intravascular bubbles were monitored by a Doppler detector placed surgically around the inferior vena cava.

No significant circulatory changes occurred prior to the beginning of gas embolism, which developed on the average at about 1.5 h after initiation of the counterdiffusion process.

With the appearance of intravascular bubbles, pulmonary arterial pressure rose and remained elevated until death. At the same time, tachycardia developed and persisted until death.

In approximately half of the animals in this series, these were the only circulatory changes until sudden hypotension and death occurred. In the rest of the animals, however, after a variable period of time, a progressive decrease in cardiac output was observed. Ultimately, systemic arterial pressure began to fall and death ensued (Figs. 1 and 2).

In both groups, concurrent with the presence of intravascular bubbles, a steady rise in hematocrit continued to the time of death.

The post-mortem examinations showed effects cited in previous studies (3,12). While all animals showed massive amounts of gas bubbles in the venous circulation and the right heart chambers, some also showed a large volume of free gas in the left ventricle and the arterial circulation, suggesting that pulmonary capacity of bubble *filtering* had been overwhelmed.

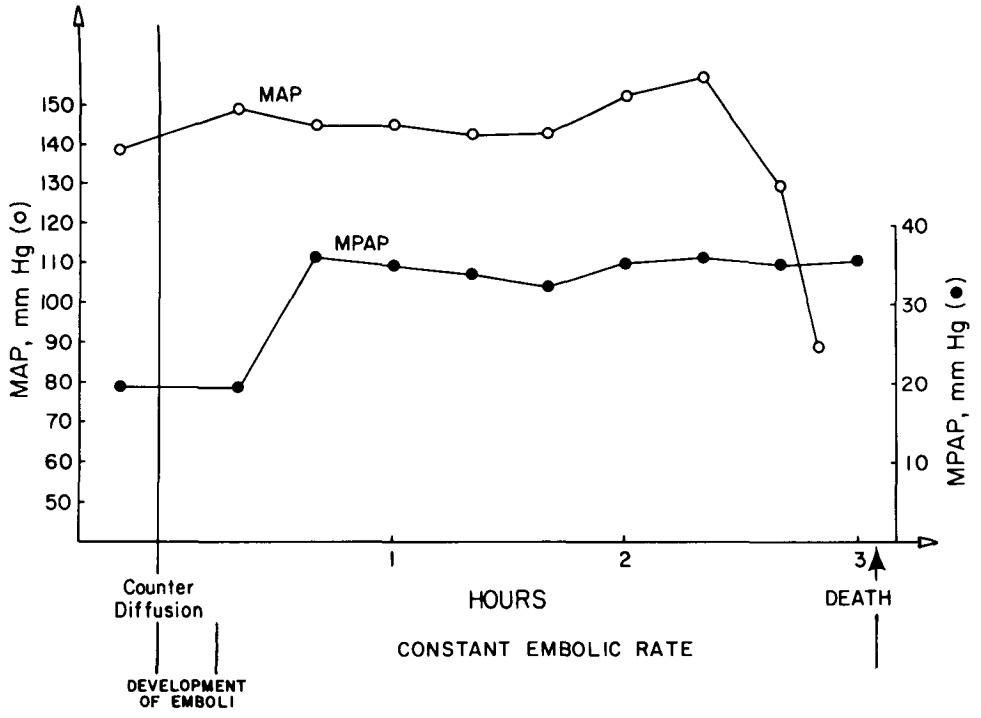



Fig. 1. Time course changes in mean systemic and mean pulmonary artery pressures in a pig breathing $N_2O$ and $O_2$ 78/22 while trunk and extremities were surrounded by helium at 1 ATA. Bubbles were detected in the inferior vena cava after 20 min of counterdiffusion. Death occurred after 3 h, 10 min of counterdiffusion.

## INTERPRETATION

From these preliminary observations, it would appear reasonable to speculate that at least two mechanisms related to the presence of bubbles in blood are operative in producing the observed deaths in these animals.

### Arterial Embolism

Although animals in this series demonstrated a remarkable tolerance to large amounts of venous gas embolism, through elimination of bubbles by the lungs, the occurrence of massive undissolved arterial gas also was documented at the time of autopsy. Possible origin of these bubbles may include de novo generation in arterial blood, or, more likely, passage through the lungs when some critical conditions develop, overcoming their filtering capacity. Whatever

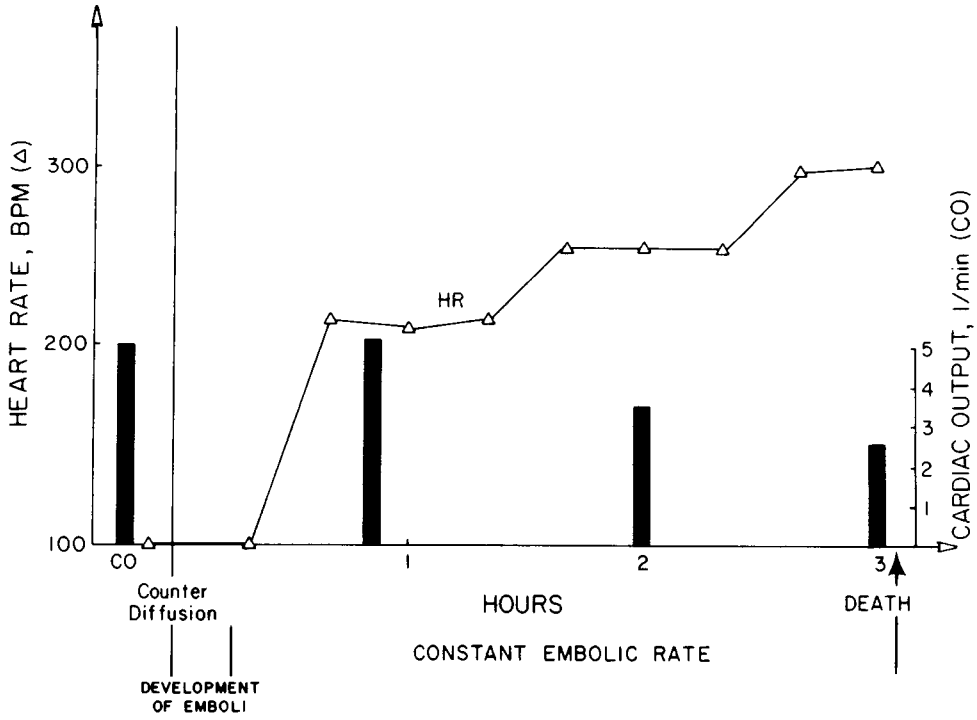



Fig. 2. Time course changes in heart rate and cardiac output in the same pig as in Fig. 1.

the mechanism, once free gas is present in arterial blood, embolism to the coronary or cerebral vessels could induce pathology of sufficient severity to produce the observed death (13).

## Reduction of Circulating Volume

The demonstration in some cases of a progessive decrease in cardiac output in the presence of tachycardia and no increase in central venous pressure, along with an increase in values of hematocrit that happened in all cases, is consistent with reduction of circulating volume due to plasma loss. A similar combination has been associated with severe decompression sickness (14,15).

As this process continues, the reduction of circulating volume may be of enough degree to induce hypovolemic shock and circulatory failure with inability to sustain life.

## SUMMARY

The nature and extent of injury induced by superficial counterdiffusion on different systems and organs, usually but not always leading to death, have

revealed the complexity and severity of this new disease process. Concurrently, many unresolved issues of particular interest and significance remain. Some of these include:

1) Failure of generation of intravascular embolism in some of the pigs so far studied. In addition, attempts to induce embolism in dogs have been, to present, consistently unsuccessful, even after 16 h of $N_2O$-He counterdiffusion at 1 ATA, and in spite of the development of large amounts of free subcutaneous gas.

2) Absence of superficial lesions on mucosal surfaces (3) and specific cutaneous areas.

3) Individual differences in susceptibility, illustrated by absence of symptoms in humans exposed to conditions that profoundly affected other subjects (1). In addition, when whole body superficial $N_2O$-He counterdiffusion in pigs at 1 ATA proved a rapidly fatal process in some cases, a few animals survived up to 26 h of continuous intravenous embolism.

As an experimental model, isobaric counterdiffusion provides the means for investigating biophysical questions, such as critical supersaturation levels and bubble growth and resolution (16). In addition, the generation of stable and readily produced gas embolism by superficial counterdiffusion in survival experiments at the surface may provide an important research tool for investigating pathophysiologic effects of in vivo venous gas embolism, superior in some respects to the uncontrollable decompression-induced embolism, and to artificial infusion of bubbles, which ignores contributions of the effects of gas dissolved in extravascular tissues. Of particular importance, this model allows systematic experimental approach to such areas as in vivo bubble activation of different plasma systems, as well as the complex and significant effects of gas embolism on pulmonary function.

Because of the many similarities between counterdiffusion-induced embolism and decompression embolism, experimental information derived from the isobaric model may result in a substantial contribution to the understanding of the pathophysiology of decompression sickness. Identification and detailed characterization of the multiple mechanisms involved in this complex pathology will then provide a rational basis for improving present techniques of its prevention and therapy.

## References

1. Lambertsen CJ, Idicula J. A new gas lesion syndrome in man induced by "isobaric gas counterdiffusion." J Appl Physiol 1975;39:434–443.
2. Lambertsen CJ, Idicula J. Cutaneous gas lesions and continuous, lethal gas embolization in animals due to isobaric inert gas counterdiffusion. Fed Proc 1974;33:455.
3. Lambertsen CJ, Idicula J, Cunnington J. Pathophysiology of superficial isobaric counterdiffusion. Philadelphia: Institute for Environmental Medicine Report, 1982.
4. Blenkarn GD, Aquadro C, Hills BA, Saltzman A. Urticaria following the sequential breathing of various inert gases at a constant ambient pressure of 7 ATA: A possible manifestation of gas induced osmosis. Aerospace Med 1971;42:141–146.

5. Graves DJ, Idicula J, Lambertsen CJ, Quinn JA. Bubble formation resulting from counterdiffusion supersaturation. A possible explanation for isobaric inert gas "urticaria" and vertigo. Phys Med Biol 1973;18:256–264.
6. Karreman G, Lambertsen CJ. Kinetics of isobaric counterdiffusion. Bull Math Biol 1977;39:587–595.
7. Imbert JP. A perfusion-limited model for isobaric counterdiffusion. Philadelphia: Institute for Environmental Medicine Report, 1975.
8. Hills BA. Supersaturation by counterperfusion and diffusion of gases. J Appl Physiol 1977;42:758.
9. Harvey CA, Lambertsen CJ. Deep tissue isobaric inert gas exchange: Predictions during normoxic helium, neon, and nitrogen breathing by men at 1200 feet sea water. In Shilling CW, Kent MB, eds. Underwater physiology VI. Bethesda: Federation of American Societies for Experimental Biology, 1979:343–358.
10. D'Aoust BG, Smith KH, Swanson HT, White R., Harvey CA, Hunter WL, Newman TS, Goad RF. Venous gas bubbles: Production by transient deep isobaric counterdiffusion of helium against nitrogen. Science 1977;197:889–891.
11. Lambertsen CJ. Advantages and hazards of gas switching: Relation of decompression sickness therapy to deep and superficial isobaric counterdiffusion. In Lambertsen CJ, ed. Decompression sickness and its therapy. Allentown: Air Products and Chemicals, Inc., 1979.
12. Cunnington JPW, Lambertsen CJ. Quantitation of spontaneous intravascular gas embolism during superficial isobaric gas counterdiffusion. Philadelphia: Institute for Environmental Medicine Report, 1981.
13. Lambertsen CJ, Cunnington JPW, Cowley JRM. The dynamics and composition of spontaneous, continuous gas embolism in the pig during isobaric gas counterdiffusion. Fed Proc 1975;34:452.
14. Bove AA, Hallenbeck JM, Elliott DH. Changes in blood plasma volume in dogs during decompression sickness. Aerosp Med 1974;45:49–55.
15. Barnard EEP, Hanson JM, Rowton-Lee MA, Morgan AG. Postdecompression shock due to extravasation of plasma. Br Med J 1966;2:154–155.
16. D'Aoust BG, Lambertsen CJ. Isobaric gas exchange and supersaturation by counterdiffusion. In Bennett PB, Elliott DE, eds. The physiology and medicine of diving. London: Baillière Tindall, 1982:383–403.

# CONDITIONS FOR HETEROGENEOUS NUCLEATION IN THE PHYSIOLOGICAL ENVIRONMENT

*P. Tikuisis and W. R. Johnson*

The genesis of bubble formation leading to decompression sickness is a problem that continues to evade many research efforts. Several models of bubble formation have been reviewed (1) and can be summarized by the following classifications: a) de novo formation, b) critical volume concepts, and c) pre-existing gas nuclei. The latter two classifications circumvent the complex question of gas phase separation. Nevertheless, a knowledge of the parameters involved in this de novo event is required to correctly model the growth of the gas phase even if the nucleation itself appears insignificant. In this paper, we examine the conditions under which heterogeneous nucleation (which is thermodynamically favorable over homogeneous nucleation) can occur in the physiological environment.

## THEORETICAL DEVELOPMENT

According to classical nucleation theory (2), there is a constant production of gas nuclei in liquids. However, unless a gas nucleus grows beyond the critical size of the liquid-gas solution, the nucleus will dissolve back into solution. The critical size is characterized by the radius, $R_c$, which is a property of the liquid-gas solution and can be expressed as (3)

$$R_c = 2\gamma/(P^T - P^A), \qquad (1)$$

where $\gamma$ is the liquid-gas interfacial surface tension, $P^T$ represents the pressure at which the gas phase is in equilibrium with the liquid-gas solution, and $P^A$ is

the ambient pressure. $P^T$ is a composite of the inert gas and any metabolic gases dissolved in the fluid or tissue. We therefore write

$$P^T = P_I^T + P_m, \tag{2}$$

where $P_I^T$ represents the inert component, hereafter referred to as the inert gas tension, and $P_m^T$ is the balance. The inert gas tension can be expressed in terms of the critical radius by combining *Eqs. 1* and *2*, i.e.,

$$P_I^T = P^A - P_m^T + 2\gamma/R_c. \tag{3}$$

The degree of gas saturation, S, is defined as the ratio of the inert gas tension over ambient pressure and therefore becomes

$$S = 1 - P_m^T/P^A + 2\gamma/R_cP^A. \tag{4}$$

To evaluate $P_m^T$, we consider the *inherent unsaturation* in living tissue, defined as the difference between the ambient pressure and the sum of dissolved gases under conditions of inert gas equilibrium (4). Assuming that the ambient composition comprises of oxygen and the inert gas, the inherent unsaturation can, therefore, be expressed as

$$\Delta p = PO_2^A - P_m^T, \tag{5}$$

where $PO_2^A$ is the ambient partial pressure of oxygen. According to the data of Hills and LeMessurier (5)), the inherent unsaturation can be approximated by

$$\Delta p = -P_v + 0.9\ PO_2^A, \tag{6}$$

where $P_v$ is the vapour pressure of water at 37°C. The expression for $P_m^T$, obtained by combining *Eqs. 5* and *6*, is therefore given by

$$P_m^T = P_v + 0.1\ PO_2^A, \tag{7}$$

which has a value of 0.0084 MPa for aqueous tissue at 37°C and at normal surface conditions.

We now consider the crevice model (6), shown in Fig. 1, for the nucleation of the gas phase. The rate of formation of a critical-sized nucleus, that is, one whose radius of curvature of the liquid-gas interface equals $R_c$, is given by

$$J = K_N \exp(-\Delta F/kT), \tag{8}$$

where $K_N$ is the rate constant, $\Delta F$ is the change in the Helmholtz potential during the formation of the critical sized nucleus, $k$ is the Boltzmann constant and T is the temperature. The rate constant, $K_N$, is given by (7)

$$K_N = kT(\pi W^3/3h \tan\alpha) \sum_i c_{io}, \tag{9}$$

where W is the radius of the mouth opening of the conical crevice, $h$ is Planck's constant, $2\alpha$ is the apex angle of the crevice and $c_{io}$ is the initial concentration of the i-th component in liquid-gas solution. The change in the

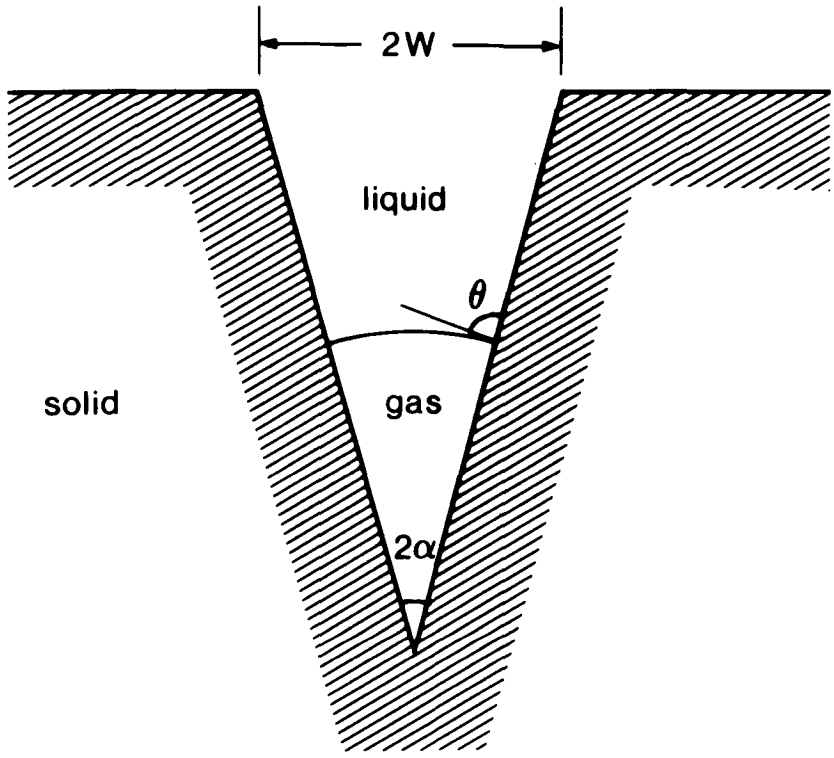



Fig. 1. Schematic of the conical crevice, where 2α is the apex angle, 2W is the diameter of the mouth opening, and θ is the contact angle between the liquid-gas interface and the crevice wall as measured through the liquid. The curvature of the liquid-gas interface of the critical-sized nucleus is defined by the critical radius, $R_c$, of the liquid-gas solution.

Helmholtz potential during the formation of the critical sized nucleus is expressed by (8)

$$\Delta F = \pi\gamma R_c\,(-2g_1/3 + 2g_2 + g_3), \tag{10}$$

where $g_1 = 2 - 3\sin(\theta-\alpha) + \sin^3(\theta-\alpha) + \cos^3(\theta-\alpha)\cot\alpha$, (11)

$$g_2 = 1 - \sin(\theta-\alpha), \tag{12}$$

and

$$g_3 = \cos^2(\theta-\alpha)\csc\alpha\cos\theta. \tag{13}$$

The rate of nucleation is quite sensitive to the change in the Helmoltz potential; that is, a small change in $\Delta F$ leads to order of magnitude changes in J as defined by *Eq. 8*. The conditions for nucleation are therefore not greatly affected by the rate itself. We choose a rate of unity to represent the onset of

nucleation in this study. Hence, from *Eqs. 8* and *10*, we obtain the expression of the critical radius for the nucleation of the gas phase in the conical crevice as

$$R_c = \{kT \ln(K_N)/\pi\gamma(-2g_1/3 + 2g_2 + g_3)\}^{1/2}. \quad (14)$$

Substitution of *Eq. 14* into *Eq. 4* will give the expression for the threshold gas supersaturation ratio, $S_n$, required for the formation of the critical sized nucleus.

## RESULTS

We consider a decompression to normal sea level values and assume a constant temperature of 37°C. In this circumstance, the expression for the threshold supersaturation ratio for nucleation, given by *Eq. 4*, becomes

$$S_n = a + b\gamma/R_c, \quad (15)$$

where $a$ equals 0.917 and $b$ equals 19.74 $MPa^{-1}$.* This reduces the number of parameters of our study to four; namely, the interfacial surface tension $\gamma$, the contact angle between the liquid-gas interface and the solid-gas interface as measured through the liquid, $\theta$, the radius of the crevice opening, W, and the apex angle in the crevice, $2\alpha$. The logarithmic dependence of $R_c$ upon W, through the rate constant $K_N$, is small and therefore the dependence of $S_n$ upon W is also small. The importance of W lies with the outgrowth or emergence of the bubble from the crevice, which is discussed further later. We will now examine the effects of the three remaining parameters on the value of $S_n$.

Figure 2 shows the values of $S_n$ plotted against $\theta$ for various values of $\gamma$. Although W and $\alpha$ are chosen to have values of 1.0 $\mu$m and 2°, respectively, the results are representative of the general dependence of $S_n$ upon $\gamma$. Low levels of gas supersaturation are required for heterogeneous nucleation only if $\gamma$ is small. Substances such as lung surfactant exhibit low values of $\gamma$ (10), but are not considered to be a "bulk" fluid. For more common fluids such as plasma, whose surface tension is about 50 dynes/cm, the sensitivity of $S_n$ upon $\theta$ is such that heterogeneous nucleation at modest gas supersaturations could only occur if the surface is hydrophobic, that is, for values of $\theta$ of 90° or greater. Note that for a half apex angle of 2°, the curvature of the liquid-gas interface in the crevice remains convex upwards, as shown for example in Fig. 1, for contact angles of less than 92°. This is true in general provided that the difference, $\theta - \alpha$, is less than 90°. If this difference approaches 90°, as is

*At high ambient pressures, the term $P_m^T/P^\wedge$ in *Eq. 4* becomes negligible and therefore the value of $a$ can be approximated by unity. In this circumstance, it can be shown that our model predicts that heterogeneous nucleation requires a specific difference between the gas tension and the ambient pressure and that this difference is independent of the ambient pressure. This feature is also characteristic of homogeneous nucleation (9).

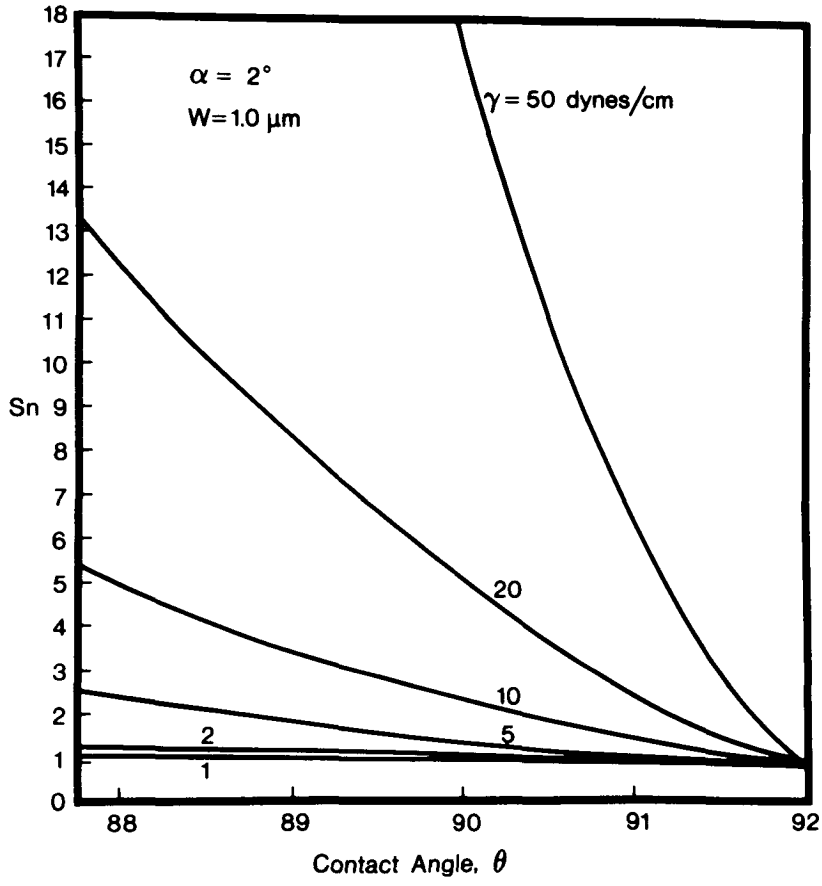



Fig. 2. Threshold gas supersaturation ratio, $S_n$, required for heterogeneous bubble nucleation as plotted against the contact angle, θ, for various values of the interfacial surface tension, γ; for a crevice with one-half the apex angle, α, equal to 2°; and a mouth opening of radius, W, equal to 1.0 μm.

shown in Fig. 2 for θ approaching 92°, then the liquid-gas interface becomes flat. Consequently, the Laplace pressure term, $2\gamma/R_c$, becomes unimportant and all curves of varying γ values converge towards a common point, that is, towards the value of *a* in *Eq. 15*. If the difference, $\theta - \alpha$, exceeds 90°, then the crevice will maintain a gas nucleus whose curvature is concave upwards. Such a configuration is thermodynamically stable under any degree of gas saturation (7), hence, the stabilizing mechanism for "pre-existing" gas nuclei, and theoretically provides a ready source for the formation of gas bubbles upon gas supersaturation. The only barrier to the emergence of such bubbles is the extent of the crevice mouth opening, which we consider further below.

The dependence of $S_n$ upon α, shown in Fig. 3, is determined by the contact angle θ. If the contact angle equals or exceeds 90°, then the value of

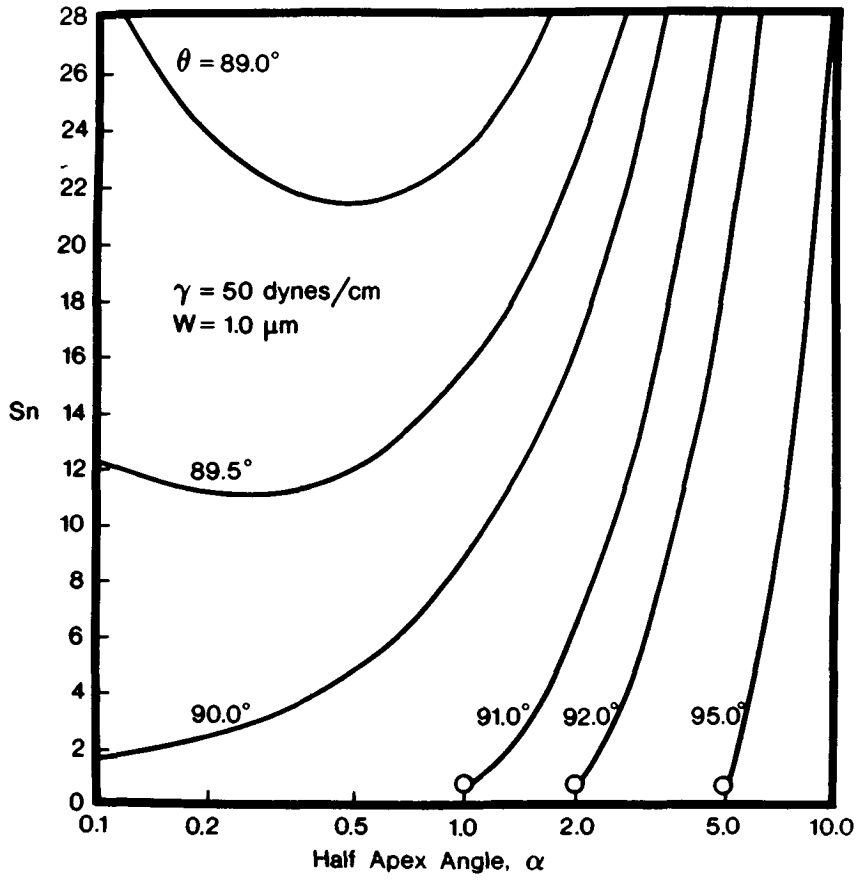



Fig. 3. Threshold gas supersaturation ratio, $S_n$, required for heterogeneous bubble nucleation as plotted against one-half the apex angle, $\alpha$, for various values of the contact angle, $\theta$; for an interfacial surface tension, $\gamma$, equal to 50 dynes/cm; and a crevice with a mouth opening of radius, W, equal to 1.0 μm.

$S_n$ is reduced by decreasing the apex angle. The *open circles* in Fig. 3 indicate the point at which the liquid-gas interface becomes flat. For contact angles of value less than 90°, there is a minimum value of the threshold gas supersaturation ratio for nucleation, which can be determined by differentiating the expression for $S_n$ by $\alpha$ and equating the result to zero. In essence, this minimum value of $S_n$ increases rapidly as the contact angle decreases in value from 90°.

Finally, we consider the emergence of a bubble from a crevice. Once the gas nucleus reaches the mouth of the crevice, the radius of the liquid-gas interface begins to decrease. This radius continues to decrease until a minimum is reached whose value is equal to the radius of the mouth opening of the crevice, i.e., W. This constraint imposes a minimum gas supersaturation

ratio for the emergence of the nucleated bubble denoted as $S_g$ and is expressed by

$$S_g = a + b\gamma/W. \quad (16)$$

Accordingly, this added constraint for bubble formation is not affected by either $\theta$ or $\alpha$. Depending upon the value of W, however, the value of $S_g$ may be less or greater than the threshold requirement for nucleation. This can be better appreciated by comparing the predictions from *Eqs. 15* and *16*. For example, for an interfacial surface tension of 20 dynes/cm, a contact angle of 90°, an apex angle of 5°, and a crevice mouth opening of 0.2 μm, the value of $S_n$ is 6.31, according to *Eq. 15*, and the value of $S_g$ is 4.86, according to *Eq. 16*. In this circumstance, the threshold gas supersaturation ratio for nucleation exceeds the supersaturation ratio required for the emergence of the bubble; hence, bubble production in this instance requires only that the nucleation threshold be met. On the other hand, if the crevice mouth opening is halved to 0.1 μm while maintaining the other parameters unchanged, then $S_g$ increases to 8.81, whereas $S_n$ increases only slightly to 6.42. Therefore, although the conditions for heterogeneous nucleation may be met in this circumstance, the bubble will not appear unless the gas supersaturation ratio exceeds 8.81. Consequently, a gas bubble may form and remain stabilized within the crevice until the ambient conditions alter, i.e., further decompression, to promote the emergence of a bubble. Figure 4 shows the gas supersaturation ratio required for the emergence of the bubble plotted against W for various values of the interfacial surface tension.

It is instructive to note the geometric relationship between $\alpha$ and W. On the one hand, heterogeneous nucleation is enhanced for small values of $\alpha$; however, for the emergence of the bubble the crevice mouth opening cannot be too small. Thus, the most favored geometry for bubble formation is a narrow yet deep conical crevice.

## DISCUSSION

The formation of gas bubbles in decompressed rats is believed to occur where the gas supersaturation ratio might be as small as 3 or 4 (11) and has been observed in guinea pigs for a gas supersaturation ratio as low as 1.69 (12). We view the results described previously as prescribing a very specific set of constraints for which heterogeneous bubble nucleation and bubble emergence can occur in the physiological environment for these ratios. Our results indicate that

1) $\theta$ must be 90° or greater;

2) $\alpha$ must be small, certainly less than 5°;

3) $\gamma$ must be relatively low compared to the interfacial surface tension value between water and air; and

4) W must be of the order of 0.1 μm (greater if $\gamma$ is large).

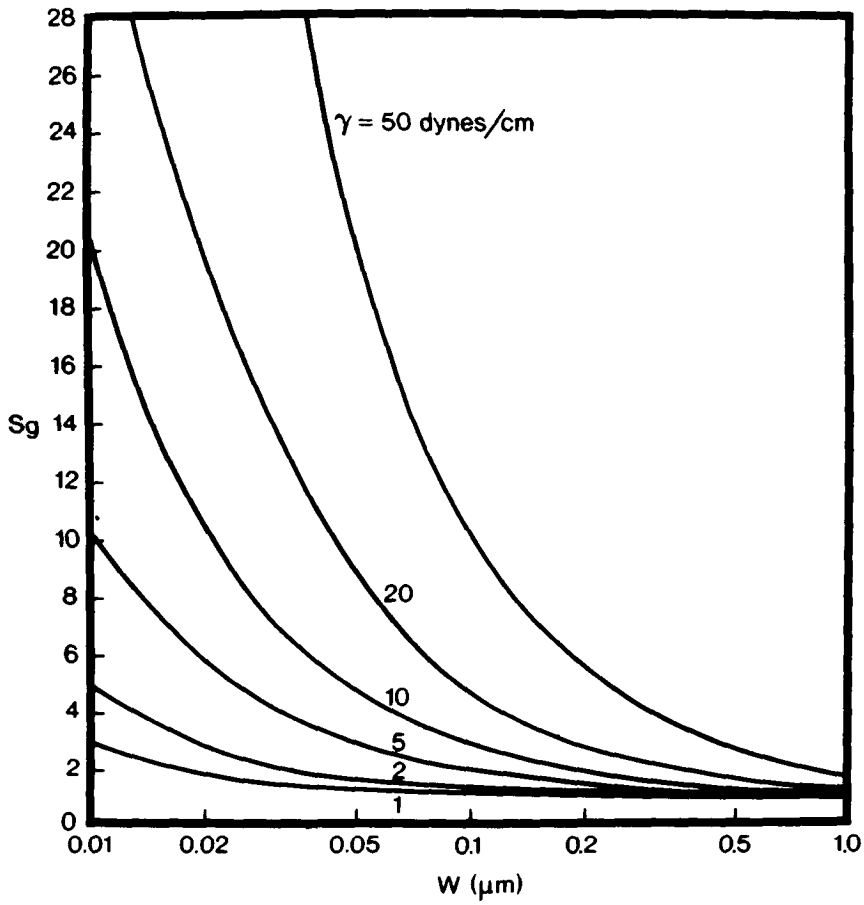



Fig. 4. Threshold gas supersaturation ratio, $S_g$, required for the emergence of a bubble as plotted against the radius of the crevice mouth opening, W, for various values of the interfacial surface tension, $\gamma$.

These conditions restrict the search for potential nucleation sites to hydrophobic surfaces forming exceptionally rough geometries that are characterized by subcellular dimensions. Thus, our attention is focused not on cellular surfaces per se, but on intercellular components, and points of cellular contact.

The "ruffled" surface geometry of certain subcellular components closely satisfies the requirements for heterogeneous nucleation as predicted by our model. For example, the membrane of mitochondria is an undulating lipid surface whose crevices have mouth openings typically less than 0.2 μm in radius and apex angles less than 2° (13). For an interfacial surface tension value of 50 dynes/cm, heterogeneous nucleation could readily occur in a conical crevice having this geometry when the gas supersaturation ratio is between 3 and 4 if the contact angle has a value of about 90.5°. However, in

order for the nucleated bubble to emerge from the crevice with this degree of gas supersaturation, an interfacial surface tension value of between 2 and 5 dynes/cm is required, as determined by *Eq. 16* and shown in Fig. 4. The hypothesis that mitochondria could be a nucleation site is reinforced by the work of Bennett (14).

Still another undulating surface where nucleation might occur is the ruffled border of the membrane of osteoclasts found in bone and represented schematically in Fig. 5. The undulations in this membrane typically have dimensions somewhat larger than those of the subcellular mitochondria (15). Therefore, we expect the gas supersaturation required for heterogeneous nucleation here to be greater than in the previous case. Indeed, for a conical crevice with an apex angle of 6° and mouth opening of 0.2 μm, and assuming a contact angle of 90.5°, our model predicts that gas supersaturation ratios

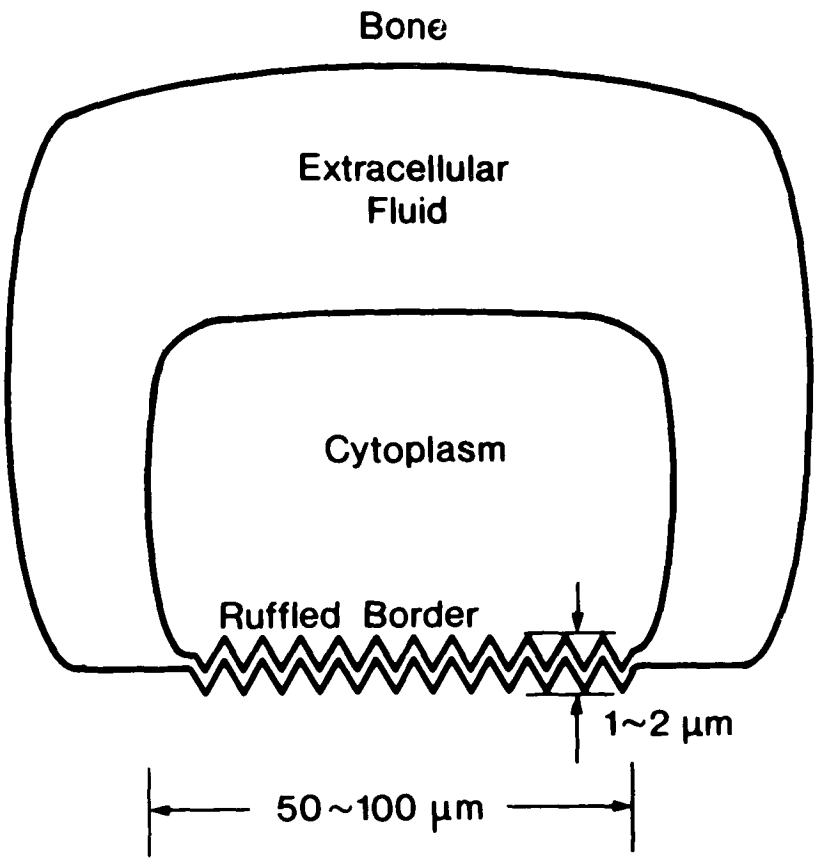



Fig. 5. Schematic of the ruffled border of the osteoclast in close contact with the bone separated by a thin layer of extracellular fluid.

ranging between 1.5 and 20.4 are necessary for interfacial surface tension values ranging between 5 and 50 dynes/cm, respectively. Emergence of the nucleated bubble for these surface tension values require minimum gas supersaturation ratios of 1.9 and 10.8, respectively. Therefore, for the assumed conditions of the ruffled border of the osteoclast, bubble production is governed by the emergence from the crevice if the interfacial surface tension has a value of 5 dynes/cm and is governed by the nucleation threshold if the value is 50 dynes/cm.

Heterogeneous nucleation within the osteoclast may cause sufficient pressure to cause bone damage. This damage could occur because the osteoclast can be modelled essentially as a closed-volume to a rapidly expanding gas bubble. The pressure in the osteoclast, once a bubble has formed, could theoretically rise to nearly the original saturation pressure (8). Therefore, if the cell lies close to the outer surface of the bone, say within a few 10's of micrometers, the bone is then subject to mechanical fracture. This prediction is consistent with recent histological observations of inner-ear bone damage in squirrel monkeys (16). Bone segments were found within the semicircular canals, a finding suggesting that these canals suffered an implosive fracture. This can be accounted for by the forces that originate in the osteoclast cells because of over-pressurization by an expanding bubble.

The required geometry for heterogeneous nucleation can also be realized in the contact between lipid membrane surfaces. Two possible candidates may be found in the Rouleaux formation of red blood cells and in the contact between adjoining cells such as those in the endothelium. In such instances where the conical crevice geometry may be present, the apex angle approaches zero at the contact point and the probability of heterogeneous nucleation at a given gas supersaturation increases. This readily can be seen in Fig. 3. The possibility of bubble emergence, however, is not exactly clear. This is exemplified in Fig. 4 where we have observed that the narrower the crevice mouth opening, the less likely will the bubble emerge. The emergence of the bubble may be resolved, however, if during the nucleation process, a distortion of the malleable cells takes place and in turn the geometry of their contact is altered in such a manner as to increase the crevice mouth opening. Thus, on one hand, close contact of cells will enhance the nucleation process, yet, on the other hand, their separation will favor emergence of the nucleated bubble. The exact mechanism for the distortion of the cells is undoubtedly complex for while the high pressure of the expanding bubble tends to separate the cells further, the surface tension of interfaces tends to draw the cells together. A bubble stabilized between two cells may emerge if the cells become separated by other means, for instance, when the Rouleaux formation of red blood cells is destroyed upon entering a high shear flow field.

Lastly, the model presented here indicates that in those locations in the physiological environment where the dimensions are quite small, with particular attention to the apex angle, heterogeneous nucleation is more likely to occur and to produce a stable bubble. Some macromolecules such as fibrinogen and globulins have dimensions of several 10's of nanometers. The

shape of these macromolecules in the blood has been investigated by numerous researchers (e.g., Ref. 17) with no definitive conclusion forthcoming. However, if the proponents of the hypothesis that fibrinogen has a convoluted shape are correct, then this particular blood protein would be worthy of investigation as a nucleation site.

In conclusion, heterogeneous nucleation and bubble emergence may readily occur during decompression in certain identifiable locations in the body. The geometrical constraints for nucleation, although severe, are satisfied in ruffled surfaces and lines of contact between surfaces. No less severe are the constraints on the surface tensions and contact angles at the candidate nucleation sites mentioned above. While most surface tensions of biological substances have been measured to be in the 10's of dynes/cm, reports of surface tensions as small as 2 dynes/cm have been reported (10). Contact angles have been measured for certain biological specimens such as bacteria (18), however, measurements for cellular membranes, as addressed in this paper, are virtually nonexistent. To completely understand the phenomena of heterogeneous nucleation and bubble emergence during decompression, one must quantify the two parameters of interfacial surface tensions and contact angle; they deserve more attention than they have received in the past.

## Acknowledgment

This study represents DCIEM publication No. 83-P-25.

## References

1. Weathersby PK, Homer LD. Current concepts of inert gas exchange and decompression. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:687–698.
2. Volmer M. Kinetics of phase formation. In: Bonhoeffer LF, ed. Chemical Reaction: Vol. IV (Translated by Intelligence Dept., AMC F–TS–7068), 1939:139–154.
3. Ward CA, Balakrishnan A, Hooper FC. On the thermodynamics of nucleation in weak gas-liquid solutions. Trans ASME 1970;92:695–704.
4. Hills BA. Decompression sickness. Vol. 1: The biophysical basis of prevention and treatment. Chichester: John Wiley and Sons, 1977:239.
5. Hills BA, LeMessurier DH. Unsaturation in living tissue relative to the pressure and composition of inhaled gas and its significance in decompression theory. Clin Sci 1969;36:185–195.
6. Harvey EN, Barnes DK, McElroy WD, Whiteley AD, Pease PC, Cooper KW. Bubble formation in animals 1. Physical factors. J Cell Comp Physiol 1944;24:1–22.
7. Fisher BA. The fracture of liquids. J Appl Phys 1948;19:1062–1067.
8. Ward CA, Johnson WR, Venter RD, Ho BN, Forest TW, Fraser WD. Heterogeneous bubble nucleation and conditions for growth in a liquid-gas system of constant mass and volume. J Appl Phys 1983;54:1833–1843.
9. Weathersby PK, Homer LD, Flynn ET. Homogeneous nucleation of gas bubbles in vivo. J Appl Physiol: Respir Environ Exercise Physiol 1982;53:940–946.
10. Enhorning G. Pulsating bubble technique for evaluating pulmonary surfactant. J Appl Physiol: Respir Environ Exercise Physiol 1977;43:198–203.

11. Vann RD, Grimstad J, Nielson CH. Evidence for gas nuclei in decompressed rats. Undersea Biomed Res 1980;7:107–112.
12. Daniels S, Eastaugh KC, Paton WDM, Smith EB. Saturation air dives: A threshold for bubble formation. Undersea Biomed Res 1982; 7(Suppl):A24.
13. Ham AW. Histology. 6th ed. Philadelphia: J. B. Lippincott, 1969:153–156.
14. Bennet RA. Fine structure of decompression sickness. In: Shilling CW, Beckett MW, eds. Underwater physiology VI. Proceedings of the sixth symposium on underwater physiology. Bethesda, MD: Federation of American Societies for Experimental Biology, 1978:595–599.
15. Ham AW. Histology. 6th ed. Philadelphia: J. B. Lippincott, 1969:407–411.
16. Landolt JP, Money KE, Topliff EDL, Nicholas AD, Laufer J, Johnson WH. Pathophysiology of inner ear dysfunction in the squirrel monkey in rapid decompression. J Appl Physiol: Respir Environ Exercise Physiol 1980;49:1070–1082.
17. Marguerie G. The shape of the fibrinogen molecule. Thrombos Haemostas (Stuttg) 1979;41:635–670.
18. Neumann AW. Contact angles and their temperature dependence: Thermodynamic status, measurement, interpretation and application. Adv Colloid Interface Sci 1974;4:105–191.

# MICROSCOPIC STUDY OF BUBBLE FORMATION NUCLEI

*D. E. Yount, E. W. Gillary, and D. C. Hoffman*

Decompression sickness is associated with such modern-day activities as deep-sea diving, flying at high altitudes in unpressurized aircraft, and EVA excursions from spacecraft. The direct cause is a reduction in ambient pressure, which results in supersaturation and in bubble formation in blood or tissue. Bubble formation, in turn, is initiated by microscopic entities, called *nuclei*, which are believed to be present not only in blood and tissue, but also in sea water, tap water, and even distilled water.

The nature of bubble formation nuclei—in fact, their very existence—is still highly controversial. If they did not exist, however, the threshold for bubble formation would be several orders of magnitude higher, and decompression sickness, as well as soda pop, would probably be unknown. The stability of nuclei is demonstrated by the fact that once a liquid has been denucleated, it remains so for extended periods (1,2). A gas filling is implied by the observations that bubble formation thresholds can be significantly raised by degassing or by a preliminary application of static pressure (1,2). Solid or liquid nuclei, containing no gas and being essentially incompressible, would not be affected.

The existence of stable gas nuclei is paradoxical. Gas phases larger than 1 μm in radius should float to the surface of a standing liquid, while smaller ones should dissolve rapidly due to surface tension. In a recent article (3), the earlier proposals for coping with this dilemma are critically reviewed, and a new model, called the *varying-permeability* or *VP model*, is introduced. According to the VP model, cavitation nuclei are stable microbubbles, that is, spherical gas phases that are small enough to remain in solution and strong enough to resist collapse; their stability is provided by elastic skins or membranes consisting of surface-active molecules. Ordinarily, VP skins are gas-

permeable, but they can become impermeable when subjected to large compressions, typically exceeding 8 atm.

By tracking the changes in nuclear radius that are caused by increases or decreases in ambient pressure, the VP model has provided precise quantitative descriptions of several bubble-counting experiments carried out in supersaturated gelatin (2-5). The model has also been used to calculate diving tables (6) and to trace levels of incidence for decompression sickness in a variety of animal species, including salmon (7), rats, and humans (8). In the investigation (4) that comes closest to the one reported here, the primitive size distribution of the objects that facilitate bubble formation in gelatin was systematically altered by passing test samples through *Nuclepore* filters with uniform pore radii of 0.18, 0.27, 0.36, 0.45, and 1.35 μm, accurate to better than ± 10%. From these data it was concluded that the radii calculated in the VP model are those of actual physical structures capable of initiating bubble formation. As in other gelatin experiments (2,3,5), the number density of the nucleating entities was found to decrease exponentially with increasing radius. This is a natural phenomenon, which can be understood and derived from statistical mechanical considerations (9).

## METHODS

On the basis of the above discussion, one might expect that aqueous media in general, and water, gelatin, blood, and tissue in particular, would contain spherical gas nuclei, that is, stable microbubbles with radii on the order of 1 μm or less. The purpose of the experiment reported here was to test this fundamental proposition visually. To eliminate any doubt as to the nature of the objects being viewed, we used both light and electron microscopes. Because nuclei are transparent, ordinary bright-field illumination was poorly suited to our needs. Phase-contrast light microscopes, on the other hand, are especially sensitive to nuclei because gas inclusions of any type produce large changes in refractive index and, hence, in the relative optical path lengths of the *direct* and *scattered* beams. The use of interference-contrast optics enhances the perceptions of depth and three-dimensionality. Dark-field illumination, in which light is scattered at oblique angles by nuclei, is also quite sensitive and produces images that are virtually free of other objects or structures. Electron microscopes permit high resolution and can be used to examine individual nuclei in detail.

Nuclear candidates were observed in distilled water, in Knox gelatin, and in agarose gelatin. Those seen in water and in fresh gelatin "squashed" between a coverslip and a glass microscope slide exhibit Brownian motion and are usually in rapid translation. In some cases, it has been possible to follow a particular candidate for several minutes, thereby demonstrating its stability and a persistence time that is at least two orders of magnitude longer than the theoretical dissolution time for an ordinary gas bubble in water (10). None of the candidate nuclei seen in water or gelatin was observed to dissolve or even to decrease in size.

Fig. 1. Photograph of a 2.5 μm agarose section obtained with an interference-contrast microscope. The shadowing of the candidates is opposite that of the surrounding gelatin, a fact implying that they are gas-filled cavities or microbubbles, rather than solid or liquid inclusions.

focus, this photo is of interest because it contains an unusually high concentration of binary objects. *Nuclear clusters* also occur, and they have a frequency on the order of 2%. Unlike osculating nuclei, the nuclei found in clusters appear to have some boundary surfaces in common and to be associated always with solid (i.e., opaque) debris.

A typical transmission electron micrograph is shown in Fig. 4. The background is homogeneous, and the nuclear candidate appears as a circular hole with clean edges. Ellipsoidal candidates are also seen with this technique, but this is believed to be the result of stretching, which sometimes occurs during the cutting of thin sections.

Most of the results reported in this paper were obtained from thin slices of agarose gelatin (11). Knox or other household gelatins (2,4,5) have the disadvantage that they become moldy after a few days and cannot be conveniently dried and sectioned. Rigid sections were a necessity in our technique for preparing electron micrographs, and although the nuclei seen in water and fresh Knox or agarose gelatin with light microscopes resembled those identified in dried sections of agarose, their rapid motion made it difficult to obtain clear photomicrographs in these substances. Finally, the number densities in agarose were some 5 orders of magnitude higher than in typical samples of distilled water, while those in water were another 1 or 2 orders of magnitude higher than in Knox gelatin. As a result of these enormous differences, the scanning time required to find a nucleus was typically 1 hour or longer in Knox gelatin and several minutes in water, whereas every agarose sample or section contained many of them.

The stiffness of the agarose test material was enhanced by *saturating* the sol with agarose powder, that is, by dissolving as much powder as possible before allowing the sol to cool. Agarose samples photographed with the phase-contrast, interference-contrast, and dark-field techniques were then allowed to dry out at room temperature for several days. This further increased the stiffness and reduced the water concentration from about 95 to 85% w/w. Sections 2.5 μm thick were cut, and no auxiliary processing, such as fixing or staining, was required. Samples destined for the transmission electron microscope, on the other hand, were subjected to a routine procedure normally applied to animal tissue. This included fixing in gluteraldehyde, a postfix in osmium tetroxide, dehydration with ethanol, and a final infiltration of the sample with epoxy resin. Sections of the desired thickness, typically 0.09 μm, were cut from hardened epoxy blocks using a diamond knife.

## RESULTS

Figure 1 is a photograph of a 2.5 μm agarose section obtained with an interference-contrast microscope. As expected, there are a number of candidate nuclei. The largest one has a radius near 1 μm. The shadowing of the candidates is opposite that of the surrounding gelatin, a position implying that they are spherical gas phases or microbubbles, rather than solid or liquid inclusions.

Figure 2 was obtained with dark-field illumination. Although the resolution in this figure is low, the contrast is high and the image is free of objects or structures other than candidate nuclei. Dark-field candidates often have halos and are reminiscent of planetary systems, such as the moons of Jupiter.

About 5% of the identified structures have been classified as *osculating nuclei*, that is, as pairs of nuclei that are just barely touching and appear still to be spherical at the point of contact. This phenomenon is illustrated in the phase-contrast photomicrograph shown in Fig. 3. Although laden with artifacts that are attached to the microscope lens and slide and are therefore out of

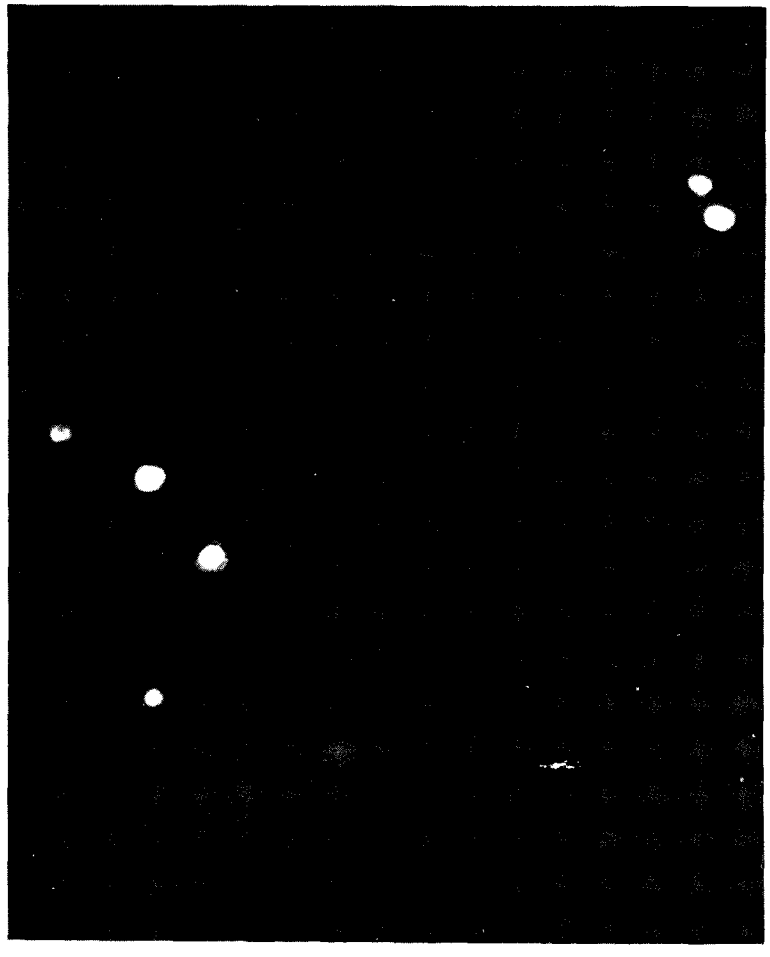


Fig. 2. Dark-field photomicrograph. Advantages of this technique are high contrast and the absence of any structures other than candidate nuclei.

A closer look at a nucleus encumbered by solid debris is provided by the electron micrograph in Fig. 5. The radius of the nucleus itself is about 0.5 μm, and surface contaminants are detectable down to a few 10's of angstroms. In no case have we observed a gas phase embedded in a crevice, as would be required by the crevice model (1), nor do the minute particles that occasionally adhere to a nuclear boundary ever cover a major fraction of the interfacial area.

The differential radial distribution of gas cavitation nuclei in agarose is plotted in Fig. 6 (12). These results were obtained with phase-contrast optics. The scanning efficiency deteriorates rapidly below 0.3 μm, and data in this region should be disregarded. Above 0.3 μm, the results can be described by

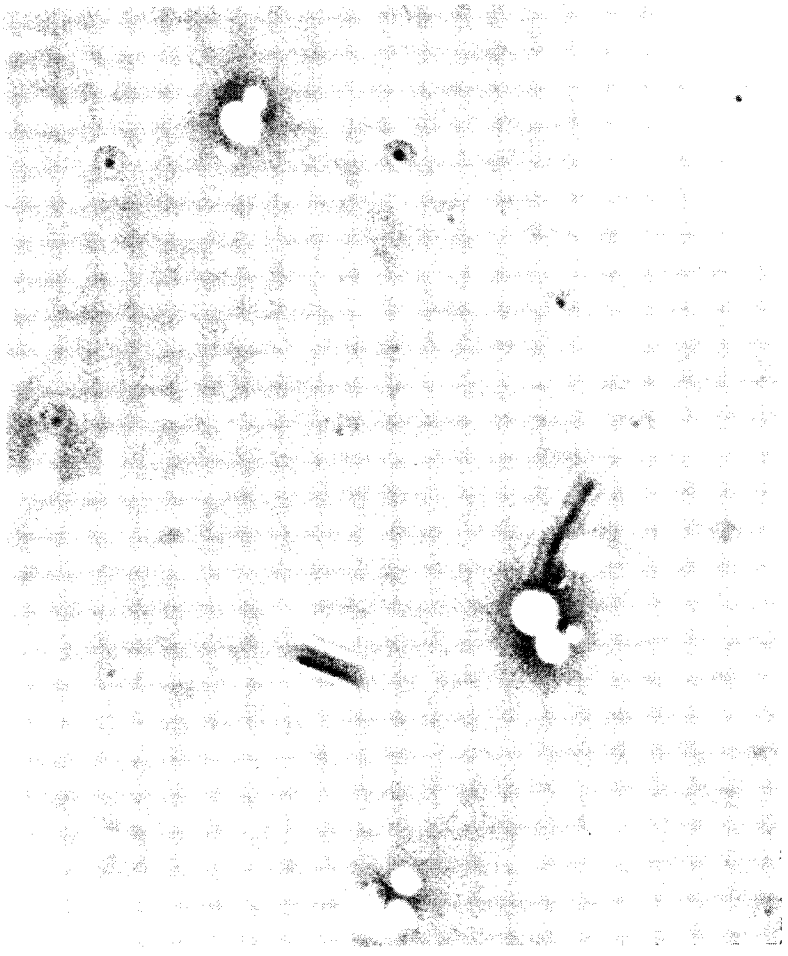


Fig. 3. Phase-contrast photomicrograph showing several examples of *osculating nuclei*. The two *caterpillars* and the *ringed dots* in this figure are artifacts attached to the microscope lens and slide.

a decaying exponential. The $\chi^2$ for 12 bins and 10 degrees of freedom is 7.9. The exponential radial distribution is a signature for bubble formation nuclei, as previously noted (3,4,5,9). Extrapolating the exponential to zero radius, we obtain a total number density on the order of $10^{10}$ nuclei/cm$^3$. The number density is 5 orders of magnitude lower in local samples of distilled water and 6 or 7 orders of magnitude lower in typical samples of Knox gelatin.

## DISCUSSION

Previous experiments with Knox gelatin have demonstrated that although some nuclei are definitely associated with the gelatin crystals, the great

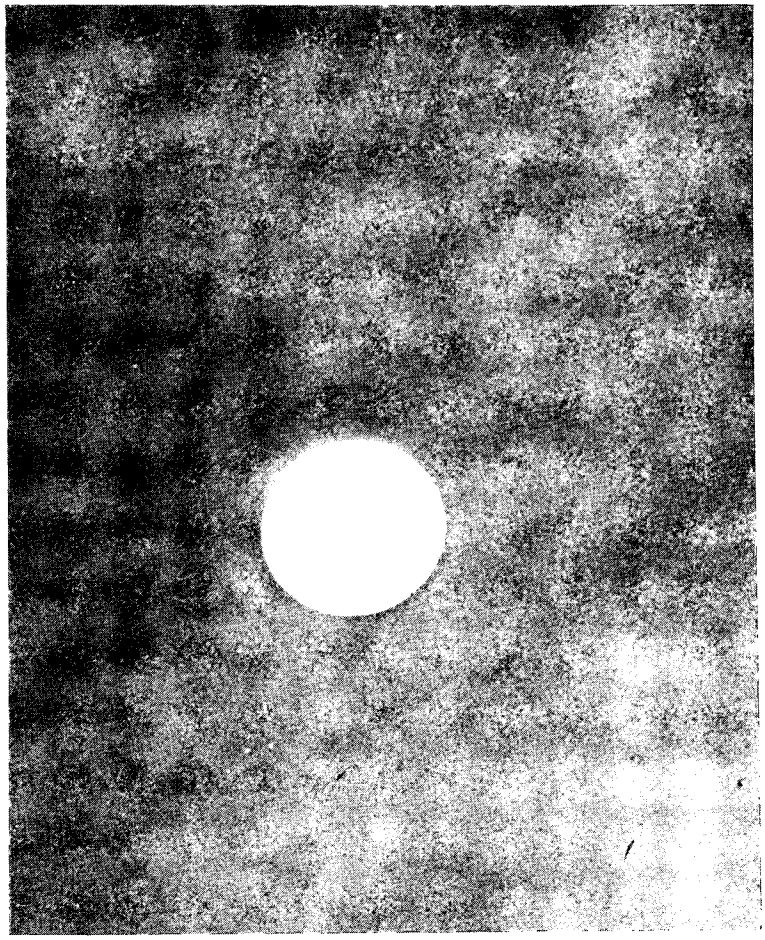


Fig. 4. Transmission electron micrograph. Most candidates detected with this technique appear as circular holes with clean edges. The surrounding medium is normally homogeneous and free of any other structures.

majority were present already in the distilled water used in mixing (2,4,5). Another relevant finding is that some (and perhaps all) specimens of Knox gelatin stock contain substances capable of eliminating nuclei (2,4,5). The microscope studies are consistent with these earlier inferences in that: candidate nuclei were found in both distilled water and Knox gelatin; the number densities were 1 or 2 orders of magnitude higher in distilled water than in Knox gelatin; and the number densities in Knox gelatin were in the range (on the order of $10^3$–$10^4$ nuclei/cm$^3$) that would be expected from the published bubble counts (2,4,5). Therefore, in Knox gelatin, and probably also in distilled water, the correspondence between supercritical nuclei and bubbles appears to be 1:1.

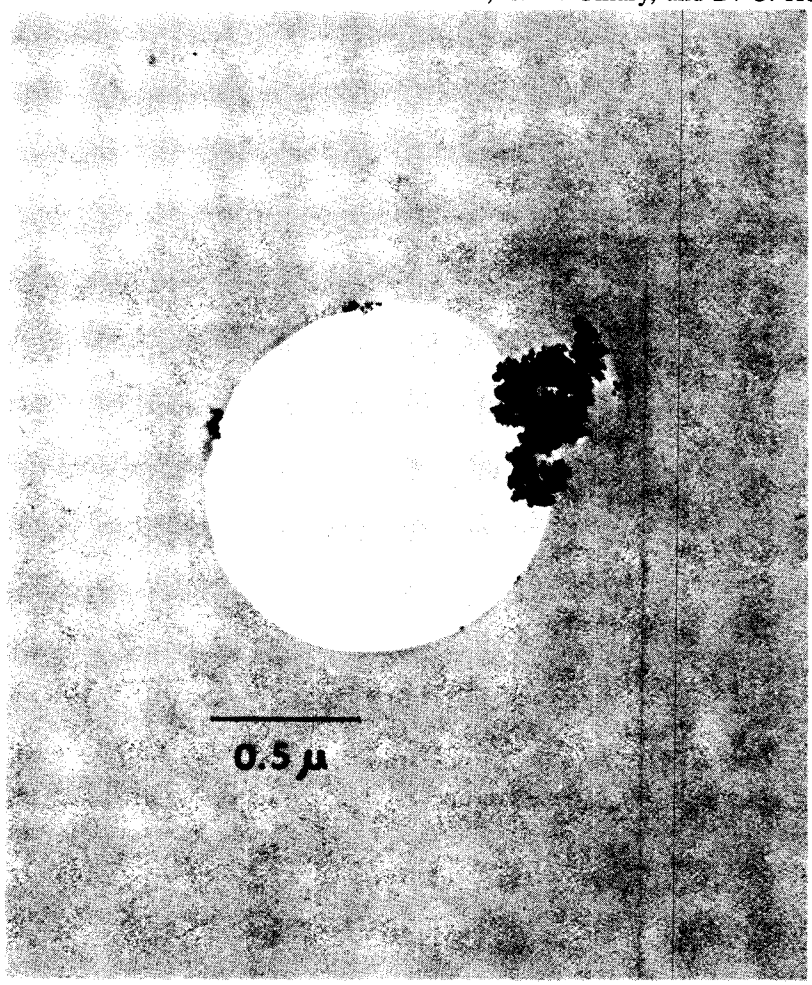



Fig. 5. Electron micrograph of a nuclear candidate encumbered by solid debris. The radius of the candidate nucleus is about 0.5 μm, and surface contaminants are detectable down to a few 10's of angstroms.

The situation in agarose gelatin must be very different. With a number density on the order of $10^{10}$ nuclei/cm$^3$, agarose is *hypernucleated*. For every gross bubble that is counted in a typical experiment, there must be a vast number of supercritical nuclei that begin to grow but quickly lose out in the severe competition for excess dissolved gas. Neighboring cavities rob gas from one another or coalesce to form larger and more viable structures (13). The direct correspondence between supercritical nuclei and gross bubbles is lost, and the ratio of number densities is no longer 1:1 but must be on the order of $10^7$:1.

The 1:1 correspondence between nuclei and bubbles in Knox gelatin can be characterized by saying that this substance is operating in a *nucleation-*

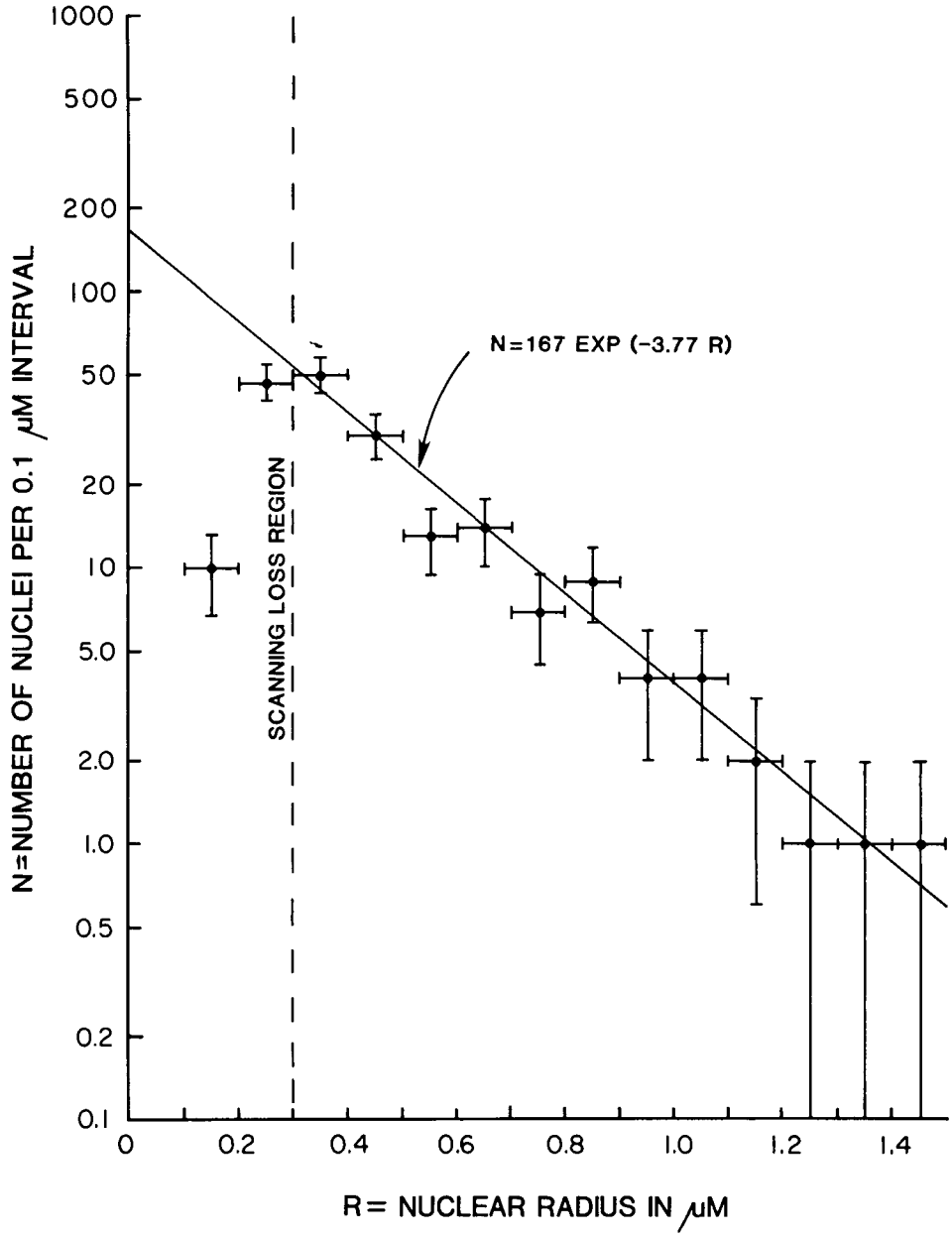



Fig. 6. Differential radial distribution of gas cavitation nuclei in agarose. The scanning efficiency deteriorates rapidly below 0.3 μm, and data in this region should be disregarded. Above 0.3 μm, the results can be described by a decaying exponential.

*limited* regime (6). An important consequence of being in this regime is that the exponential size distribution of the nuclei can be correctly deduced by applying the VP model to bubble counts generated by various pressure schedules (3). Agarose gelatin, on the other hand, is operating in the *phase-equilibration* regime (14) in which nucleation is so profuse that virtually all of the excess dissolved gas comes rapidly out of solution, and the number of gross bubbles is not determined by nucleation per se but by other factors, such as the speed with which supersaturation is induced, the stiffness of the surrounding medium, and the like. The nuclei in agarose again manifest the exponential distribution, as in Fig. 6, but this fact cannot be immediately verified by analyzing a set of bubble counts and pressure schedules.

The observations of osculating nuclei and of clusters, though unexpected, have a simple explanation in the VP model via the tendency of surfactant films to attract one another and form bilayers similar to those which stabilize soap bubbles in air. It should be pointed out, however, that for two or more bubbles aggregating in a homogeneous liquid, there is theoretically no stable configuration involving an extensive septum (13). This suggests that osculating nuclei are, in fact, just barely touching and that the solid debris, which seems always to be present in nuclear clusters, is an essential component of these more complex systems.

The accretion of solid debris by ordinary gas bubbles is a well-known phenomenon and has been used for many years to separate mineral ores from gangue by *flotation*. This process can be controlled and significantly enhanced by adding appropriate surfactants (15). Attached particles and surfactant coatings may also account, respectively, for the neutral buoyancy and long persistence in sea water of microbubbles with radii up to 60 μm (16). The tendency of bubble formation nuclei to accrete solid debris was inferred already from the gelatin filtration experiment and is discussed especially in the two appendices of Ref. 4.

The main conclusion of this microscopic investigation is that typical samples of water and gelatin contain stable microbubbles with radii on the order of 1 μm or less and with an exponential size distribution. Precisely the same conclusion had been reached earlier on the basis of bubble-counting experiments (2,4,5), and a nucleation model, called the *varying-permeability* or *VP model*, had been developed (3) and elaborated (9) to describe the subject entities. Although the VP model appears also to be useful in calculating diving tables (6) and in tracing decompression data on salmon, rats, and humans in the nucleation-limited regime (7,8), it has not yet been determined whether VP nuclei actually exist in vivo.

As a partial and very preliminary answer to this question, we invite the interested reader to spend some time scanning micrographs, such as those that appear each month in *The Journal of Cell Biology*. Most of the issues that we have examined do contain at least one photograph with at least one candidate nucleus, that is, a circular (or spherical) object with a blank center and a radius on the order of 1 μm or less. Specific examples are evident in Fig. 5 of Ref. 17 and in Fig. 5*a* of Ref. 18. Such objects, if they are mentioned at all,

may be referred to by the authors as gas inclusions, pits, artifacts, and the like. Mostly, they are ignored or eliminated in selecting photographs for publication. At this stage of our investigations, we cannot prove that any of the candidates we have seen in micrographs are actually bubble formation nuclei, but certainly the VP model would be far less credible if no candidates could be found.

## Acknowledgments

It is a pleasure to thank our colleagues Ed Beckman, Nancy Morrison, Jon Pegg, and Birch Porter for assistance and for useful suggestions and comments during the course of this investigation. This work, carried out under "The Physics of Gas Bubbles: Medical Applications" Project (HP/R-4), is a result of research sponsored in part by the University of Hawaii Sea Grant College Program under Institutional Grant Number NA81AA-D-00070 from NOAA Office of Sea Grant, U.S. Department of Commerce.

## References

1. Harvey EN, Barnes DK, McElroy WD, Whiteley AH, Pease DC, Cooper KW. Bubble formation in animals. I. Physical factors. J Cell Comp Physiol 1944;24:1–22.
2. Yount DE, Strauss RH. Bubble formation in gelatin: a model for decompression sickness. J Appl Phys 1976;47:5081–5089.
3. Yount DE. Skins of varying permeability: A stabilization mechanism for gas cavitation nuclei. J Acoust Soc Am 1979;65:1429–1439.
4. Yount DE, Yeung CM, Ingle FW. Determination of the radii of gas cavitation nuclei by filtering gelatin. J Acoust Soc Am 1979;65:1440–1450.
5. Yount DE, Yeung CM. Bubble formation in supersaturated gelatin: A further investigation of gas cavitation nuclei. J Acoust Soc Am 1981;69:702–708.
6. Yount DE, Hoffman DC. Decompression theory: A dynamic critical-volume hypothesis. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:131–146.
7. Yount DE. Application of a bubble formation model to decompression sickness in Fingerling salmon. Undersea Biomed Res 1981;8:199–208.
8. Yount DE. Application of a bubble formation model to decompression sickness in rats and humans. Aviat Space Environ Med 1979;50:44–50.
9. Yount DE. On the evolution, generation, and regeneration of gas cavitation nuclei. J Acoust Soc Am 1982;71:1473–1481.
10. Epstein PS, Plesset MS. On the stability of gas bubbles in liquid-gas solutions. J Chem Phys 1950;18:1505–1509.
11. D'Arrigo JS. Improved method for studying the surface chemistry of bubble formation. Aviat Space Environ Med 1978;49:358–361.
12. Yount DE, Gillary EW, Hoffman DC. Microscopic identification of bubble formation nuclei. In: Hoyt JW, ed. ASME cavitation and polyphase flow forum 1982. New York: The American Society of Mechanical Engineers, 1982:6–9.
13. Yount DE. A model for microbubble fission in surfactant solutions. J Colloid Interface Sci 1983;91:349–360.
14. Hills BA. Vital issues in computing decompression schedules from fundamentals. I. Critical supersaturation versus phase equilibration. Int J Biometeor 1970;14:111–131.
15. Adamson AW. Physical chemistry of surfaces. 3rd ed. New York: John Wiley & Sons, 1976:459–474.
16. Mulhearn PJ. Distribution of microbubbles in coastal waters. J Geophys Res 1981;86:6429–6434.

17. Jackson WT, Doyle BG. Membrane distribution in dividing endosperm cells of haemanthus. J Cell Biol 1982;94:637–643.
18. Wang RJ, Wissinger W, King EJ, Wang G. Studies on cell division in mammalian cells. VII. A temperature-sensitive cell line abnormal in centriole separation and chromosome movement. J Cell Biol 1983;96:301–306.

# DECOMPRESSION THEORY: A DYNAMIC CRITICAL-VOLUME HYPOTHESIS

*D. E. Yount and D. C. Hoffman*

As emphasized by Hills (1,2), there are two basically different approaches to decompression optimization. The first is to devise a convenient calculational method and then modify it empirically until it is in reasonable agreement with the available data. The second is to develop a theoretical model from fundamental physical and physiological principles and then attempt to quantify its response to changes in exposure pressure. A key issue in either case is the identification of the proper decompression criterion.

The empirical approach is illustrated by the method of Haldane (3). The Haldane decompression criterion is expressed as a pressure ratio, which has been interpreted a posteriori as a supersaturation limit for the formation of bubbles whose mere presence is assumed to cause symptoms (4). Alternatively, the ratio could represent a critical volume of separated gas or a critical degree of embolism that the body can tolerate (5). This second possibility was mentioned already in Ref. 3; however, since it is not rigorously compatible with the assumptions of exponential gas exchange and of symmetric gas uptake and elimination, it cannot properly be regarded as a bona fide part of the Haldane scheme (1,2).

The theoretical approach is illustrated by the method of Hills (6), which is based on the principle of *phase-equilibration.* In Hills' regime, bubble formation is assumed to be so profuse in the relevant tissues that all gas in excess of equilibrium is "dumped" into the gas phase within a few minutes after a pressure reduction. If one further assumes that the volume of separated gas is critical, the result is not a pressure ratio but a *zero-supersaturation* criterion for decompression (1,2).

The physiological circumstances implicit in the Haldane method (4) represent the "best case" in the sense that little or no gas has come out of solution.

The circumstances invisioned by Hills (1,2) correspond to the "worst case" because the volume of separated gas is maximal. In addition, the pressure gradient for eliminating gas via the circulation, essentially the supersaturation $P_{ss}$, is maximal for Haldane and minimal for Hills.

Evidently, the best-case and worst-case calculational methods of Haldane and of Hills, founded respectively on no gas release and on complete phase equilibration, lie at opposite ends of the bubble formation or nucleation spectrum (2). The truth, we believe, lies somewhere in between. There is ample evidence that bubble formation does occur routinely in asymptomatic Haldanian decompressions (7), and there is also ample evidence that the total volume of released gas at the onset of mild decompression symptoms is much smaller than would be required by phase equilibration. Rubissow and Mackay (8), working with rats, have found that following initial decompressions, 2–10 bubbles with diameters of 2–5 $\mu$m are present per $mm^3$ in fatty tissue. This corresponds to a volume of gas released into bubbles, which is less than $10^{-5}$ of that still in solution. More recently, Hills (9) has estimated that 17% of the dissolved gas was released in guinea pigs decompressed from 4 atm abs to 2.21 atm abs, while 21% was released in going from 4 atm abs to 1 atm abs. The corresponding decreases in nitrogen washout rates were only 7 and 15%, respectively.

With the development of a detailed mathematical model describing bubble formation in aqueous media (10), it is now possible to quantify various degrees of nucleation and place any given dive profile at a more realistic position on the nucleation scale. The methods of Haldane and of Hills may then be regarded as limiting special cases of a more general decompression theory that should someday be applicable to the whole range of hyperbaric and hypobaric situations.

In the remainder of this paper, we report on our first attempts to calculate a comprehensive set of diving tables by applying nucleation theory. The computational algorithms are summarized in the next section, and results are discussed in the section that follows. A promising feature of the new tables is that they give sensible prescriptions for a wide range of diving situations, yet employ a small number of parameters and a single set of parameter values. All of the calculations reported here were carried out on an ordinary home computer (Radio Shack TRS-80 with 48K memory).

## METHODS

In previous applications of our nucleation model to decompression sickness (11–13), we were dealing mainly with rudimentary pressure schedules in which the subjects were first saturated with gas at some elevated pressure $P_1$ and then supersaturated by reducing the pressure from $P_1$ to the final setting $P_2$. The data in such experiments are most easily presented by plotting the combinations of supersaturation versus exposure pressure ($P_{ss} \simeq P_1 - P_2$ versus $P_1$), which yield a given morbidity, for example, a 50% probability of

contracting decompression sickness. To describe these data, we assumed that lines of constant morbidity were also lines of constant bubble number $N$ (11–13). The bubble number, in turn, was assumed to be equal to the number of spherical gas nuclei with initial radii $r_o$ larger than some minimum radius $r_o^{min}$ (10). This approach was remarkably successful, partly because the schedules involved were so simple—representing, as it were, a type of controlled experiment in which most of the variables in the problem were fixed.

Our naive assumption of constant nucleation or constant bubble number does not encompass the full range of conditions covered by modern diving tables. That is, it yields a set of tables which, though they may be very safe, do not track conventional tables in their global behavior and often require total ascent times that would generally be considered excessive by the commercial diving industry. Treating the conventional tables as valid experimental data, we have been forced to develop a more comprehensive decompression criterion.

The first step has been to replace *constant bubble number* with a *critical-volume hypothesis*, thereby assuming that signs or symptoms will appear whenever the total volume V accumulated in the gas phase exceeds some designated critical value $V_{crit}$. Although $V_{crit}$ itself is fixed for all of our diving tables, gas is continuously entering and leaving the gas phase. In this sense, our decompression criterion is dynamic, rather than static as in other applications of the critical-volume point of view (14).

The idea that gas is continuously leaving the gas phase is suggested by our previous work (11–13), which seems to imply that there is a bubble number $N_{safe}$ that can be tolerated indefinitely, regardless of the degree of supersaturation $P_{ss}$. From this, we deduce that the body must be able to dissipate free gas at a useful rate that is proportional both to $N_{safe}$ and to $P_{ss}$. A possible rationale is provided by physiological studies demonstrating that so long as its capacity is not exceeded, the lung is able to continue functioning as a trap for venous bubbles (15).

Another implication of our present investigation is that in practical diving tables (and especially in surface-decompression procedures), the actual number of supercritical nuclei $N_{actual}$ is allowed temporarily to exceed the number that can be tolerated indefinitely $N_{safe}$. This permits the volume of the gas phase to inflate at a rate that is proportional to $P_{ss}$ $(N_{actual} - N_{safe})$. In our present formulation, the increase in gas-phase volume continues until $P_{ss}$ is zero. At this point, usually long after the dive has ended, the net volume of released gas has reached its maximum value $V_{max}$, which must be less than $V_{crit}$ if signs and symptoms of decompression sickness are to be avoided.

Our computation of a diving table begins with the specification of six nucleation parameters. These are the surface tension $\gamma$, the nuclear skin compression $\gamma_C$ (10), the minimum initial radius $r_o^{min}$ (10), the pressure $p^*$ at which the skins become impermeable to gas (10), the time constant $\tau_R$ for the regeneration of nuclei crushed in the initial compression (16), and a composite parameter $\lambda$, which is related to $V_{crit}$ and determines, in effect, the amount by which the actual bubble number $N_{actual}$ can exceed the safe bubble number $N_{safe}$.

$N_{actual}$ is much larger than $N_{safe}$ for short dives, but the two are nearly equal for dives of long duration.

From the given set of parameter values, the program calculates a preliminary estimate of $P_{ss}$ that is just sufficient to probe the minimum initial radius $r_o^{min}$ and hence to produce a number of bubbles equal to $N_{safe}$. In the permeable region of the model, the nuclear radius $r_1^{min}$ following an increase in pressure from $P_o$ to $P_1$ can be obtained from the equation (10)

$$(1/r_1^{min}) = (1/r_o^{min}) + (P_1 - P_o)/2(\gamma_C - \gamma). \quad (1)$$

Regeneration of the nuclear radius is allowed to take place throughout the time $t_R$ after $P_1$ is reached. This is a complex statistical-mechanical process (16), which we have chosen to approximate via an exponential decay with the regeneration time constant $\tau_R$:

$$r(t_R = r_1^{min} + (r_o^{min} - r_1^{min})[1 - \exp(-t_R/\tau_R)]. \quad (2)$$

The supersaturation of $P_{ss}^o$ that is just sufficient to probe $r_o^{min}$ is then found from (10)

$$P_{ss}^o = 2(\gamma/\gamma_C)(\gamma_C - \gamma)/r(t_R). \quad (3)$$

Holding $P_{ss}^o$ fixed, the program next calculates a decompression profile and the total decompression time $t_D$. From $t_D$ and the constant

$$\beta_o = 2(\gamma_C - \gamma)/r_o^{min}, \quad (4)$$

a new value $P_{ss}^{new}$ is obtained which will probe a new initial radius $r_o^{new}$ that is smaller than $r_o^{min}$ and hence will result in a number of bubbles that is larger than $N_{safe}$.

In principle, the revised bubble number $N(r_o^{new})$ can be found by assuming that the integral radial size distribution of spherical gas nuclei in vivo is a decaying exponential (16,17),

$$N(r_o^{new}) = N_o \exp(-\beta_o S r_o^{new}/2kT), \quad (5)$$

where S is the skin area occupied by one surfactant molecule in situ, $k$ is the Boltzmann constant, and T is the temperature. In practice, however, the absolute bubble number $N$ and the net gas volume V are not explicitly determined since the arbitrary normalization $N_o$ of the nuclear size distribution cancels out.

After several pages of mathematical manipulation, we have derived a simple formula for $P_{ss}^{new}$ that takes into account the critical volume $V_{crit}$, the exponential radial distribution $N(r_o^{new})$, and the inflation of the gas phase—essentially the time integral of $P_{ss}$ $(N_{actual} - N_{safe})$. The result, which must be recalculated for each tissue half time H, is

$$P_{ss}^{new} = P_{ss}^o\{1 + \lambda/[(\beta_o + P_1 - P_o)(t_D + H/0.693)]\}, \quad (6)$$

where S, $k$, and T have been absorbed into the composite *critical-volume* parameter $\lambda$. Using the respective values of $P_{ss}^{new}$ for each "tissue compartment," the program determines a more severe decompression profile, which

will yield updated values of $t_D$ and $P_{ss}^{new}$. After several iterations, $t_D$ and $P_{ss}^{new}$ converge, implying that $V_{max}$ now differs from $V_{crit}$ by an acceptably small amount.

The uptake and elimination of inert gas by the body is assumed to be exponential, as in conventional tables (18). Water vapor pressure and the dissolved partial pressures of oxygen and carbon dioxide are calculated in the manner described in Ref. 19. The net contribution of these "active" gases is nearly constant at 102 mmHg for inspired oxygen pressures up to about 2 atm abs. This limit is not reached for air decompression tables at ambient pressures below about 10 atm abs. The half times H for the various tissue compartments are 1, 2, 5, 10, 20, 40, 80, 120, 160, 240, 320, 400, 480, 560, and 720 min. The onset of impermeability, $p^* = 9.2$ atm abs, is high enough so that nearly all of our air decompression tables lie in the "permeable" or "linear" region of our nucleation model (10).

Because the model predictions depend only upon the ratios $\gamma/\gamma_C$ and $2\gamma/r_o^{min}$, the value of $\gamma$ is essentially arbitrary (10,13). To be definite, however, we have set $\gamma = 17.9$ dyn/cm (20). With this choice, the values of the remaining four parameters are $\gamma_C = 257$ dyn/cm $r_o^{min} = 0.775$ μm, $\tau_R = 20{,}160$ min, and $\lambda = 5000$ fsw/min. These were found by requiring that the total decompression times in our tables resemble those in the TEKTITE saturation dive (21) and in the U.S. Navy (22) and Royal Naval Physiological Laboratory (23) manuals. In other words, all of the results reported in this paper were obtained by optimizing the values of only four nucleation parameters, $\gamma_C$, $r_o^{min}$, $\tau_R$, and $\lambda$.

Depths and pressures are usually given in feet of sea water (33 fsw = 10 msw = 1 atm = 2 atm abs, etc.) for convenience in making comparisons with the TEKTITE, USN, and RNPL reference schedules. For similar total decompression times, the set of tables generated in this study is expected to yield smaller total bubble volumes and therefore to be safer. None of the tables has as yet been tested on either animal or human subjects.

## RESULTS

In this section, the salient features of a number of diving tables using air as the breathing mixture are compared. The VPM and USN (22) profiles for an *exceptional exposure* involving greater than normal risk are shown in Fig. 1. In both cases, the descent and ascent rates are 60 fsw/min, and the 3.33 min required to reach 200 fsw is counted as part of the 60-min bottom time. The total decompression times are similar, the important difference being the deeper *first stop* of the VPM table, 130 fsw versus 60 fsw for USN. This is a persistent feature of the literally hundreds of comparisons we have made of VPM tables with a variety of conventional tables now in use. Our calculations indicate that the longer "first-pull" of these conventional tables results in a larger supersaturation $P_{ss}$, in a larger bubble number $N$, and ultimately in a larger maximum volume of released gas $V_{max}$.

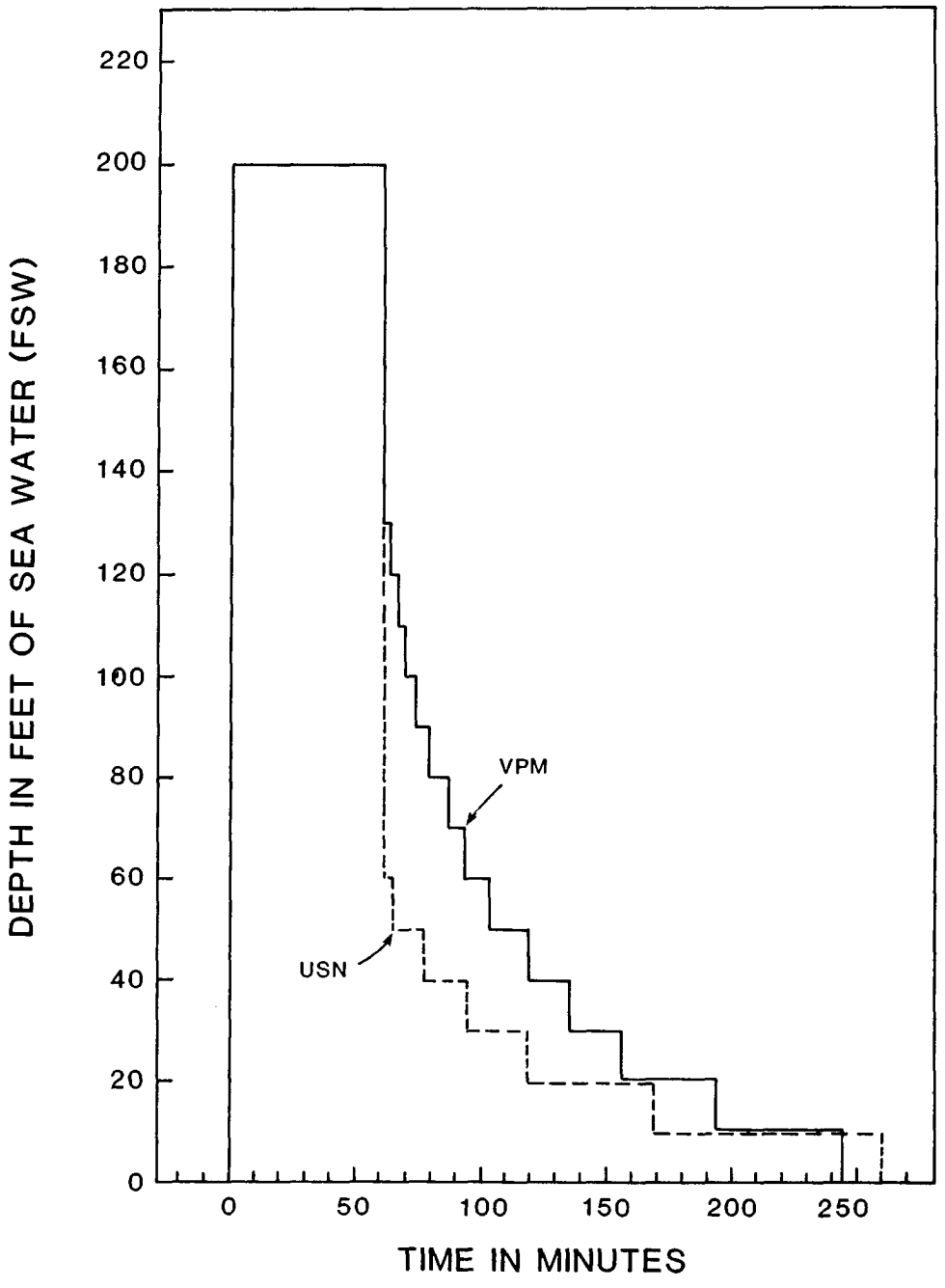



Fig. 1. Varying-permeability model (VPM) and U.S. Navy (USN) decompression profiles for a 60-min dive to 200 fsw. The longer "first-pull" of conventional tables results in a larger supersaturation $P_{ss}$, a larger bubble number $N$, and ultimately in a larger maximum volume of released gas $V_{max}$.

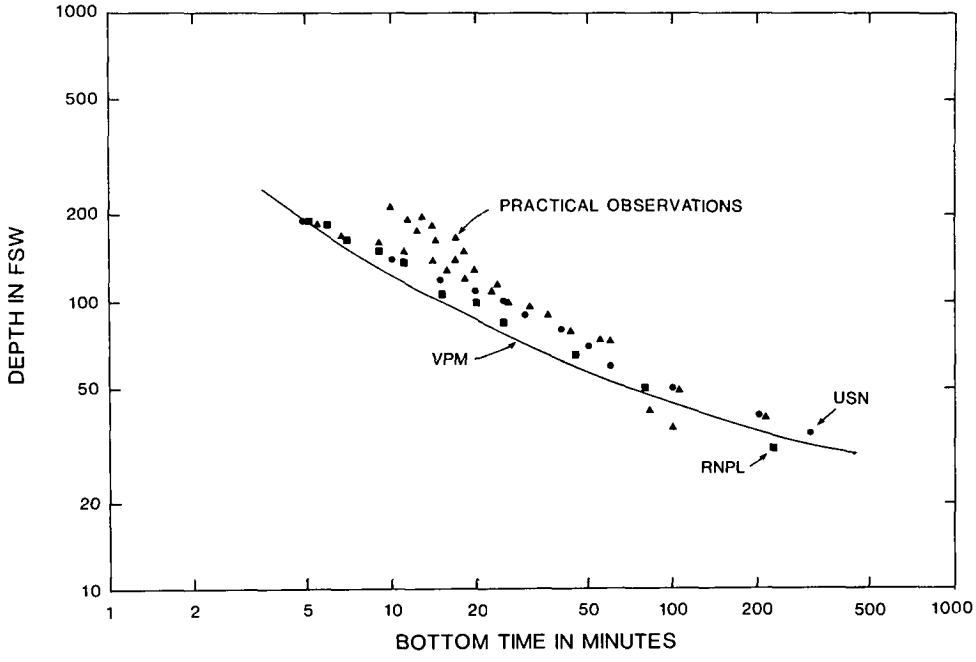



Fig. 2. Comparison of varying-permeability model (VPM), U.S. Navy (USN), and Royal Naval Physiological Laboratory (RNPL) *no-stop* decompressions with various practical observations, i.e., combinations of depth and bottom time that yielded no symptoms or only the mildest symptoms. The VPM *curve* lies just below the USN and RNPL recommendations at all but one RNPL point and therefore serves as a safe, tight, and useful lower bound.

Figure 2 compares VPM, USN (22), and RNPL (23) *no-stop* decompressions, along with various "practical observations" compiled by Leitch and Barnard (24). Although there are some differences in this plot in the rates of descent and ascent and in the exposure conditions (24), the absence of prolonged decompression stages makes this type of "data" nearly independent of the overall surfacing strategy. The VPM curve lies just below the USN and RNPL recommendations at all but one RNPL point (230 min at 33 fsw), and over the entire range, it serves as a safe, tight, and therefore useful lower bound. The fact that the VPM curve is a bit low in this case reflects the general conservatism of the tables we have prepared. A bolder, more aggressive set of tables could, of course, be computed by simply adjusting the values of the nucleation parameters.

Total ascent times for VPM, USN (22), and RNPL (23) are plotted as a function of the bottom time at 200 fsw in Fig. 3. The VPM curve lies close to the USN points for bottom times that extend all the way from 5 to 360 min. The large difference in USN and RNPL total ascent times (often more than a factor of 2) illustrates the wide divergence in opinion that still exists, even among highly respected investigators in the diving field.

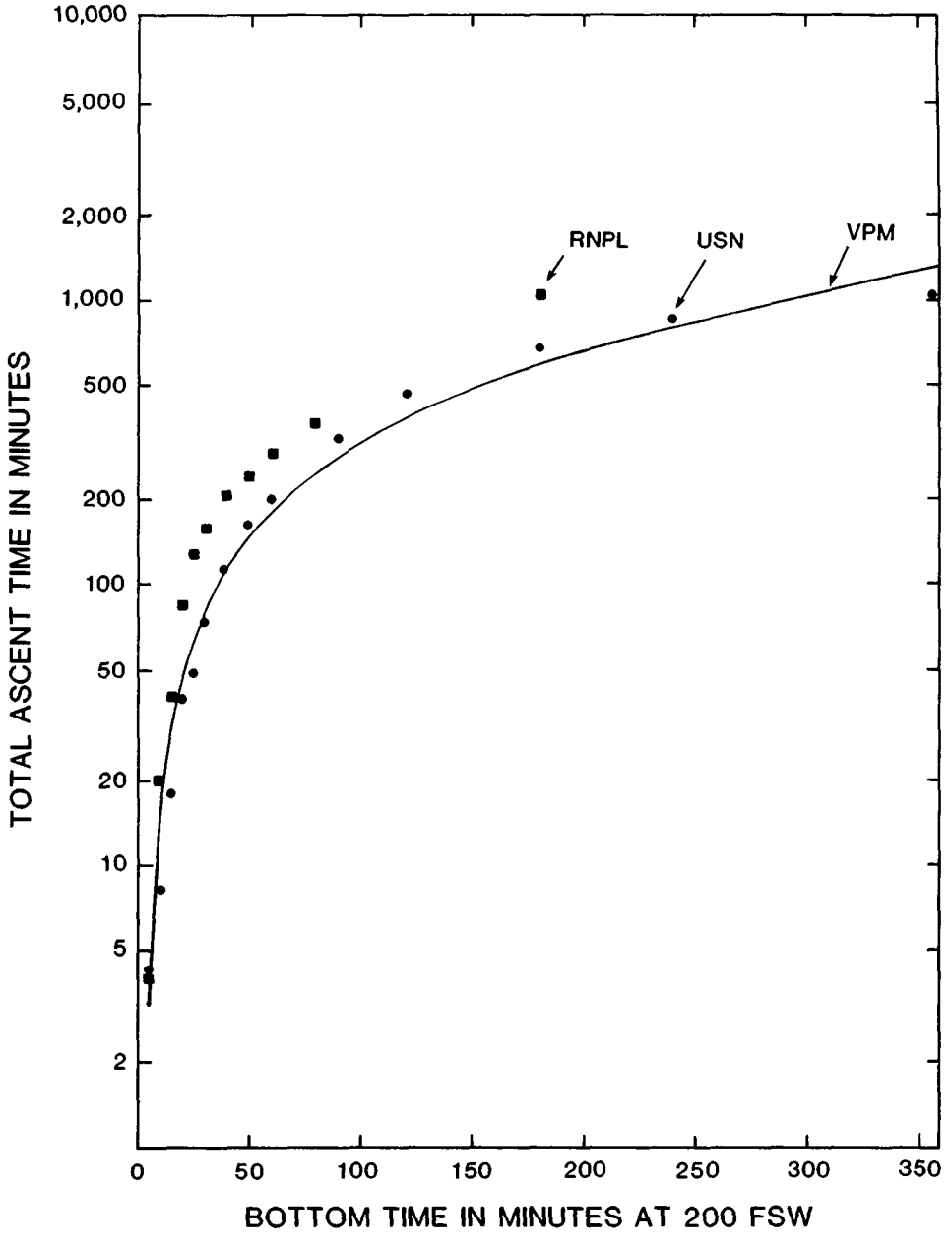



Fig. 3. Total ascent times versus bottom times at 200 fsw for VPM, USN, and RNPL decompression tables. The total ascent times for USN and RNPL often differ by more than a factor of 2.

One very practical reason for attempting to optimize decompression procedures from first principles is the hope that if a correct global theory can someday be formulated, it will then be possible to relate and describe the whole range of decompression experience with a small number of equations and parameter values. Instead of "titrating" a handful of "volunteers" to develop a new table or determine a new *M-value* (22), a method which necessarily has limited statistical accuracy, one will be able to use an already calibrated theory to interpolate or extrapolate, thereby bringing to bear the full statistical weight of a much larger data base. This idea is illustrated in Fig. 4, which summarizes total ascent times versus bottom times for VPM decompressions from air dives to 60, 100, 200, and 300 fsw.

As a second illustration of the global approach, Fig. 5 connects the no-stop decompressions in Fig. 2 with the 14-day, 100-fsw TEKTITE saturation dive (21). The latter has been used by humans without incident. However, the close agreement apparent in this graph is partly fortuitous because the TEKTITE stops were 5 rather than 10 fsw apart, and the breathing gas was a normoxic oxygen-nitrogen mixture rather than air. In addition, both air and pure oxygen were breathed during various stages of the TEKTITE decompression. A more precise comparison is given in Table I, where the VPM schedule was calculated for a 14-day exposure to the 126-fsw equivalent air depth of the TEKTITE dive.

By replacing our earlier assumption of constant bubble number with a dynamic critical-volume hypothesis, we have succeeded in preparing a comprehensive set of air diving tables which, though untested, appear in all respects to be quite reasonable. It should not be forgotten, however, that the constant-bubble-number criterion did work well in those rudimentary cases in which it was first applied (11–13). This raises the question of whether our new and different criterion can also describe these special situations. The answer is affirmative, suggesting that our tables obey a kind of *correspondence principle* in which *critical volume* becomes equivalent to *constant bubble number* in the limit of a nucleation-dominated regime, i.e., a regime in which $N_{actual}$ approaches $N_{safe}$ and the allowed supersaturation $P_{ss}$ is determined directly by $r_o^{min}$.

An illustration of the critical-volume $\leftrightarrow$ critical-nucleation correspondence for humans is provided by Fig. 6. The rudimentary cases referred to in this figure, in the previous paragraph, and also at the begining of the METHODS section are those in which the subjects are first saturated with gas at some elevated pressure $P_1$ and then supersaturated by reducing the pressure from $P_1$ to the final setting $P_2$. In experiments with human subjects, $P_1 - P_2$ is usually defined as the greatest pressure reduction that can be sustained without the onset of decompression sickness. To simulate this condition with our tables, we have selected dives with bottom times of 720 min and have taken $P_2$ to be the depth of the first decompression stop. This provides a reasonable approximation to a single-step decompression in the nucleation-dominated regime because, in this limit, the rate at which gas is permitted to come out of solution is just slightly larger than that which the body can dissipate and therefore tolerate indefinitely.

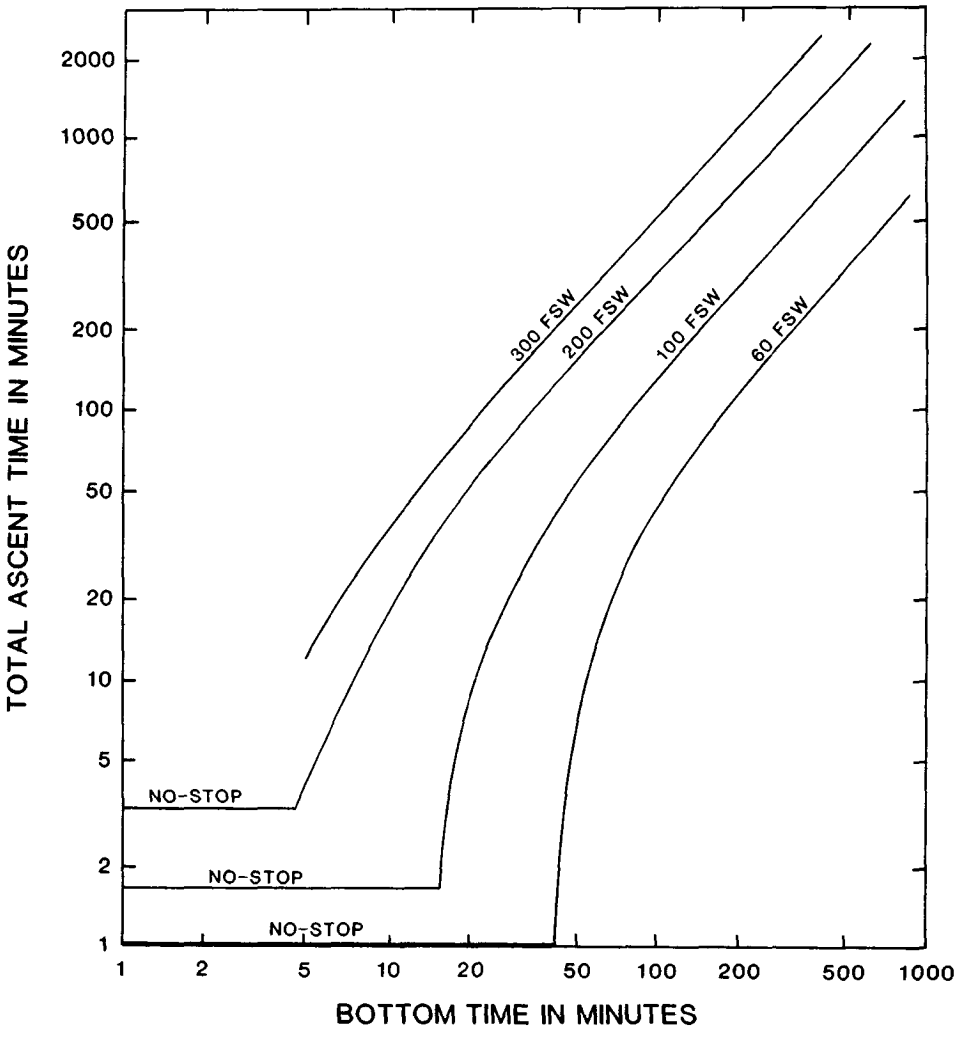



Fig. 4. Total ascent times versus bottom times for VPM at depths of 60, 100, 200, and 300 fsw. This figure and the one which follows illustrate how a large range of decompression experience can be described by a global theory using a small number of equations and parameter values.

In the permeable region of our nucleation model ($P_1 < p^* = 9.2$ atm abs), this procedure yields a linear relationship,

$$P_1 = 1.372\ P_2 + 0.335 \text{ atm abs}, \tag{7}$$

which has a correlation coefficient of better than 0.999 for the eight combinations of $P_1$ and $P_2$ which were used. Similar expressions,

$$P_1 = 1.375\ P_2 + 0.52 \text{ atm abs} \tag{8}$$

and

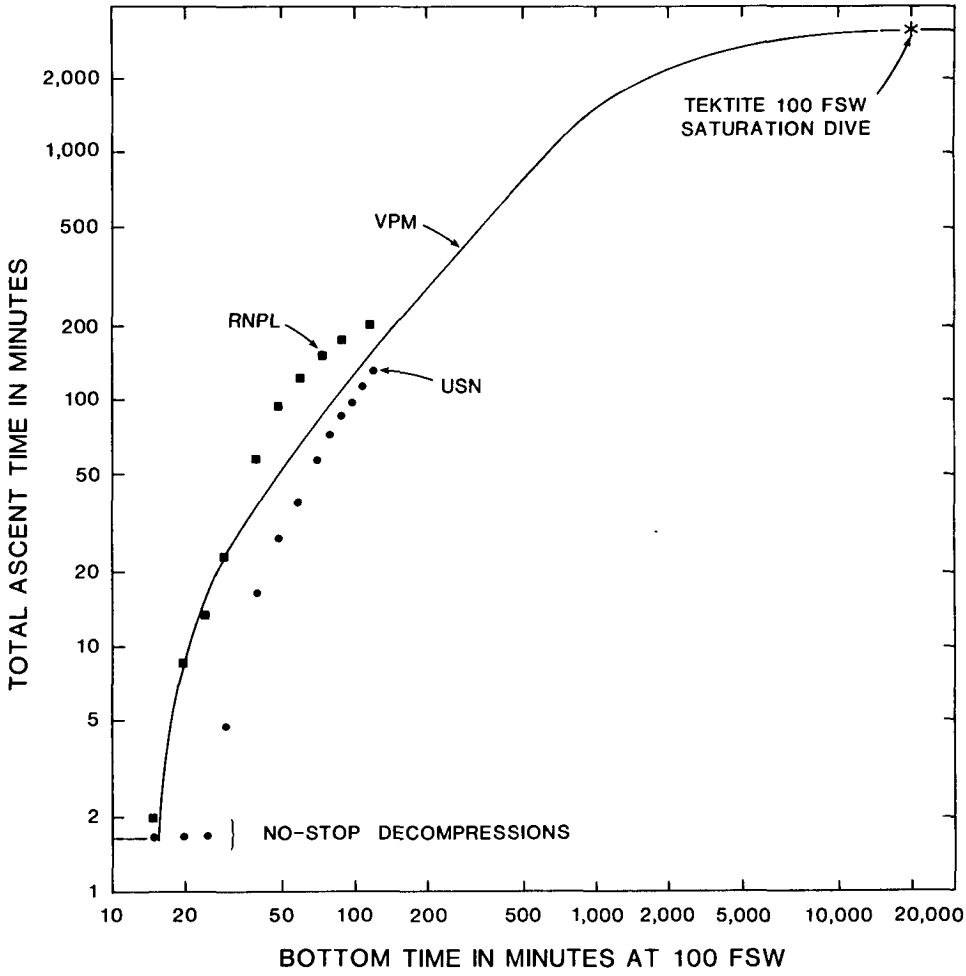



Fig. 5. Total ascent times versus bottom times at 100 fsw for VPM, USN, RNPL, and TEKTITE decompression tables. All of the VPM schedules reported in this paper were computed with the same values of the four adjustable nucleation parameters $\gamma_C$, $r_0^{min}$, $\tau_R$, and $\lambda$.

$$P_1 = 1.366\ P_2 + 0.56 \text{ atm abs}, \tag{9}$$

have been extracted by Hennessy and Hempleman (14) from, respectively, the USN and RNPL tables. As can be seen in Fig. 6, the *three straight lines* are nearly parallel, and VPM is 0.1 to 0.2 atm lower than USN and RNPL. The fact that these lines are similar to the isopleths of constant bubble number presented for the permeable region in Refs. 11, 12, and 13 verifies the above mentioned correspondence for this rudimentary case. The *no-stop threshold,* $P_1 = 1.87$ atm abs and $P_1 - P_2 = 0.87$ atm, was obtained by averaging the values of $P_1 = 1.90$ atm abs, $P_1 - P_2 = 0.90$ atm measured by Hempleman

TABLE I

Comparison of the 14-day, 100-fsw TEKTITE Decompression Table with the Equivalent VPM Schedule

| Depth (fsw) | Time at Stop (min) TEKTITE | Time at Stop (min) VPM |
|---|---|---|
| 100-90 | 10 air | |
| 90 | 60 air | |
| 85 | 90 air | 21 air |
| 80 | 100 air | 157 air |
| 75 | 110 air | 163 air |
| 70 | 120 air | 168 air |
| 65 | 360 air | 175 air |
| 60 | 140 air | 181 air |
| 55 | 160 air | 188 air |
| 50 | 160 air | 196 air |
| 45 | 10 oxy | 204 air |
| | 150 air | |
| 40 | 130 air | 213 air |
| 35 | 20 oxy | 222 air |
| | 150 air | |
| 30 | 360 air | 234 air |
| 25 | 30 oxy | 246 air |
| | 150 air | |
| 20 | 150 air | 259 air |
| 15 | 50 oxy | 273 air |
| | 120 air | |
| 10 | 160 air | 291 air |
| 5 | 60 oxy | 309 air |
| | 110 air | |
| TOTAL | 2960 | 3502 |
| | (+170 oxy) | |
| | (3130) | |

(25) with those of $P_1 = 1.83$ atm abs, $P_1 - P_2 = 0.83$ atm found by Kidd, Stubbs, and Weaver (26). The VPM result is $P_1 = 1.71$ atm abs, $P_1 - P_2 = 0.71$ atm. The *altitude bends threshold* plotted in Fig. 6, namely, $P_1 = 1.00$ atm abs, $P_1 - P_2 = 1.00 - 0.40 = 0.60$ atm, was calculated from the value of $P_2 = 7550$ m $= 307$ mmHg $= 0.40$ atm abs determined by Gray (27). The VPM limit of $P_1 = 1.00$ atm abs, $P_1 - P_2 = 0.52$ atm is again slightly lower. The extrapolations of the lines for USN and RNPL (14) are both slightly higher than the experimental no-stop and altitude bends thresholds plotted in this figure.

## DISCUSSION

We are aware that this investigation, though promising, can be criticized on a number of grounds. The most serious, we believe, is the fact than none

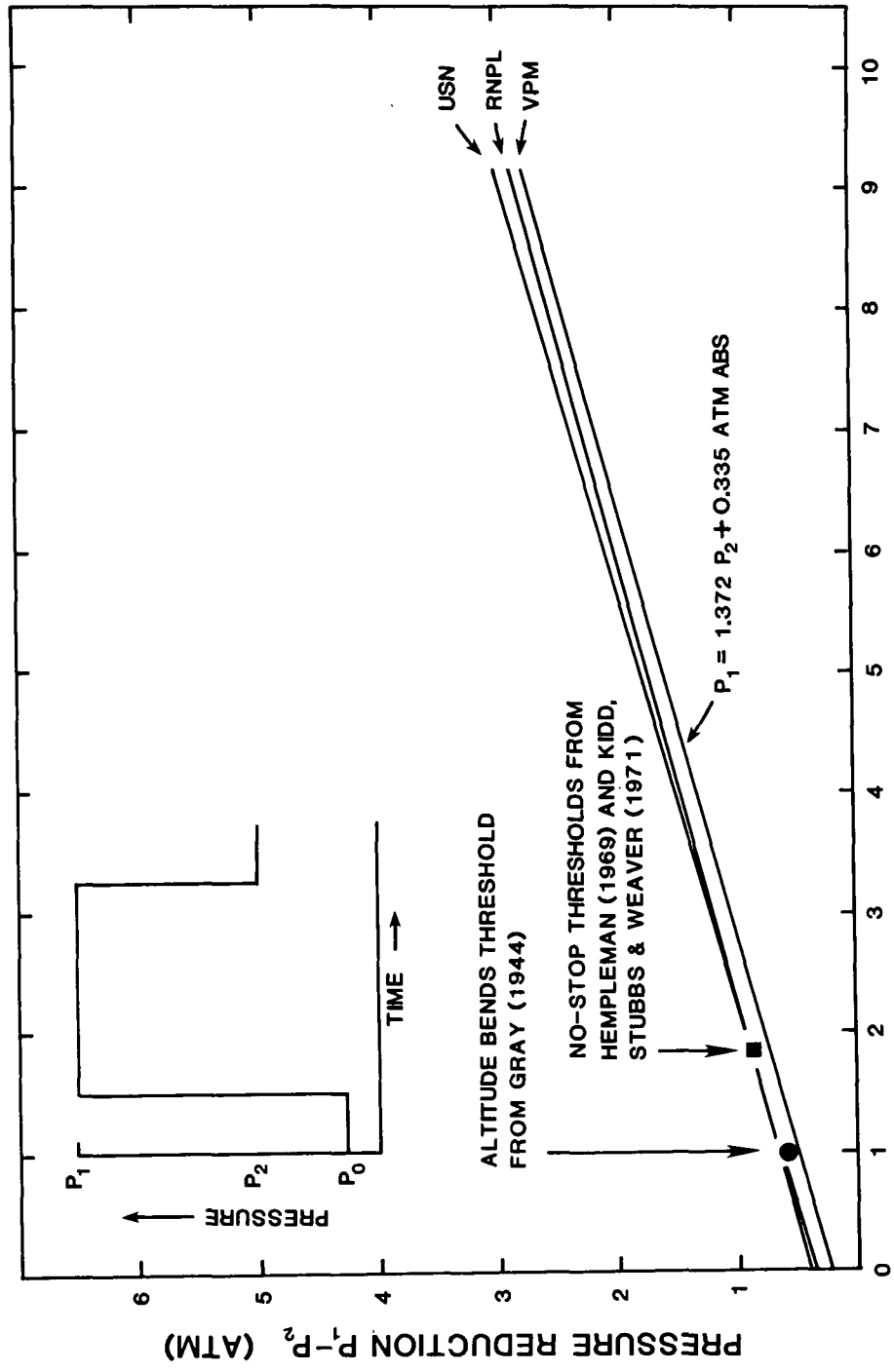



Fig. 6. Allowed pressure reduction $P_1 - P_2$ versus exposure pressure $P_1$ for USN, RNPL, and VPM air diving tables. In the limit of a nucleation-dominated regime, lines of constant critical volume are also isopleths of constant $_1$ bubble number.

of our diving tables have been tested. Unfortunately, we have neither the resources nor the support to sustain such an effort. Our immediate goal, therefore, was not to produce an operational set of diving tables but instead to determine whether a reasonable and comprehensive set of such tables could be computed from our nucleation model using a modest number of assumptions, equations, and parameter values. The answer, quite obviously, is yes.

Our operational definition of *reasonable and comprehensive* is: *similar, both in scope and in total decompression time, to other tables now in use.* A possible criticism here is that some of the reference tables are not very safe, and we may be trying too hard to match them, for example, by abandoning our original goal of zero or constant (but physiologically insignificant) bubble number. Alternatively, we may be losing a chance to shorten decompression obligations and improve diving efficiency. This is a matter of judgment in which we have decided to begin by accepting the whole range of diving experience, including conventional tables, as useful experimental data. Greater safety and/or efficiency may then become feasible both through an improved decompression strategy, as in Fig. 1, and through the unification and "smoothing" which result when a global theory is applied to a broad data sample. What is not a matter of judgment, but an early conclusion of this investigation, is the fact that any set of tables based on zero or constant bubble number is likely to be very different in global behavior from other tables now in use.

Another criticism is that we have said very little about the physiological processes that presumably underlie our mathematical equations. We take oxygen and carbon-dioxide into account and assume a reasonable range of tissue half times, but many other details are overlooked. We make no distinction, for example, between "fatty, loose tissue" and "watery, tight tissue" (14), nor do we state explicitly where the bubbles form or how they grow, multiply, or are transported. Finally, we say nothing about such factors as solubility, diffusion versus perfusion, tissue-deformation pressure, or tissue-specific differences in surface tension. Our response to criticisms of this type is that most of the omitted processes are poorly understood, and their inclusion at this stage would serve only to complicate the model and increase the number of undetermined parameters.

As a by-product of this investigation, we have gained a better understanding of practical decompression tables now in use. We believe, for example, that profuse bubble formation is permitted by such tables, particularly during dives of short duration. Meanwhile, the number of primary bubbles, i.e., bubbles formed directly from nuclei rather than from other bubbles (28), is allowed to vary widely. The common assumption (3,5,6,14) that the volume of released gas is critical seems still to be viable providing allowance is made for the body's ability to dissipate free gas at a useful rate (15). Since gas is continuously entering or leaving the gas phase, optimal decompression is defined by a *dynamic critical-volume hypothesis* requiring that the *net* volume of free gas be always less than the threshold value $V_{crit}$.

## Acknowledgments

We are especially grateful to Gilbert Grenié of the École Centrale des Arts et Manufactures, Chatenay-Malabry, France, who visited the University of Hawaii for several months in 1980 and played a key role in the initial stages of this investigation. Other visitors from the École Centrale who have participated in this program are Bernard Rémy in 1981, Gildas Herjean in 1982, and Philippe Mazas in 1983. We also wish to thank our colleagues Ed Beckman, Claude Harvey, Jon Pegg, and Birch Porter for useful suggestions and comments. This work, carried out under "The Physics of Gas Bubbles: Medical Applicatons" Project (HP/R–4), is a result of research sponsored in part by the University of Hawaii Sea Grant College Program under Institutional Grant Number NA81AA–D–00070 from NOAA Office of Sea Grant, U.S. Department of Commerce.

## References

1. Hills BA, Vital issues in computing decompression schedules from fundamentals. I. Critical supersaturation versus phase equilibration. In J Biometeor 1970;14:111–131.
2. Hills BA. Zero-supersaturation approach to decompression optimization. In: Hong SK, ed. International symposium on man in the sea. Bethesda: Undersea Medical Society, Inc., 1975:179–189.
3. Boycott AE, Damant GCC, Haldane JS. The prevention of compressed air illness. J Hyg (Camb) 1908;8:342–443.
4. Workman RD. Calculation of decompression schedules for nitrogen-oxygen and helium-oxygen dives. Washington, DC: U.S. Navy Experimental Diving Unit, 1965. (NEDU report no. 6–65)
5. Behnke AR. Decompression sickness following exposure to high pressures. In: Fulton JF, ed. Decompression sickness. Philadelphia: W. B. Saunders & Co., 1951:53–89.
6. Hills BA. A thermodynamic and kinetic approach to decompression sickness. Ph.D. Thesis. Adelaide: Library Board of South Australia, 1966.
7. Spencer, MP, Campbell SD. Development of bubbles in venous and arterial blood during hyperbaric decompression. Bull Mason Clinic 1968;22:26–32.
8. Rubissow GJ, Mackay RS. Decompression study and control using ultrasonics. Aerosp Med 1974;45:473–478.
9. Hills BA. Effect of decompression per se on nitrogen elimination. J Appl Physiol: Respir Environ Exercise Physiol 1978;45:916–921.
10. Yount DE. Skins of varying permeability: a stabilization mechanism for gas cavitation nuclei. J Acoust Soc Am 1979;65:1429–1439.
11. Yount DE. Responses to the twelve assumptions presently used for calculating decompression schedules. The Seventeenth Undersea Medical Society Workshop, Decompression theory. UMS Publ. No. 29WS(DT) 6–25–80. Bethesda: Undersea Medical Society, Inc., 1978:143–160.
12. Yount DE. Application of a bubble formation model to decompression sickness in rats and humans. Aviat Space Environ Med 1979;50:44–50.
13. Yount DE. Application of a bubble formation model to decompression sickness in fingerling salmon. Undersea Biomed Res 1981;8:199–208.
14. Hennessy TR, Hempleman HV. An examination of the critical released gas volume concept of decompression sickness. Proc R Soc Lond Biol 1977;197:299–313.
15. Butler BD, Hills BA. The lung as a filter for microbubbles. J Appl Physiol: Respir Environ Exercise Physiol 1979;47:537–543.
16. Yount DE. On the evolution, generation, and regeneration of gas cavitation nuclei. J Acoust Soc Am 1982;71:1473–1481.
17. Yount DE, Gillary EW, Hoffman, DC. Microscopic study of bubble formation nuclei. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda, Undersea Medical Society, Inc., 1984:119–130.
18. Yount DE, Strauss RH. Decompression sickness. Am Sci 1977;65:598–604.
19. Yount DE, Lally DA. On the use of oxygen to facilitate decompression. Aviat Space Environ Med 1980;51:544–550.

20. Davson H. A textbook of general physiology. London: Churchill, 1964:185.
21. Beckman EL, Smith EM. TEKTITE II: medical supervision of the scientists in the sea. Texas Reports on Biol and Med 1972;30:155–169.
22. U.S. Navy diving manual (NAVSHIPS 0994–001–9010), U.S. Navy Department. Washington: U.S. Government Printing Office, 1970.
23. Air diving tables. Royal Naval Physiological Laboratory, Alverstoke, Hants. London: Her Majesty's Stationary Office, 1968.
24. Leitch DR, Barnard EEP. Observations on no-stop and repetitive air and oxynitrogen diving. Undersea Biomed Res 1982;9:113–129.
25. Hempleman HV. British decompression theory and practice. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving and compressed air work. Baltimore: Williams & Wilkins, 1969:331–347.
26. Kidd DJ, Stubbs RA, Weaver RS. Comparative approaches to prophylactic decompression. In: Lambertsen CJ, ed. Proceedings of the fourth symposium on underwater physiology. New York: Academic Press, 1971:167–177.
27. Gray JS. Aeroembolism induced by exercise in cadets at 23,000 feet. Washington, DC: UA Natl Res Council, 1944. (Comm Aviat Med report 260)
28. Yount DE. Multiple inert-gas bubble disease: a review of the theory. Twenty-second ONR/UMS workshop on isobaric inert gas counterdiffusion. Bethesda: Undersea Medical Society 1982;90–125.

# MICRONUCLEI AND BUBBLE FORMATION: A QUANTITATIVE STUDY USING THE COMMON SHRIMP, *Crangon crangon*

*S. Daniels, K. C. Eastaugh, W. D. M. Paton, and E. B. Smith*

The symptoms of decompression sickness are a direct consequence of gas bubble formation in body tissues. The driving force for such separation is the supersaturation generated by the lowering of ambient pressure below the tension of dissolved gases. Even when symptoms are absent, a substantial number of "silent" bubbles are often formed which may be safely eliminated.

The mechanism of bubble formation has still to be understood and the literature abounds with models and hypotheses, reflecting the complexity of gas separation in vivo. The decompression procedures used today are largely adaptations of Haldane's original empirical observation that divers could safely ascend from 10 msw without symptoms occurring, regardless of the duration of the dive or the rate of ascent (1). As a result of this empirical approach, the procedures do not remove entirely the possibility of bubble formation, and until the underlying principles of bubble inception are fully understood, such decompression procedures are potentially hazardous.

The supersaturation required to produce de novo nucleation in previously denucleated water lies between 100 and 1000 atm (2). If the body behaved like denucleated water, and was totally free of a gas phase, then do novo cavitation could not account for the bubbles arising on decompression, since the levels of supersaturation generated in diving are very much lower than 100 atm. An alternative explanation for their presence is growth from previously existing *micronuclei*, which we may define as small, undetectable masses of gas, existing by virtue of some stabilizing mechanism. These nuclei would serve as *centers* for bubble inception. The point of distinction between a nucleus and a bubble is quite arbitrary and impossible to define in terms of size or shape, but

in this discussion we shall define a bubble as a means of separated gas macroscopic enough to exist without a stabilizing surface.

Indirect evidence for micronuclei comes from both in vitro and in vivo studies. Harvey pretreated water with a hydrostatic pressure of over 1000 atm for up to half an hour, and subsequently could not generate bubbles in it either by decompression or by developing mechanical supersaturations by irradiating with high frequency sound waves (3). The pretreatment had supposedly "crushed" the nuclei back into solution, thus providing no centers for bubble formation or growth. Yount achieved similar results using gelatin, and determined that pre-existing micronuclei were responsible for 99.9% of bubble formation in this in vitro system (4). Knapp's (5) extensive study into the tensile strength of water showed that pressures as low as 20 to 30 atm produce definite increases in the tensile strength. The duration of the pressurization did not appear to be a contributing factor in these studies. The effect of pretreatment was found to last some weeks if the liquid was sheltered from any contamination that could reintroduce nuclei to the system. This finding suggests that nuclei cannot be generated spontaneously in vitro.

The first evidence for the existence of micronuclei in vivo came from Evans and Walder's work (6) using translucent shrimps. They found that hydrostatic pressure pretreatment with 400 atm for 2 min reduced by a highly significant factor the number of shrimps in which bubbles were observed on subsequent decompression. This finding suggested that a compressible phase does exist in biological tissue.

The nature of micronuclei is a matter of great speculation. Harvey's model of micronuclei was that they are undissolved pockets of gas adhering to solid particles (3). Since the "sticking" of gas to surfaces is a matter of contact angles, acutely-angled hydrophobic cracks or crevices were invoked as the stabilizing surfaces. Gas trapped in such a crevice would have its boundary concave to the liquid, a situation that would lower the internal gas pressure and thus the tendency for the nucleus to dissolve.

An alternative model invokes an organic "skin" surrounding a free gas nucleus in the bulk of the liquid or solid (7). A small nucleus without such a skin would be crushed into solution rapidly as a result of the high internal pressure due to surface tension. The presence of the skin would not only have the surfactant effect of lowering the surface tension, which would lower the internal pressure, but perhaps more importantly, would effect a mechanical barrier to diffusion, which would provide rigidity to the nucleus. Yount has improved upon this model by attributing varying permeability to the skins; at radii below a certain critical radius, the skins are impermeable, and the nuclei are mechanically stabilized against their internal crushing pressures (8).

The origin of micronuclei is similarly an issue of much controversy and speculation. The direct contact of a hydrophobic surface with a gas phase would immediately result in their formation. However, in vivo, the situation is undoubtedly not always as simple as this. Nuclei could form as a result of localized mechanical supersaturations generated, for example, on muscle contraction, where a pressure pulse arises. During the brief negative components

of the pulse, cavities could be formed; these cavities would immediately be filled with gas molecules, which up until then would have been dissolved in the surrounding tissues. Similarly, in areas of constricted vasculature, a cavity could arise as the increased velocity at the constriction would effect a localized lowering of pressure. The interfaces thus formed would immediately attract surfactant molecules and the nuclei would develop their stabilizing skins. The possibilities are almost endless, and it is impossible to predict which one, if any, plays the more important role.

Despite the uncertainties surrounding their nature and origin, the susceptibility of micronuclei to hydrostatic pressure pretreatment provides a means to explore some of their properties. The common shrimp, *Crangon crangon*, has been used to allow a direct study of decompression-induced bubble formation in vivo.

## METHODS

The shrimps, caught off the South Coast, weighed between 0.1 and 2.0 g. Under suitable illumination, any bubble formed inside the shrimps could be directly observed with a microscope. The shrimps were decompressed, four (and sometimes two) at a time, in a perspex decompression cell connected via a three-way tap to a reservoir previously evacuated to the required subatmospheric pressure. All decompressions were made into this reservoir and were thus effectively instantaneous. Initially, bubble formation in the shrimps was studied for 1 h postdecompression but 15 min was found to be a sufficient period of observation. The site and time of each bubble inception was noted, and its postinception behavior followed during this 15-min period. It was possible to detect most bubbles with radii >15 microns and all bubbles with radii >25 microns. The majority of bubbles formed grew to radii >100 microns.

In experiments involving hydrostatic pressure pretreatment, the pretreatment was carried out in a sealed, constant-volume bomb (5 cm diameter, 16 cm deep, internal dimensions), in which the pressure was hydrostatically increased to the required level. Shrimps undergoing pretreatment were placed in the bomb in small plastic bags of sea-salt solution, able to transmit the applied pressure.

Results were analyzed using the distribution-free Wilcoxon ranking test for two samples (9), and probability values quoted are the comparisons with the appropriate control series. Although there was great agreement between the nonparametric Wilcoxon method and the Student's *t*-test as regards the criterion of significant or not significant, the Wilcoxon method was considered more secure.

### General Description of Bubble Formation in Shrimps

Well over 90% of the bubbles forming in the shrimps appeared in the cephalothorax. Bubbles forming in the gut were invariably eructated very soon

after their inception. Such bubbles are not strictly internal and were not included in the overall bubble counts. Many bubbles also formed underneath the carapace of both the cephalothorax and the abdomen. Such bubbles were often dislodged by an abrupt movement of the shrimp and were similarly treated as external. Only those bubbles that formed and remained were included in the overall bubble counts. Invariably these bubbles were small and spherical when they first appeared and usually grew to a maximum size within a few minutes. Larger bubbles were also formed by the coalescence of two or more smaller ones; they were often oval or cylindrical in shape, sometimes reaching a millimetre or more across and becoming visible to the naked eye.

On returning the shrimps to atmospheric pressure at the end of the 15-min observation period, all bubbles were seen to dissolve. The shrimps were always entirely bubble-free within 8 min.

In general, shrimps exhibited a remarkable tolerance to bubble formation. Shrimps given a decompression equivalent to rapid surfacing from 90 msw survived in more than 90% of cases, despite occasional extensive bubble formation. Of the shrimps that died following decompression, death did not appear directly related to the number of bubbles formed; often a shrimp would survive after severe bubbling, and other times a shrimp would die after only a few bubbles had been observed.

There was no immediately apparent relationship between a shrimp's weight and the number of bubbles it formed following a given severity of decompression.

## RESULTS

### Effect of the Severity of the Decompression

The effect of varying decompression ratios on the bubble count was investigated to provide control counts for later series of experiments. The

TABLE I

Control Bubble Counts for Various Decompression Ratios

| Decompression Ratio | No. | Mean Bubble Count ± SE |
|---|---|---|
| 1.5:1 | 12 | 0.1 ± 0.1 |
| 2:1 | 12 | 0.3 ± 0.2 |
| 4:1 | 12 | 0.5 ± 0.3 |
| 6:1 | 34 | 3.2 ± 0.5 |
| 8:1 | 12 | 3.7 ± 1.9 |
| 10:1 | 20 | 3.6 ± 0.8 |
| 12:1 | 10 | 4.6 ± 0.8 |

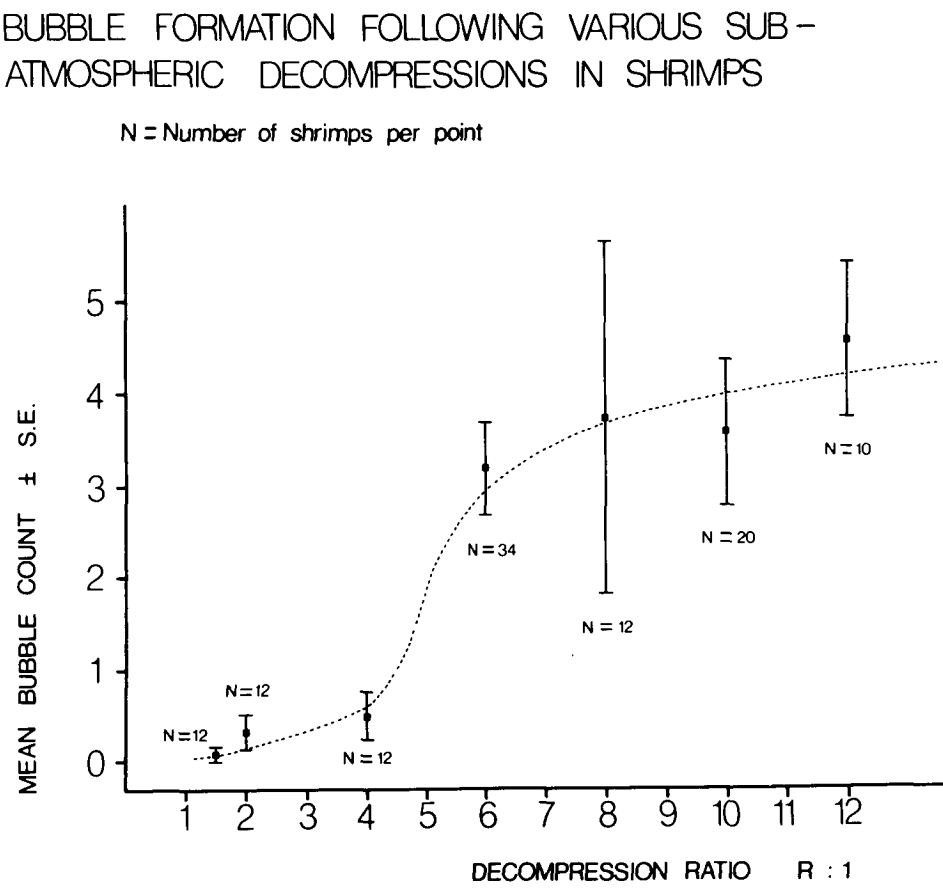



Fig. 1. Bubble counts after various subatmospheric decompressions in the common shrimp, *Crangon crangon*. The decompression ratios are expressed relative to atmospheric pressure. Each *point* represents the mean bubble count for the number of shrimps given and the *error bars* show the SEM. The *line* was drawn by eye.

results can be seen in Table I and graphically illustrated in Fig. 1. The ratios are those between atmospheric pressure and the altitude pressures to which the shrimps were instantaneously decompressed. As can be seen, a few bubbles appeared at ratios lower than Haldane's empirically derived *safe* ratio of 2:1.

As expected, the mean number of bubbles per shrimp increases with increasing severity of decompression, the most dramatic increase occurring betwen ratios of 4:1 and 6:1. After that, the mean bubble count appears to level off. The actual volume of separated gas could well continue to increase for the higher decompression ratios, with the increase distributed among the bubbles already formed. The apparent maximum bubble count may indicate that the shrimps' reservoir of micronuclei is depleted on the more severe decompressions.

## Effect of Hydrostatic Pressure Pretreatment

Shrimps were hydrostatically compressed to pressures ranging between 25 and 400 bars. The duration of the pretreatment was varied from 10 s to 10 min. After pretreatment they were decompressed by a ratio of 10:1. The interval between the end of the pretreatment and the decompression was 5 min, practically the minimum time required to remove the shrimps from the bomb and transfer them to the decompression cell. The results can be seen in Table II and in Fig. 2.

The effect of the duration of pretreatment is quite marked. For each level of compression, excepting the 400-bar treatments, increasing the duration of pretreatment reduced the bubble count. All pretreatments of 10 min duration produce highly significant reductions, and the finding that pressures as low as 25 atm reduce the bubble count by a factor of as much as 5 aroused speculation that even lower levels of compression might produce a similar reduction if the duration of the pretreatment was made even longer. Accordingly, 12 shrimps were given a 1-h pretreatment at 10 bars. The mean bubble count on a subsequent 10:1 decompression (2.583 ± 0.679 SE) was lower than the control, as seen in Fig. 2, though the reduction is not statistically significant (P = 59%). Exposures to 10 bars for 2 h were tried but the mortality after these exposures was high.

TABLE II

Effect of the Duration and Magnitude of Pressure Pretreatment

No. of shrimps per pressure/time experiment = 20
Decompression Ratio 10:1
Time Lag 5 min

| Pretreatment (atm) | Duration | Mean Bubble Count ± SE | P (%) |
|---|---|---|---|
| Control | — | 3.6 ± 0.8 | — |
| 25 | | 2.6 ± 0.8 | 23 |
| 50 | | 1.7 ± 0.4 | 8 |
| 100 | 10 s | 2.0 ± 0.6 | 16 |
| 200 | | 1.2 ± 0.4 | 2 |
| 400 | | 0.4 ± 0.2 | <1 |
| 25 | | 2.2 ± 0.7 | 12 |
| 50 | | 1.6 ± 0.4 | 6 |
| 100 | 2 min | 1.0 ± 0.3 | <1 |
| 200 | | 0.4 ± 0.3 | <1 |
| 400 | | 1.4 ± 0.6 | 2 |
| 25 | | 0.7 ± 0.3 | <1 |
| 50 | | 1.1 ± 0.5 | <1 |
| 100 | 10 min | 0.3 ± 0.2 | <1 |
| 200 | | 0.3 ± 0.1 | <1 |
| 400 | | 0.5 ± 0.2 | <1 |

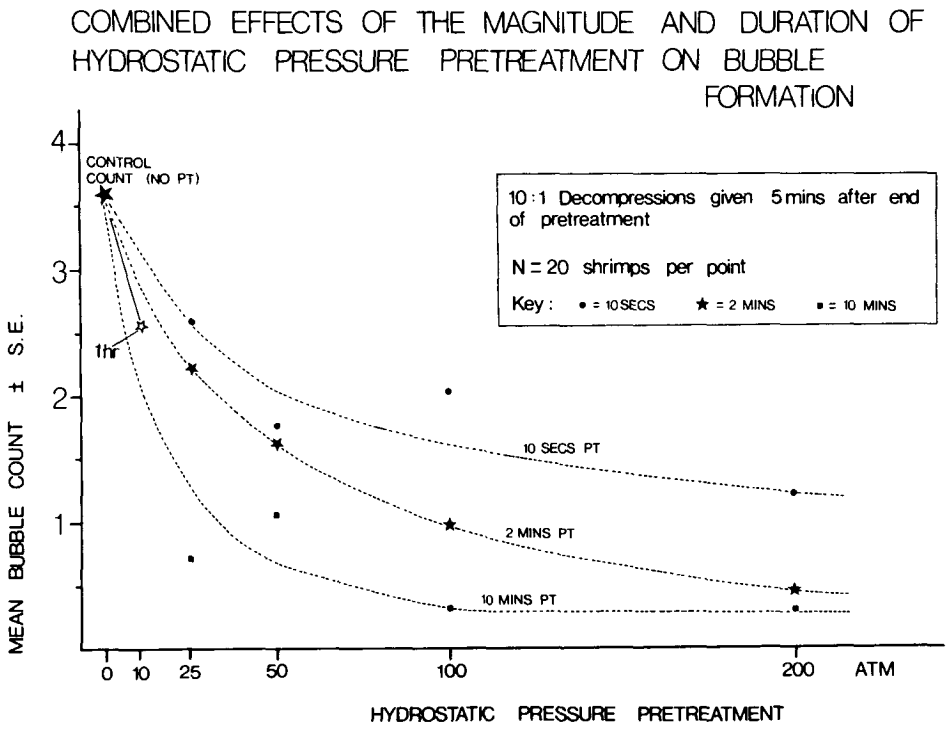



Fig. 2. Bubble counts in shrimps after various durations and magnitudes of pressure pretreatments. Mean bubble counts are shown with each *point* the mean of 20 shrimps. The *lines* were drawn by eye. For details of the SE's see Table II. In all cases the decompression was in the ratio 10:1.

To determine whether such pretreatments genuinely affect micronuclei removal and are not due to any changes in oxygen partial pressure in the shrimps brought about by changed metabolic rates resulting from the pressurization, we investigated bubble formation in dead shrimps. Shrimps were killed by immersion in three different solutions: a) M/100 "MS-222 Sandoz"; b) M/10 000 Phenyl Methyl Sulphonyl Fluoride (PMSF); and c) M/100 Copper Sulphate. Control bubble counts in shrimps killed by a lethal dose of the anesthetic, MS-222, were no different from those observed with live animals, and pretreatment with 200 atm for 2 min rendered a similar reduction in bubble count. The immersion in PMSF results in increased cellular respiration and hence reduces dissolved oxygen tension. Bubble counts in shrimps killed this way were lower than in live shrimps, but pretreatment still produced a significant decrease in count. Bubble counts from shrimps killed by copper sulphate were only marginally different from the live animal counts and, again, pretreatment significantly reduced this bubble count.

It can be concluded that changes in dissolved oxygen tension may affect the bubble count, but not so much as to totally account for the decreases

observed following pressure pretreatment. The effects of a decreased oxygen tension and pressure removal of micronuclei appear to be additive in their reduction of bubble counts, but the bubble counts in shrimps in which the oxygen tension must be very low are still significantly lower in those that have been pretreated than in those that have not.

For pretreatment durations of 2 min and longer, the mean bubble count following compression to 400 bars was higher than those following 200-bar compression; this finding indicates that some other effect of increased hydrostatic pressure might be manifest above 200 atm. The deformation or damage of some larger molecules present in living organisms (e.g., proteins) due to such extreme pressures could result in the creation or the exposure of hydrophobic surfaces. New micronuclei could be formed; this formation might explain the increased bubble counts following 400-atm pretreatment.

MacDonald (10) has reported changes in locomotor activity and related changes in oxygen consumption of marine invertebrates at increased hydrostatic pressures, a condition that could be analogous to the high pressure neurological syndrome (HPNS) observed in higher species. However, he did not report any fatalities even of crustacea that had been raised to 500 atm, although his most rapid rates of compression were slow compared to those used in this study. All pretreatments performed in our work involved rapid rates of change in pressure; compression was done in less than 10 s and decompression even faster.

As noted previously, shrimps did not survive 2 h at 10 bars. This may have been due to an exhaustion of oxygen in the limited volume of salt solution in the plastic bags, although a control group of shrimps not subjected to pressure did not die after 2 h in the same volume of oxygenated salt solution. MacDonald (10) reported a 45% increase in oxygen consumption due to increased locomotor activity at pressures up to about 100 atm, so oxygen exhaustion remains a possibility.

To investigate any possible long-term effect of pressure per se, shrimps were exposed to each of the 15 different exposures discussed in the last section, and their condition monitored for 24 h after treatments ended. There was no evidence that pressure had had any permanent adverse effect on the animals.

## The Regeneration of Micronuclei

Evidence from in vitro studies (5) would suggest that once removed, micronuclei will not spontaneously regenerate if the liquid is kept at rest and free from contaminations that could reintroduce nuclei to the system. However, these conditions can hardly be met in vivo. If pressure pretreatment depletes the body's supply of micronuclei, are they regenerated and, if so, what is the time course for their regeneration?

To investigate this, we performed a series of experiments in which the shrimps were given a pressure pretreatment already known to significantly reduce the bubble count on decompression 5 min later (200 bars for 2 min).

The shrimps were then left for various "time lags" longer than 5 min before being given a 10:1 decompression. The bubble counts were made as usual following the decompressions. The results are given in Table III and are graphically illustrated in Fig. 3.

These results show that micronuclei do regenerate in vivo, with a half time of 8–10 h. The regeneration is substanatially complete after 16 h. The mechanism for this regeneration is at present unknown, although experiments to ascertain the effect of the level of locomotor activity are in progress.

## CONCLUSIONS

The crushing of micronuclei by hydrostatic compression is as dependent on the duration of the treatment as on the pressure itself; a longer duration of a given pretreatment will destroy more nuclei than a shorter duration of the same treatment. Low pressures (25 atm) are sufficient to reduce subsequent bubble formation by as much as 80% if the duration of the pretreatment is 10 min. Shorter durations require higher pressures to produce the same effect, but even a brief 10-s treatment will reduce the bubble count by as much as 66% if the exposure pressure is 200 atm. Once destroyed, micronuclei are regenerated in vivo, and the time course for their regeneration would appear to be of the order of 8–10 h.

It would appear that all nuclei in the shrimp are "activated" following decompressions more severe than 8:1 because the total number of bubbles per

TABLE III

Regeneration of Micronuclei

| Pressure Pretreatment<br>Decompression Ratio | | 200 bars for 2 min<br>10:1 | | | |
|---|---|---|---|---|---|
| Time Lag | No. | Mean Bubble Count ± SE | | | P (%) |
| 5 min | 20 | 0.4 | ± | 0.3 | <1 |
| 30 min | 20 | 0.9 | ± | 0.3 | <1 |
| 1 h | 20 | 0.9 | ± | 0.3 | <1 |
| 2 h | 20 | 1.2 | ± | 0.5 | 1 |
| 4 h | 20 | 0.8 | ± | 0.4 | <1 |
| 5 h | 20 | 1.0 | ± | 0.3 | <1 |
| 6 h | 20 | 1.3 | ± | 0.3 | 3 |
| 7 h | 20 | 1.4 | ± | 0.3 | 4 |
| 8 h | 20 | 2.1 | ± | 0.5 | 16 |
| 10 h | 20 | 2.2 | ± | 0.4 | 25 |
| 16 h | 20 | 2.9 | ± | 0.6 | 70 |
| 24 h | 20 | 3.7 | ± | 0.8 | 93 |
| 32 h | 20 | 3.4 | ± | 0.7 | 84 |
| 48 h | 20 | 3.6 | ± | 0.6 | 42 |
| Control | 20 | 3.6 | ± | 0.8 | — |

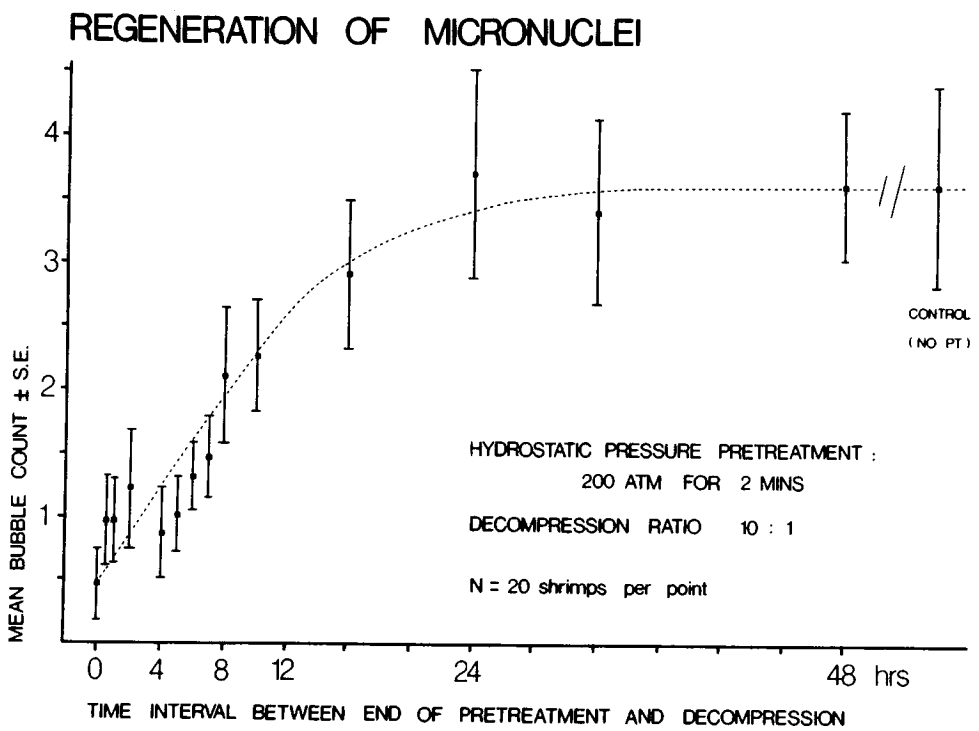



Fig. 3. Bubble counts in shrimps, after decompression in the ratio 10:1 and with 200-bar pressure pretreatment for 2 min, for various times between pretreatment and decompression. Each *point* is the mean of 20 experiments and the *error bars* show the SEM. The *line* was drawn by eye.

shrimp reaches a maximum at this ratio. The greatest change in the number of bubbles is for decompression ratios between 4:1 and 6:1. It may be that the very rapid change in the number of bubbles is a reflection of two populations of nuclei with different stabilizing features. Some nuclei can be activated at ratios lower than Haldane's empirically derived *safe* ratio of 2:1.

This work has provided further evidence for the existence of micronuclei in vivo. Contrary to the suggestion by Harvey (3) that gas micronuclei are unnecessary for bubble formation under conditions that might be important in the body, these results would imply that nuclei do account for a large proportion of the bubbles resulting from decompression.

## Acknowledgments

We thank the MRC and the Wellcome Trust for research support.

## References

1. Boycott AE, Damant GCC, Haldane JS. The prevention of compressed air illness. J Hyg 1908;8:342–443.

2. Harvey EN, McElroy WD, Whitely AH, Warren GH, Pease DC. Bubble formation in animals III. The analysis of gas tension and hydrostatic pressure in cats. J Cell Comp Physiol 1944;24:117–132.
3. Harvey EN. Physical factors in bubble formation. In Fulton JF, ed. Decompression sickness. Philadelphia: W. B. Saunders Co., 1951:90–114.
4. Yount DE, Strauss RH. Bubble formation in gelatin; a model for decompression sickness. J Apply Phys 1976;47:5081–5089.
5. Knapp RT. Cavitation and nuclei. Trans Am Sec Mech Eng 1958;1315–1324.
6. Evans A, Walder DN. Significance of gas micronuclei in the aetiology of decompression sickness. Nature 1969;222:251–252.
7. Fox FE, Herzfeld KF. Gas bubbles with organic skin as cavitation nuclei. J Acoust Soc Am 1954;26:984–989.
8. Yount DE. Skins of varying permeability; a stabilization mechanism for gas cavitation nuclei. J Acoust Soc Am 1979;65:1429–1439.
9. Wilcoxon F. Biometrics 1947;3:119.
10. MacDonald AG. Locomotor activity and oxygen consumption in shallow and deep sea invertebrates exposed to high hydrostatic pressure and low temperature. In Lambertsen CJ, ed. Underwater physiology V. Proceedings of the fifth symposium on underwater physiology V. Bethesda: Federation of American Societies for Experimental Biology, 1976:405–419.

# INVITED REVIEW: INERT GAS EXCHANGE AND COUNTERDIFFUSION IN DECOMPRESSION SICKNESS AND DIVING MEDICINE

*B. G. D'Aoust*

As a reviewer of this session, I must confess considerable dismay at the task at hand. I am asked to present an overview of the very core of the decompression problem: the two areas that are inseparable in considering decompression sickness, whether one approaches it from a clinical or theoretical perspective, are gas exchange and bubble formation. Counterdiffusion is just one aspect of gas exchange—a most important one, of course. These questions have occupied many astute investigators for many years and while there now seems to be more of a consensus as to what the important issues are (also reflected in the papers in this session), one would not always guess that this consensus exists from perusing the literature of the day. We often appear to be reinventing the wheel: ignoring other's work—particularly that of some of the older statesmen of the enterprise—and being rather more preoccupied with theory than experiment. We also frequently indulge in what I will call "semantic inflation," a curious process that seems to be a combination of "explanation by naming" and paradigmatic thinking (1). In short, we seem to have a new set of follies to avoid as well as the old ones, although these, unmasked, are really just the old ones with modern trimmings.

One is more likely to fall into these traps when espousing a pet theory or interpreting new and intriguing data to support it, and so I have decided to resist, as much as possible, the temptation to work the presentations in this session into my own ideas: so that I might share with you some thoughts on past and future strategies in research that can be expected to produce definitive results in the future. Counterdiffusion may play a critical role.

What follows is part review, and I hope, part prologue for the next few years of work. You will recognize as much concern for the style and approach

brought to bear on the problems at hand as for the central questions themselves. This is a subject that always intrigued my doctoral chairman, Professor Per Scholander, who, although less interested in diving than comparative physiology, contributed much to this field through his basic studies and techniques.

In keeping with this theme and in recognition of the great debt I owe Professor Scholander for his constant concern for and willingness to discuss these issues as well as his profound effect on all who worked with him, I wish to dedicate this review to his memory.

And finally, because what you hear today cannot help but be some of the reflected light from your own efforts, I want to acknowledge the vital interaction with my colleagues, their contributions (and sometimes confrontations!) in shaping these thoughts.

## THE CONSENSUS

It is one of the paradoxes of research that both consensus and debate are essential, so let us start with what is generally agreed to be the case. It is generally agreed that separated gas—or changes in an existing gas phase—is the primary etiological agent in decompression sickness. Without reviewing the evidence pro and con for this assumption, I'll point out that one must demonstrate the production of fat emboli from a decrease in hydrostatic pressure when no gas is present if one is to assign a primary role for fat emboli or thrombi. This fact, then, allows the assumption that the fundamental understanding needed is of gas exchange and bubble formation and resolution in the human body.

The interrelationships of these two issues are summarized in Fig. 1, where both gas exchange and supersaturation are illustrated. The figure is explained in detail in the figure legend. It will be familiar to most of you under the familiar name of the "Workman model" (2); the Multiple Parallel Exponential (MPE) model; the Haldane approach (3); the "pragmatic" approach (4); and so on. It depicts four different hypothetical tissue half times, two of them having shaded areas representing the integral of supersaturation and time following decompression. It therefore summarizes graphically many of the current issues of decompression, gas exchange rates, critical supersaturations, bubble growth rates, the (assumed) stability of supersaturation, and, by implication, the slowest tissues.

Fifteen years ago, one might have argued that the then-current understanding of gas exchange and decompression sickness had depended less upon available experimental methods and techniques than upon the logical intellect of J. S. Haldane. Further refinements supporting his approach came clustered at several points in recent history. The early studies of Behnke et al. (5) pointed out the validity of the exponential approach and were later supported by the seminal mass spectrometer studies of Jones (6). The appearance of the analog computer in the early 60's and the coemergence of control theory into

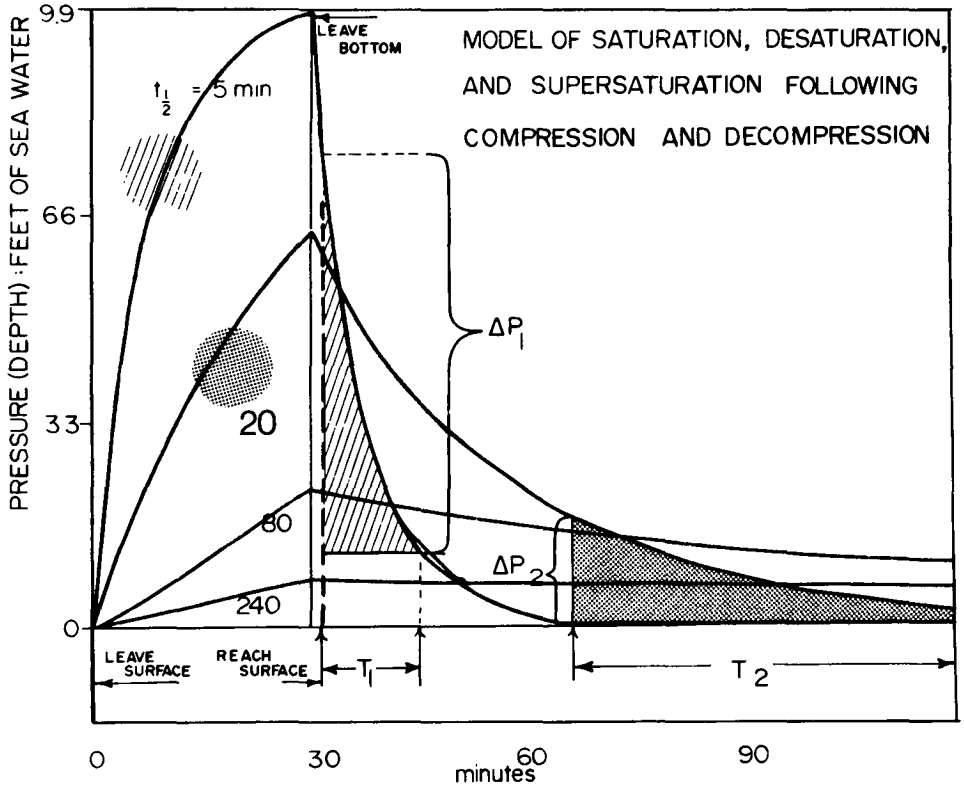



Fig. 1. Gas exchange model used in multiple, parallel exponential (MPE) approaches to decompression. Four hypothetical tissue half times are shown during saturation and desaturation after a bottom time of 30 min. Following arrival at the surface (*vertical dotted line*) two different periods of time are shown for a 5- and a 20-min tissue; the figure illustrates the different types of *stress* on tissues that exchange gas at different rates. The mathematical approximation is compensated by the conceptual convenience of the scheme, and its versatility in adjusting empirical decompression approaches in a consistent fashion. The *cross-hatched* areas under the desaturation curves for the 5- and 20-minute tissues illustrate conceptually what has become tacitly accepted in decompression table formulation and practice, namely, that the faster tissues can "withstand" a higher supersaturation for a shorter time, whereas the slower tissues (in the model) must be allowed less supersaturation because they unload their gas more slowly and therefore are subject to a supersaturation stress for a longer time (*compare* $T_1$ and $T_2$). The areas *under each curve* are purposely shown as approximately equal to illustrate the concept of the product of time and supersaturation as a potential ascent criterion. It must be understood that the entire process of gas phase separation, bubble growth, resolution, and gas elimination from the entire body takes place independently of any projections of the model, but in some cases and possibly even in some tissues is approximately described by the model. Thus, it provides chiefly conceptual guidance rather than predictive accuracy.

physiology prompted several years of fashionable (but not always enlightening) studies of gas exchange in anesthesiology (7,8), which proved to have some benefits in teaching and theoretical analysis. The advent of the commercially available biomedical mass spectrometer in the late 60's further refined some of the experimental data but provided little if any improvement in the predictability of decompression models.

This was largely because of the two unknowns of diving physics: the degree of supersaturation causing bubbles and the actual degree of saturation or desaturation of various tissues at different times. This uncertainty led to alternative hypotheses: a) that one could treat the process just as easily as if bubbles were always being decompressed (9,10) rather than supersaturation; b) that one could treat gas exchange as a simple slab diffusion model (11)—an elegantly simple approach that had the merit of needing less computation yet provided almost identical results to those of the parallel exponential approach provided that supersaturation thresholds were adjusted.

The acceleration of offshore oil exploration increased both the depth and length of deep diving and brought out the weaknesses in all existing assumptions and models. Not surprisingly, research activity increased and in the course of studies at both Duke University and the University of Pennsylvania, the phenomenon of supersaturation induced by gas sequencing or breathing a soluble gas, or both, while surrounded by a less soluble gas was discovered and vigorously studied by a number of laboratories (12–15). This finding has revealed an extremely powerful research tool for testing theories of gas exchange, perfusion, supersaturation development and decay, and decompression sickness.

Counterdiffusion allows the development of supersaturation and therefore bubbles in the body without the need to change the hydrostatic pressure. This then circumvents one of the most serious difficulties inherent in research on decompression sickness, namely, that of the unknown effects of decompression on the cardiovascular system. Inducing bubbles without decompression has revealed that low supersaturations will produce bubbles, that an undersaturation can be produced by proper gas sequencing, and that supersaturation can, in many cases, be quasi-stable in the body for considerable periods of time (15). In summary, the technique has revealed not only the errors of past approaches but also their common truths.

Five of the six papers in this session deal with essentially four different aspects of these issues and illustrate not only different strategies of approach but also different views as to which particular current issues require resolution to improve understanding, predictability, and operational safety and versatility of diving practices. The four areas represented cover the basic biophysics of diving including bubble formation and stability, gas exchange, counterdiffusion, and the phenomenon of adaptation and variability in response. These last two are arbitrarily lumped together here because of my suspicion that they are very much related. It is reassuring to see investigators continuing to explore the problem of variability in response and adaptation because this is one of the phenomena which, rather than frustrating one's attempts to produce nice, crisp

reproducible models, may be used as a means to reveal the physiological processes affecting gas exchange, bubble formation and resolution. In fact, it is this phenomenon as much as any other that has kept us all confused, to say nothing of occupied, over the past 10 years.

Today's presentation on this subject by Eckenhoff and Hughes (16) provides an excellent example of how studying the variability using objective techniques can clearly eliminate certain interpretations of the phenomenon, i.e., the possibility that an important aspect of the adaptive phenomena is the volume of gas released as venous gas emboli. Their results do not support such an assumption and suggest the limitations of the strictly physical interpretations of adaptation.

On the other hand, increased understanding of the strictly physical aspects of the problem are now resulting from the work represented by Yount and Hoffman (17) in this session. Authors Yount and Hoffman (17) emphasize Hills' (18–19) identification of essentially two distinct approaches one can take in designing decompression tables: fitting reality to convenience empirically and fitting reality to theory conveniently! Although such paraphrasing does no justice to many elegant and innovative attempts and partial successes that have resulted in the past, it does perhaps serve to point out the source of much of the confusion in the area and the philosophical differences in style and approach that have gone into this work. In fact, many approaches to decompression tables reveal differences in response to demonstrated facts rather than differences in opinion as to what the facts really are, or what criteria should be used. This problem is understandable if we remind ourselves that decompression table formulation is a practical rather than a scientific endeavor (4). Certainly, many new and scientifically intriguing phenomena have been observed in the course of practically motivated tests.

The discovery of *isobaric counterdiffusion* is a case in point. When the Philadelphia group first described this phenomenon at the Fifth Symposium in 1972 (13), researchers soon recognized that it constituted a powerful research tool with importance and operational potential far exceeding its significance as a hazard, which, in any case, was easily avoided when recognized. Both *steady state isobaric counterdiffusion* and *transient isobaric counterdiffusion* (20) provide a means further to investigate gas exchange in vivo in a qualitative manner, and it is likely that this new technique will eventually lead to more quantitative gas exchange models; it has already facilitated comparisons that have revealed the merits of the perfusion dependent models—a theoretically "fleshed out" version of the MPE approach (4). Although the approach has not yet been successfully used to establish either the average time constants of gas exchange or the critical supersaturation thresholds of the body, initial results, as so often happens, were promising in both areas (15), as is shown in Figs. 2 and 3.

Figure 2 summarizes what we hoped in 1977 might typify the idealized use of the counterdiffusion approach. Notice the intriguing coincidence of the maximum in bubble count rate with an approximate 40-min $N_2$ exchange half time. Although later studies proved to make this hope seem just a bit naive—

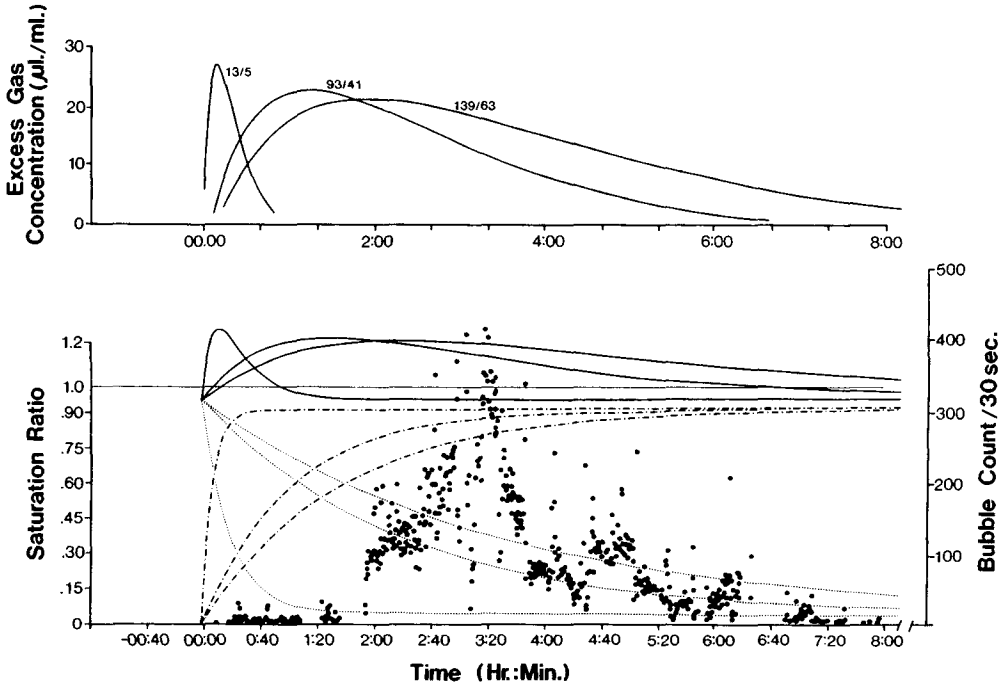



Fig. 2. Composite diagram of one of the first transient counterdiffusion experiments carried out in 1976 by the Virginia Mason Group. The experimental data shown as *small dots* represent the bubble counts detected in the posterior vena cava using a surgically implanted Doppler cuff and bubble counter. For comparison of the time of appearance of bubbles with the time of maximum supersaturation, three hypothetical tissue half-time pairs were computed for helium and nitrogen half times. That is, it was assumed a) that any particular location in the body has a characteristic nitrogen and helium half time, and b) that these do not bear a constant ratio to each other but differ according to the rate at which the tissues exchange gas. Thus, the ratio of the 13:5 nitrogen-helium half time is 2.6, whereas the ratio of the 139:63 nitrogen-helium pair is 2.21. This difference attempts to take into account the possibility of a greater or lesser effect of diffusion rate in contributing to the gas-exchange half time of any point in the body. The total inert gas tension, i.e., nitrogen and helium, is computed after a sudden gas switch at 198 fsw imposed on awake goats, which were saturated at that pressure of nitrogen. The total environmental gas change required less than 5 min. Although the total inert gas tensions are plotted against time after the gas switch in the *lower figure*, the *top figure* plots the transient change in calculated total excess dissolved gas. Notice that the time of the maximum value of excess dissolved gas *leads* the trend in total gas tension. This fact must be considered in accounting for bubble growth and resolution under these conditions. The *clusters of dots* show the timing of bubble counts after the gas switch. Notice in particular that the maximum in bubble count rate occurs at approximately 3 h, 20 min after the gas switch, or 6 half times for a 40-min tissue (the *abcissa* is divided into 40-min increments). (Reprinted from Ref. 15, with permission from *Science*)

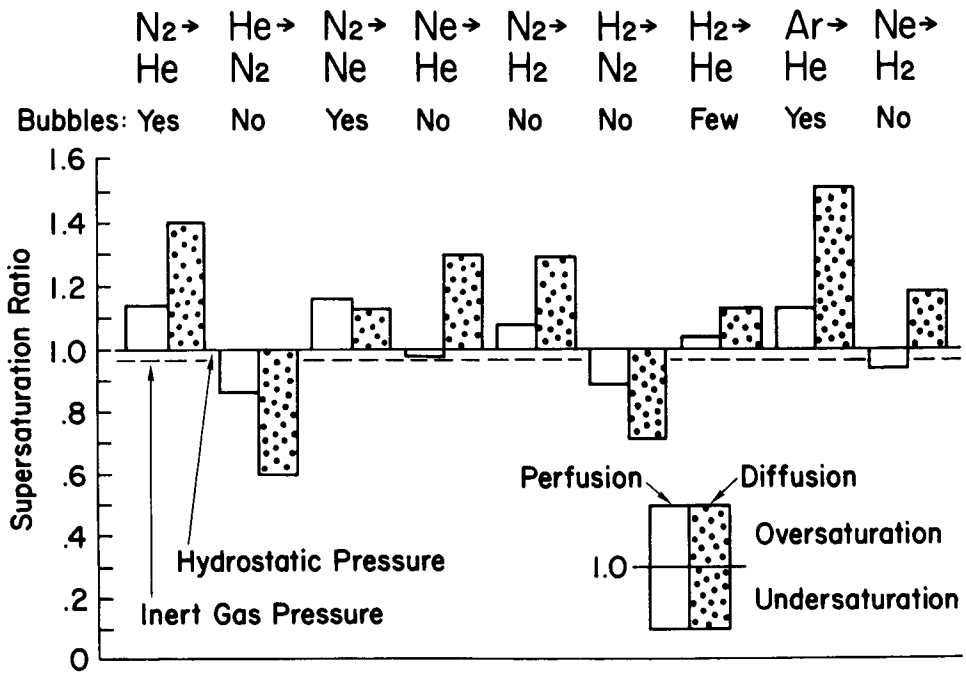



Fig. 3. Summary comparison of calculations of diffusion vs. perfusion-dependent supersaturation when produced by a gas sequence illustrated at the *top* of the figure. These are based on the results of a large number of transient isobaric counterdiffusion experiments carried out by the Virginia Mason Group from 1976 to 1982. All combinations of gas switches shown at the *top* of the figure were experimentally tested for the production and the relative numbers of bubbles after a gas switch (like that described in Fig. 2). The presence or absence of bubbles is indicated by the words *"Yes"* or *"No"* immediately under the gas pair. The convention is that the gas indicated to the *left of the arrow* was the gas on which the animals were saturated and this gas was suddenly replaced in the chamber by the new gas. The histograms *shaded with dots* represent the degree of supersaturation or undersaturation calculated to have been produced according to a diffusion dependent model, and the *open histograms* represent the same calculation for a perfusion-dependent model. Notice that in most cases the histograms "point" in the same direction meaning that both models predict the same qualitative result. The exceptions are two and occur for the neon-to-helium experiment and the neon-to-hydrogen experiment—*neither* of which produced bubbles. This is consistent only with the perfusion-dependent model in that it predicts undersaturation and the diffusion-dependent model predicts supersaturation. This finding is taken as good evidence that the supersaturation causing the bubbles detected at the central venous location is related to a perfusion-dependent process.

revealing as they did a great deal of individual variability—we were able to demonstrate the utility of the technique in proving the undersaturation concepts first pioneered by Keller in 1962 (21,22) and in a later series of experiments using isobaric counterdiffusion and Doppler bubble detection to test simplified perfusion vs. diffusion assumptions. These experiments provided strong evidence for the validity as well as utility of the perfusion-dependent approach. A summary of this work appears in Fig. 3 and indicates the potential for both research and operational extension of *No-Decompression* limits (details are described *in the figure legend*).

Thus, the MPE model of gas exchange "fits in" with an understandable tendency to acknowledge the great amount of spatial and temporal ranges in tissue solubility, perfusion, and vascularity and, in the view of many, still can provide the most useful approximation of gas exchange. On the other hand, it presents a logical trap, frequently pointed out by proponents of the diffusion models (9,11,19,22,23) in that many initial bubble formation sites are obviously extracellular and probably a result of diffusion limitations! Also, any objective look at mammalian anatomy reveals the experimental difficulty of verification of either model in any usable manner. This brings us to the problem of the criteria that are to be used to verify theories of gas exchange and, inevitably, to the problem of bubble formation.

Three of the papers in this session deal with this relatively "hot" area. Although the basic concepts of silent "bubbles" have been around since Haldane formulated his approach and the verification of gas micronuclei has been with us for approximately 40 years, because of Harvey's (24) work, there has been a resurgence of interest in the last 10 years—largely because of the 1969 work of Evans and Walder (25). More recently, Yount and co-workers (26–28), Vann and Clark (29), Daniels et al. (30), Gerth and Hemmingsen (31), and Tikuisis and Johnson (32) have studied the dynamics of gas micronuclei, their origins, stability, lifetimes, and resolution. It is particularly gratifying to see today's modern methods being brought to bear on these fundamental problems. Authors Daniels et al. (30), Yount and Hoffman (17), and Tikuisis and Johnson (32) represent some of the most vigorous contemporary contributors to this area of bubble formation, stability, and resolution, and I believe that the merging of their findings will lead to a new level of theoretical insight and practical improvement in decompression models.

## PERSPECTIVES FOR THE FUTURE

Having given you my "once over lightly" version of where we are and how we got there, I want to complete my stated purpose of considering perspectives for the future. The questions that seem critical at this stage follow.

In regard to bubble formation: what is the course, stability, distribution, and size range in the body of the microgas nuclei whose existence is supported by both theory and experiment?

Of the vascular gas emboli that are now detected by Doppler ultrasound, what proportion have their origins as gas micronuclei, and what proportion are more appropriately treated accordingly to the "crack" model as introduced by Harvey (24) and further reported here by Tikuisis and Johnson(32)?

In a similar way, what proportion of the extravascular gas bubbles in decompression have their origin as micronuclei vs. "crevices," and how are their populations and distributions related to tissue function?

Is it possible that many of the physiological responses to decompression could result from physicochemical changes in cell organelles or membranes, or both, as a result of expansion of inter- or intracellular microgas nuclei or crevices, and if so, could such changes explain some of the adaptive as well as variable phenomena that are observed?

And finally, are there processes in the body that actually generate stable gas micronuclei, such as muscular contraction, cell growth, fluid (Bernoulli) flow, radiation, local chemical trauma such as peroxidation, complement cascade, and other cell-killing mechanisms?

In regard to gas exchange: at what point in decompression rate does the cardiovascular system "see" the effects of decompression so that gas elimination ceases to be symmetrical with uptake? Studies in our laboratory with dogs and goats (33,34) have demonstrated that known stressful decompression in these animals can retard gas elimination. The question remains whether currently acceptable decompression rates slow down gas elimination as shown by D'Aoust et al. (33). Although calculations show that the sizes of released bubbles, and so the critical volume of gas released, is insufficient to significantly change the amount of gas dissolved, as pointed out by Yount and Hoffman (17) in this session, it is still possible that a very small amount of gas-phase separation or volume expansion can drastically affect the cardiovascular system or microcirculation, or both, in a tissue bed and so retard gas elimination. Elucidating the mechanism of physiological "amplification" of these responses must be an important part of future work. Indeed, it is remarkable that, as pointed out by Pisarello et al. (35), massive venous gas emboli failed to alter cardiovascular parameters in half of their subjects, although this failure may have been the result of the anesthesia.

Do the species differences in susceptibility to steady state isobaric counterdiffusion encountered by the Philadelphia group reflect chiefly compositional differences (i.e., fat content) and its effect on total gas concentrations, or is this difference more related to mechanical considerations? If so, the total gas concentration as much as the partial pressure becomes one of the important parameters in decompression table calculation. No previous models have used this parameter although it has been suggested by D'Aoust and Smith (36).

Is there a fundamental physical reason for the variability in response to decompression (or, put another way, to bubble formation), and could it be a manifestation of variations in gas saturation state or elimination rates, or alternatively, is it a variation in nuclei numbers or critical thresholds? Stated still another way, which parameter is more constant in the human body: gas exchange rate or critical thresholds? We still have no way of knowing!

Finally, but perhaps most important and significant, what are the processes of adaptation? Are they related to spent nuclei or bubble sites; reduction in sympathetic or autonomic threshold of response; reduced perfusion (on the repetitive dive) to the affected area; or, as one throws up one's hands, all of the above?

And what, you may ask, are my suggestions for answering these questions? As I see it, the first ingredient is a considerably increased degree of cooperation as well as collaboration between groups. In the salad days of funding of biomedical research, it is perhaps understandable as well as affordable that more alternative models, theories, and approaches proliferated. It is equally inevitable that we must now bring the best of the last 10 years together to focus on the difficult questions in as cooperative a fashion as possible. We must extend investigations of gelatin into in vivo work such as reported by Daniels et al. (30) in this session, and earlier by Evans and Walder (25), and extend them ultimately into mammalian systems. I saw some elegant preparations at a Microcirculation Congress in 1979; I believe they would have great potential for "marrying" the two conjunctive problems of gas exchange and bubble formation, and at the same time would be amenable to the techniques of counterdiffusion. I wrote for more information seeking collaboration, but received no answer. Many of you have had similar experiences.

On the scholastic side, we must pay more attention to the fashionable follies of the era. In many cases, progress is being held back as much by the unwillingness to cooperate as by artificially inflated pressures of the "least publishable unit" (LPU), discussion of which I leave to others in more secure surroundings. Consider, if you will, how many times you have seen glaring omissions of relevant citations in the reference list of a paper when it is obvious that the authors know better? Such "not invented here" tactics in the long run help neither the authors responsible nor the readers.

Finally, we need to pay more attention to what constitutes *scientific research*. The distinction between searching out that which is truly not known vs. fleshing out existing theories becomes cloudy in a field such as this, where the benefits are practical and the very basic aspects generally understood. Nevertheless, it deserves our attention if we are to call this activity *scientific research*. The pre-eminence of the paradigmatic approach to many areas of research as described by Thomas Kuhn (1) in the early 60's has often suffocated innovation, creativity, and even the practical application of these gifts by many investigators. And when the paradigmatic type of thinking—the norm in peer review committees and councils—is brought to bear on questions of merit or funding, or both, the results are hardly constructive. It is just as counterproductive to ignore a good experiment because it will be difficult to test as it is to carry out a mediocre one because it is easy—or worse, because it fits the budget and is not controversial.

To avoid these pitfalls, we must all at least recognize them. Beyond that, surely the recognition that we are all more dependent on one another (as shown by papers in this session) than we are in competition should help to insure the kind of atmosphere necessary. For my part, I've enjoyed participat-

ing in this session, and I hope that my concerns find some support from this group.

## References


1. Kuhn ST. The structure of scientific revolutions. Chicago: University of Chicago Press, 1962.
2. Workman RD. Calculation of decompression schedules for nitrogen-oxygen and helium-oxygen dives. Washington DC: U.S. Navy Experimental Diving Unit, 1965. (NEDU report no. 6–65)
3. Boycott AE, Damant GCC, Haldane JS. Prevention of compressed air illness. J Hyg (Camb) 1908;8:3442–3443.
4. Schreiner HR, Kelly PL. A pragmatic view of decompression. In Lambertsen CJ, ed. Underwater physiology IV. Proceedings of the fourth symposium on underwater physiology. New York, Academic Press, 1971:205.
5. Behnke AR, Willmon TL. Gaseous nitrogen and helium elimination from the body during rest and exercise. Am J Physiol 1941;131:619–636.
6. Jones HB. Respiratory system: Nitrogen elimination. In: Glasser, ed. Medical physics. Vol. II. Chicago: Year Book Publishers, 1951:855–871.
7. Kety SS. The theory and applications of the exchange of inert gas at the lungs and tissues. Pharm Rev 1951;3:1–41.
8. Papper EM, Kitz RJ. The uptake and distribution of anesthetic agents. New York: McGraw-Hill, 1963.
9. Hills BA. A thermodynamic and kinetic approach to decompression sickness. Ph.D. Thesis. Adelaide: Library Board of South Australia, 1966.
10. Hills BA. Decompression sickness. Vol. 1: The biophysical basis of prevention and treatment. New York: John Wiley and Sons, 1977.
11. Hempleman HV. Decompression theory: British practise. In Bennett PB, Elliott DH, eds. The physiology and medicine of diving and compressed air work. 2nd ed. London: Baillière, Tindall, 1975:331–347.
12. Blenkarn GD, Aquardo C, Hills BA, Saltzman HA. Uticaria following the sequential breathing of various inert gases at a constant ambient pressure of 7.7 ATA. A possible manifestation of gas induced osmosis. Aerosp Med 1971;42:141–146.
13. Idicula J, Graves DJ, Quinn JA, Lambertsen CJ. Bubble formation resulting from the steady counterdiffusion of two inert gases. In: Lambertsen CJ, ed. Underwater physiology V. Proceedings of the fifth symosium on underwater physiology. Bethesda: Federation of the American Societies for Experimental Biology, 1976:335–340.
14. Graves DJ, Idicula J, Lambertsen CJ, Quinn J. Bubble formation in physical and biological systems: a manifestation of counterdiffusion in composite media. Science 1973;179:1139–1140.
15. D'Aoust BG, Smith KH, Swanson HT, White R, Harvey CA, Hunter WL, Neuman TS, Goad RF. Isobaric counterdiffusion; experimental production of venous gas emboli at five atmospheres with diving gases. Science 1977; 197:889–891.
16. Eckenhoff RG, Hughes JS. Acclimatization to decompression sickness. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society 1984:93–100.
17. Yount DE, Hoffman DC. Decompression theory: A dynamic critical-volume hypothesis. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:131–146.
18. Hills BA. Vital issues in computing decompression schedules from fundamentals. I. Critical supersaturation versus phase equilibration. Int J Biometeorol 1970;14:111–131.
19. Hills BA. Zero-supersaturation approach to decompression optimization. In: Hong SK, ed. International symposium on man in the sea. Bethesda: Undersea Medical Society, Inc., 1975:179–189.
20. D'Aoust BG, Lambertsen CJ. Isobaric gas exchange and supersaturation by counterdiffusion. Chapt 15. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving and compressed air work. London: Ballière Tindall, 1982.

21. Keller H. A method of deep diving with fast decompression by alternating different inert gases. Rev Physiol Subaq Med Hyp 1968;1:127–129.
22. Buhlmann AA. The use of multiple inert gases in decompression. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving and compressed air work. 2nd ed. London: Ballière Tindall, 1975.
23. Hempleman HV. The unequal rates of uptake and elimination of tissue nitrogen gas in diving procedures. London: Medical Research Council, Royal Navy Personnel Research Committee, 1960.
24. Harvey EN. Physical factors in bubble formation. In: Fulton J, ed. Decompression sickness. Philadelphia: W. B. Saunders, 1951:90.
25. Evans T, Walder DN. Significance of gas micronuclei in the aetiology of decompression sickness. Nature (Lond) 1969;222:251–252.
26. Yount DE, Strauss RH. Bubble formation in gelatin; a model for decompression sickness. J Appl Phys 1976;47:5081–5089.
27. Yount DE. Skins of varying permeability: a stabilization mechanism for gas cavitation nuclei. J Acoust Soc Am 1979;65:1429–1439.
28. Yount DE. Application of a bubble formation model to decompression sickness in Fingerling salmon. Undersea Biomed Res 1981;8:199–208.
29. Vann RD, Clark HG. Bubble growth and mechanical properties of tissue in decompression. Undersea Biomed Res 1975;2:185–194.
30. Daniels S, Eastaugh KC, Paton WDM, Smith EB. Micronuclei and bubble formation: A quantitative study using the common shrimp *Crangon crangon*. In Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea' Medical Society, Inc., 1984:147–157.
31. Gerth WA, Hemmingsen EA. Gas supersaturation thresholds for spontaneous cavitation in water with gas equilibration pressures up to 570 atmospheres. Z Naturforsch 1976;31A:1711–1716.
32. Tikuisis P, Johnson WR. Conditions for heterogeneous nucleation in the physiological environment. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:107–118.
33. D'Aoust BG, Smith KH, Swanson HT. Decompression induced decrease in nitrogen elimination rate in awake dogs. J Appl Physiol 1976;41:348–355.
34. D'Aoust BG, Swanson HT, White R, Dunford R, Mahoney J. Central venous bubbles and mixed venous nitrogen in goats following decompression. J Appl Physiol: Respirat Environ Exercise Physiol 1981;51:1238–1244.
35. Pisarello J, Fried M, Fisher DG, lambertsen CJ. Superficial isobaric counterdiffusion gas lesion disease: Effects leading to mortality. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:101–106.
36. D'Aoust BG, Smith LS. Bends in fish. Comp Biochem Physiol 1974:49A:311–321.

# Part III

## DECOMPRESSION SICKNESS, OSTEONECROSIS, AND OTHER DIVE-RELATED DISORDERS

# SCUBA DISEASE REVISITED(?)

*M. E. Bradley and R. C. Bornmann*

In 1970, Edmonds (1) reported a self-limited respiratory disease afflicting students at the Royal Australian Navy Diving School. He ascribed this disease to aspiration of small amounts of sea water during dives preceding the onset of the syndrome. The syndrome reported by Edmonds bears a strong resemblance to a disease known as *scuba disease*, which was prevalent between 1955 and 1959 in U.S. Navy diving operations in tropical and subtropical climates. Scuba disease was shown to be the result of aspiration of gram-negative organisms, especially *Pseudomonas*. The source of this infection was found to be grossly contaminated scuba hoses and regulators.

This paper, therefore, focuses attention on the similarities of these two disease outbreaks and emphasizes the potential hazard of diving in warm, humid environments with underwater breathing equipment that is not cleaned regularly and adequately.

## METHODS AND RESULTS

Edmonds reported a series of 30 cases of disease that occurred in Royal Australian Navy divers at the RAN Diving Training School at Sydney. These cases occurred during a 9-month period, and were ascribed to aspiration of sea water. Edmonds dubbed this disease, *A Saltwater Aspiration Syndrome*. The information about this disease is excerpted from Edmonds report (1).

Information concerning scuba disease was gleaned from U.S. Navy reports and correspondence during 1956–1960 and from a publication by Kavanagh et al. (2). Scuba disease was prevalent in U.S. Navy divers at the Underwater Swimmers School at Key West, Florida, during the period of 1955–1959. A review of the records of this school revealed that every class

experienced this disease, with attack rates of 10–75%. During the 7-month period from April to October 1958, 43 divers were afflicted. Comparison of the symptoms, physical and laboratory findings, and clinical courses of these outbreaks follows.

## Dive History and Prodromal Period

Edmonds reported that 90% of afflicted divers provided a history of aspiration. Over 90% reported an immediate postdive cough. This was followed by a latent period with an average duration of 2 h (range 0–15 h) during which these individuals were asymptomatic.

The reports of scuba disease in the Key West divers do not specifically mention the occurrence of aspiration of sea water. One report states, however, that "since the compressed air was completely dry, breathing this air was irritating enough to elicit comment by the majority of the swimmers. For relief, many swimmers swallowed water and allowed a small amount of water to enter the intake air hose, so that the air would become moisturized as it passed through the hose." From this description it is likely that many of these divers did indeed aspirate some quantity of sea water. Postdive, these divers were essentially asymptomatic for an average period of 24 h (range: 12–48 h).

## Initial Symptoms

The incidence of initial symptoms in these two patient populations following the prodromal period is reported in Table I. Dyspnea and production of sputum were not prominent in patients with scuba disease. Edmonds, however, reported that 73% of his patients had dyspnea, and 66% produced some sputum. Individuals from both groups of patients reported retrosternal discomfort or pain.

TABLE I

The Incidence of Initial Symptoms of the Saltwater Aspiration Syndrome and Scuba Disease

| Symptoms | Saltwater Aspiration (%) | Scuba Disease (%) |
|---|---|---|
| Chills | 77 | 91 |
| Anorexia, Nausea, Vomiting | 80 | 94 |
| Cough | 66 | 48 |
| Malaise | 53 | 79 |
| Aching | 33 | 34 |
| Headache | 66 | 58 |

## Physical and Laboratory Findings

The positive findings of physical examination and laboratory studies in both groups of patients are summarized in Table II. Measurements of vital capacity and $FEV_{1.0}$ in Edmond's patients showed an average decrease of 0.7 L early in the course of the disease. Measurements of arterial blood gases were obtained in two patients; they were 76 mmHg in one patient and 40 mmHg in the other patient.

Measurements of pulmonary function were not obtained for those patients with scuba disease. Extensive serological, viral, bacteriological, rickettsial, and fungal studies, which were essentially negative, were conducted, however.

## Clinical Course

Edmonds reported that "signs and symptoms usually reverted to normal in six hours and rarely persisted beyond 24 hours unless the case was of considerable severity." Abnormalities in chest x-rays also usually disappeared in 24 h, but in severe cases remained for a week.

Scuba disease generally ran its course in 24–48 h, and patients were fit again in 72 h. In November 1958, one of the students who had this disease developed a fulminating bronchopneumonia and died. At the time of autopsy, culture studies produced heavy growths of various species of *Pseudomonas* from the lung, liver, and kidney. No fungal or viral agents were recovered.

TABLE II

Physical Examination, Findings, and Laboratory Studies in the Saltwater Aspiration Syndrome and Scuba Disease

| Findings | Saltwater Aspiration (%) | Scuba Disease (%) |
|---|---|---|
| Fever | In 50% of patients mean of 100.6°F (in one instance as high as 104°F) | In 100% of patients mean of 100.7°F (in one instance as high as 105°F) |
| Lungs | 50% had "evidence of crepitation" or rhonchi | 25% had scattered rales or rhonchi |
| Chest X-Ray | 50% had areas of patchy consolidation or increased respiratory markings | 15–20% had patchy areas of consolidation, punctate miliary infiltrates, or increased hilar markings |
| White Blood Cell Count & Differential | Mild leukocytosis (not in excess of 20 000), with a shift to the left in a few cases | Mild leukocytosis (median of 12 000; not in excess of 17 000), with a shift to the left in a few cases |

### Diving Conditions

Underwater breathing equipment used by those patients with the Saltwater Aspiration Syndrome was the CABA (open-circuit scuba) or the SSBA (a surface-supplied system that is essentially a scuba device with "bail-out bottles." The climate of Sydney is temperate with cool (50°F) winters and warm-hot (80–90°F) summers with high humidity.

The breathing gear used by personnel who had scuba disease was the double-hosed Cousteau-Gagnan Aqua-Lung in vogue in the late 1950's. The climate of Key West, the major locus of this disease, is subtropical with a high average temperature and humidity.

## DISCUSSION

As can be seen in the preceding section, there are strong similarities between the Saltwater Aspiration Syndrome described by Edmonds (1), and scuba disease prevalent in the late 1950's at Key West, Florida. The three major differences between these two disease processes appear to be a) the length of the prodromal period (2 h for saltwater aspiration versus 24 h for scuba disease); b) the lesser incidence of dyspnea and production of sputum in patients with scuba disease; and c) the length of illness (6–24 h for saltwater aspiration versus 24–72 h for scuba disease).

Edmonds (1) hypothesized that the disease process that he observed in his patients was the clinical analogy to aspiration of sea water in animal models (3). He stated that some aspects of the clinical presentation might be the result of hypothermia coupled with hypoxemia. It is disturbing, however, that there is a total absence of subsequent confirmatory reports of a Saltwater Aspiration Syndrome in other diver populations. While Edmonds thought that decompression sickness and pulmonary barotrauma should be considered in the differential diagnosis of the Saltwater Aspiration Syndrome, he did not consider scuba disease. We deem it likely that the Saltwater Aspiration Syndrome is in fact a form of scuba disease.

There are indications that scuba disease was very prevalent throughout diver populations who used scuba gear during the period of 1955–1960. The same disease was observed in Underwater Ordnance Units at Key West and Pearl Harbor, Hawaii, in Underwater Demolition Team divers operating in the Virgin Islands, and in civilian scientist divers at a U.S. Navy laboratory in San Diego, California. A report from a Cuban physician during this time period describes a similar *flu-like* illness that occurred in college students following use of scuba gear while on vacation in Cuba.

Because of the magnitude of scuba disease, together with the stimulus provided by the death of one of these divers, an extensive epidemiological and health hazards survey was initiated. Surveys for possible toxic chemicals, metals, and gases were negative, as were surveys for viral, rickettsial, and most fungal agents. Samples obtained from the scuba regulators, hoses, and

mouthpieces, however, yielded very heavy growths of *Pseudomonas* species (Figs. 1,2), together with Fusarium oxysporum and Fusarium solanum. This study implicated *Pseudomonas* species as the primary etiological agent. Release of endotoxins from *Pseudomonas* entering the respiratory and/or gastrointestinal tracts was considered to be a major factor in the pathogenesis of this illness.

*Pseudomonas,* like most gram-negative bacteria, contains potent endotoxins. Consequently, invasion of the blood stream by these organisms and their subsequent lysis can evoke a syndrome similar to that seen when some of the older, less-purified typhoid vaccines were administered. This endotoxemic syndrome consists of chills, fever, headache, malaise, and even shock, as well as other manifestations. The pathogenesis of scuba disease may be as follows: gram-negative bacteria or their lysis products enter the respiratory and/or gastrointestinal system of the diver. They are eventually absorbed into the systemic circulation where lysis of the intact bacteria releases the endotoxin. If the amount of endotoxin released exceeds the detoxification capacity of the liver, the result is the endotoxic syndrome. If the exposure is minimal or gradual, the detoxification mechanism is adequate, or becomes enhanced and suppresses the syndrome.

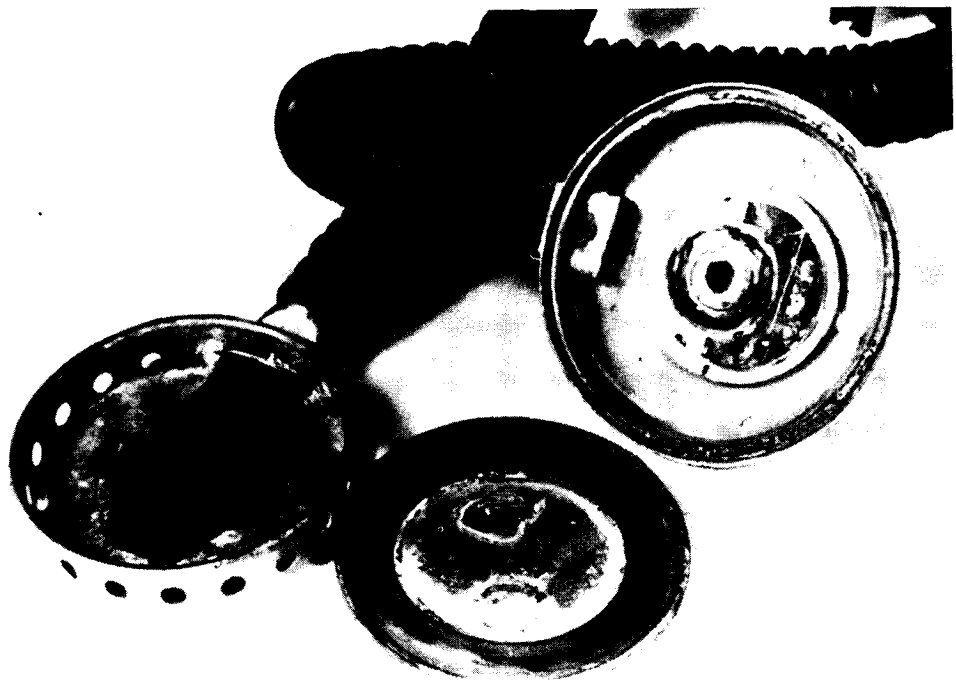


Fig. 1. Cousteau-Gagnan two-stage regulator.

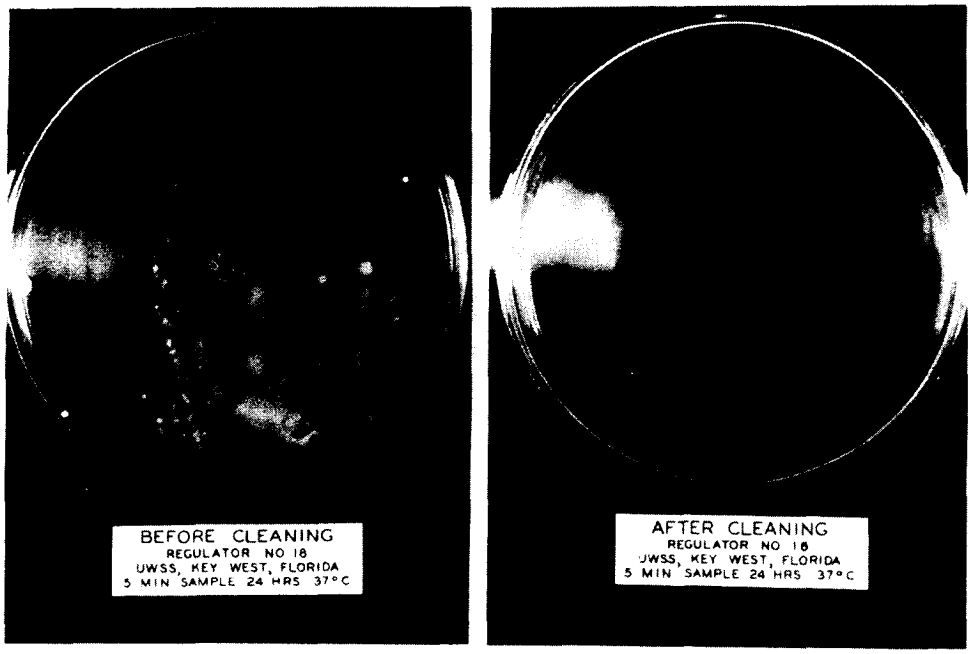



Fig. 2. Air samples Cousteau-Gagnan scuba regulator: *before cleaning* and *after cleaning*.

As a result of the scuba disease survey, rigorous, regular cleaning and decontamination procedures for scuba gear were initiated (Fig. 3). This action was promptly followed by the virtual disappearance of scuba disease in U.S. Navy diving personnel at that time.

In the past decade, episodes of otitis externa, caused by *Pseudomonas aeruginosa,* have frequently disrupted saturation dives conducted by the military and commercial diving communities. Alcock (4) performed a microbiological examination of the external ear canals of 58 divers and found only one (2%) who harbored *Pseudomonas*. *Pseudomonas* is a commensal in the bowel and on the skin of many healthy people, however, and multiplies rapidly in warm, humid environments. During the saturation dives, *Pseudomonas* was isolated at some stage in 82% of the 58 divers. Frequent decontamination of the interior of chambers and regimens of prophylactic ear treatment have substantially reduced the incidence of otitis externa during saturation dives (4,5).

Cleaning and decontamination of underwater breathing equipment is not routinely performed in the saturation diving environment. It is worrisome that Daily et al. (6) found detectable levels of endotoxins in the blood of four divers during a 1500-ft dive. They postulated that the possible route for access of endotoxins into the systemic circulation may have been via the peritoneal cavity. Entry in the oropharynx, respiratory tract, and the gastrointestinal

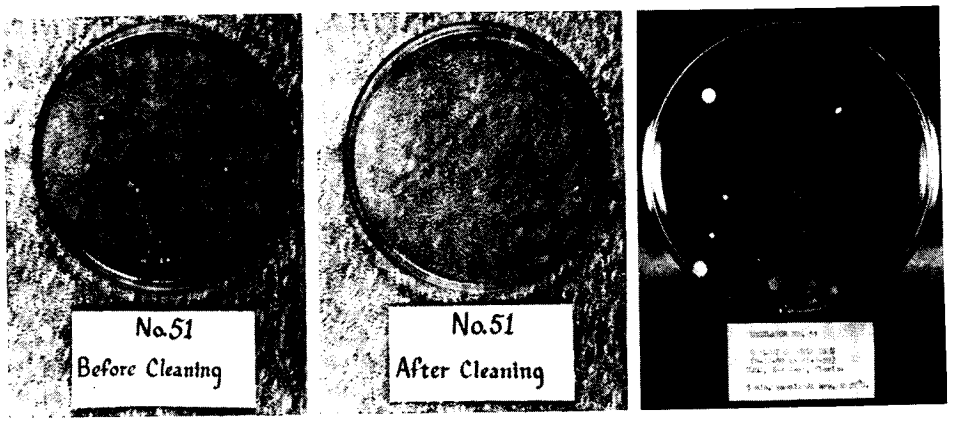



Fig. 3. Air samples Cousteau-Gagnan scuba regulator. *Left to right: before cleaning; after cleaning*; and *following storage for 42 days.*

system from contaminated underwater breathing equipment, however, is more likely.

In the past 15 years, the problem of nosocomial infections has been a major problem in the hospital setting (7) and is especially great in respiratory and surgical intensive care units. Since the treatment of nosocomial gram-negative infections carries a high morbidity and mortality, and since present methods of treatment are not very satisfactory, the emphasis in clinical settings has been prevention of the disease.

Scuba disease remains a potential health hazard for recreational, military, and commercial divers, especially when diving in warm, humid climates and in saturation diving environments. Regular cleaning and decontamination of underwater breathing equipment is imperative. Medical personnel involved in the training and treatment of divers must be aware of the potential incidence of this disease.

## Acknowledgments

This work was supported by NMRDC Task No. M0099PN.01B.0004. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting views of the Navy Department or the Naval Service at large. The outstanding editorial work of Ms. Maureen Miller is greatly appreciated.

## References

1. Edmonds C. A saltwater aspiration syndrome. Military Med 1976;141:779–785.

2. Kavanagh AJ, Halverson CW, Jordan CJ, Lachapelle NC, Sanborn WR, Emmons CW, Siess EE. A scuba syndrome. Connecticut Med 1963;27(6):315–318.
3. Halmagyi DFL, Colebatch HJH. Ventilation and circulation after fluid aspiration. J Appl Physiol 1961;16:35–40.
4. Alcock SR. J Hyg 1977;78:350–395.
5. Raymond LW, Spaur WH, Thalmann ED. Prevention of divers' ear. Br Med J 1978;1(6104):48.
6. Daily OD, Uddin DE, Walker RI, Spaur WH. Endotoxemia during hyperbaric stress. Undersea Biomed Res 1978;5(Suppl):37.
7. Hedley-Whyte J, Burgers GE III, Feeley TW, Miller MG. Problems of nosocomial pulmonary infections. In: Applied physiology of respiratory care. Boston: Little, Brown and Co., 1976:37–54.

# AN ACTUAL APPROACH TO PREVENT DECOMPRESSION SICKNESS IN COMPRESSED AIR WORKERS

*Y. Mano, M. Shibayama, A. Ishiyama, S. Takahashi, and H. Maeda*

Hempleman (1) pointed out the importance of the nitrogen gas diffused in the human body. The bubbles in the tissues originate from the pre-existing nuclei, and the gas initially diffuses into the gas nuclei in the tissue and is then frothed out by decompression depending on the difference between the inside tension of the gas nuclei and the surrounding tension of the tissue.

Mammalian gelatin samples were first used according to this theory by LeMessurier in 1972 (2) as a model in the etiology of decompression sickness; further research has been carried out mainly by Beckman and co-workers (3) and Yount et al. (4). Recently, Mano et al. have evaluated the relative effectiveness of different kinds of decompression tables in reducing bubble formation using agarose gels under rigorously controlled conditions. Preliminary reports on this work have already been presented (5 – 7).

We extracted data from our many treatment histories of both Type 1 (over 300) and Type 2 (119) bends cases for which there were exact records of the exposure depth, time lapse, decompression procedure, and bends onset. We simulated those conditions of each case in a laboratory chamber and analyzed the relation between the number of bubbles and bends onset (7).

Based on these basic studies of agarose gel bubbles, we applied these bubble-counting techniques in an experiment in a caisson field from March to September, 1982.

## MATERIALS AND METHODS

We manufactured disposable counting cells of 0.3 mL agarose gel. Each group of compressed air workers carried six counting cells with them and

counted the mean number plus or minus the standard deviation of gel bubbles during and after decompression (Fig. 1).

The gel cells were made as follows. A stock solution containing 1.0 mM of Tris buffer [(hydroxymethyl) - aminomethane, $NH_2C(CH_2OH)_3$] was prepared first. The pH was adjusted to 7.4 in each experiment by adding a small quantity of HCl. The buffered solution was heated to 85±0.3°C, and thereafter a highly purified agarose powder (Bio-Rad, High Gel Temperature, Lot. No. 21674 [moisture content = 7.75%; sulfur content <0.3%]) was added to the solution (0.7% W/W) and agitated carefully for 20 min. After the temperature decreased to 80°C within 5 min, the agarose solution was poured into the plastic disposable counting cells previously made acid-free, of which each volume of the gel amounted to 0.31 g (0.309 mL). Three cells were put together for future use, saturated with water vapor, sealed tightly by press-through packaging (PTP), and stocked at room temperature (Fig. 1). The PTP gells are acceptable for use within 6 months unless the seal is broken.

Compressed air workers perforate the PTP by two small pinholes and bring it with them into the compressed air work field; in this way these gel cells are physically exposed to the same pressure exposure and decompression

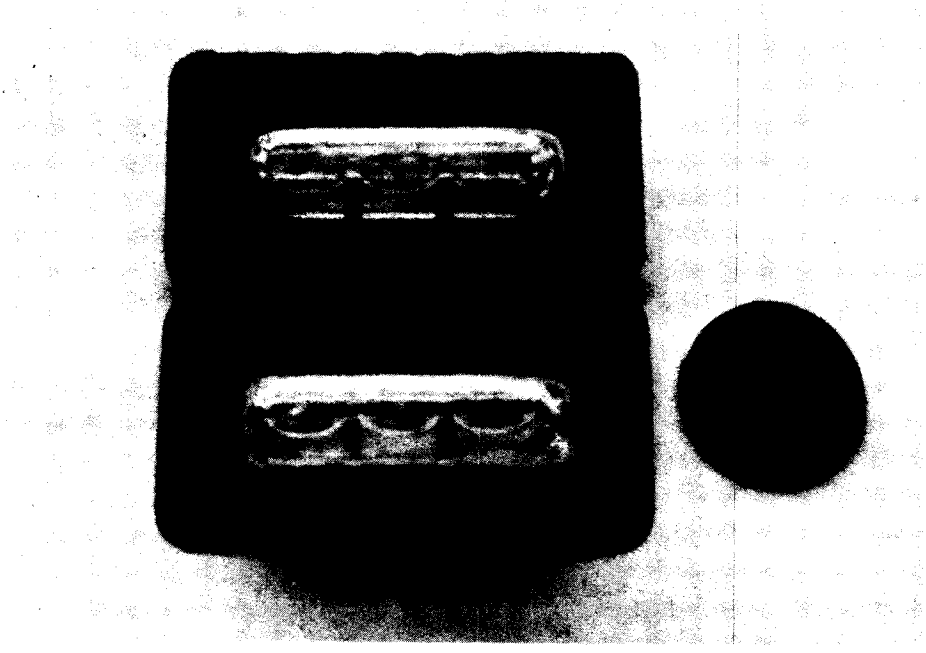


Fig. 1. Agarose bubble counter (lockmate).

procedure of the workers. The workers can count the number of bubbles in each cell with their naked eye during and after decompression. Using this method, the workers themselves can evaluate through the bubble-counting technique whether their decompression procedure may physically be suitable for their compressed working pressure and time.

Each decompression profile was exactly recorded in a self-registering pressure gauge.

## RESULTS

The caisson field was $33 \times 16$ m square and the maximum depth from the surface was 39.5 m. This caisson was immersed in the ocean and the mud volume excavated was 32 000 $m^3$. The working pressure in the caisson was between 2.2 and 4.2 ATA. The daily bottom time was within 6 h and the Japanese standard decompression table was used for the decompression, but Blackpool tables were also adopted at times (8).

The experiment using agarose gels was run from March 3 to September 13, 1982. The number of counting cells totalled 1722. There were 13 limbs bends and no Type 2 bends throughout this caisson work, but some workers forgot to take the cells with them; therefore we could not enter all exposure cases and bends cases into the population. Because of this problem, the number of subjects and bends cases was limited to only workers who had been clearly tested by an agarose gel model. That number of workers was 1757, and the number of bends was 5. Three of the bends cases were limb bends of knee joints, two were skin rashes, and there were no Type 2 bends. The incidence of decompression sickness was 0.29%, and in only 0.17% of the cases the recompression treatment was needed.

The relation between bends cases and the number of bubbles is described in Table I. Related to the number of bubbles: there were no bends if the mean number of bubbles was less than $10.0 \pm 2.5$ per sample; 1.16% of bends when the mean number of bubbles was $15.0 \pm 2.5$; 2.35% when the mean number of bubbles was $20 \pm 2.5$; and 3.33% when the mean number of bubbles was $25 \pm 2.5$. This regression line between bends incidence and the number of bubbles was obtained by the equation:

$$y = 0.2236 \times - 2.203, \; r = 0.9991$$
$$(10 \leqq \text{number of bubbles} \leqq 25).$$

The evaluation of decompression procedure that must be recorded by regulation was done in each bends case, and it was recognized that every procedure was deviated from the standard decompression table and shortened intentionally. It became clear that the number of bubbles would be lower than 10 if workers obeyed the regulation (Fig. 2). The number of bubbles for *Bends case #4* was $19.7 \pm 1.9$, but would have become $8.0 \pm 1.5$ if the worker had obeyed the regulation.

The daily variation of the number of bubbles looks like the teeth of a saw (Fig. 3), and bends onset appeared at the top at any cases (Fig. 4). Com-

TABLE I

Relation Between Bends Cases and the Number of Bubbles

| No. of Bends Cases | No. of Bubbles $\bar{x} \pm SD$ | Situation of Bends Onset | | | | |
|---|---|---|---|---|---|---|
| | | Onset Date (month/day) | Bottom Pressure (ATA) | Working Time (min) | Decompression Time (min) | Symptom |
| 1 | 19.8 ± 3.1 | 5/15 | 3.30 | 313 | 82 | Skin Rash (the breast) |
| 2 | 17.3 ± 4.0 | 6/13 | 3.80 | 317 | 109 | Skin Rash (the abdomen) |
| 3 | 20.8 ± 7.7 | 6/20 | 3.78 | 300 | doubtful (recorder trouble) | Left Knee Bends |
| 4 | 19.7 ± 1.9 | 6/24 | 3.70 | 279 | 62 | Right Knee Bends |
| 5 | 14.5 ± 2.5 | 6/25 | 3.65 | 145 | 66 | Right Knee Bends |

pressed air workers checked the number of bubbles by themselves during decompression and shortened the previously decided stopping time at 1.6 or 1.3 ATA if the number was counted at the lower level and the number rose, then they started their first stop at the deeper depth and gradually prolonged the decompression time on the next day.

Accidents occurred on May 23 and 24 (*see* Fig. 4) when the electric current was cut off and the compressed air work had to be broken off. The workers had to be decompressed immediately without obeying the regulation, so the number of bubbles was increased. The workers entered the recompression chamber in an effort to prevent bends. This bubble-counting technique provided a warning of bends onset to compressed air workers.

## DISCUSSION

The number of bubbles is one of the initial factors to bends onset but there are wide individual differences and other factors. We can state the relation between the number of bubbles and the physical pressure changes, and this technique can teach us whether the pressure exposure and the following decompression procedure must physically be profitable or not. The number of bubbles formed physically increases according to the bottom time and is in proportion to the saturated pressure (Fig. 5).

We can recognize the bubble formations within 1 min after decompression, count the number definitely 3 min after decompression, and decide upon

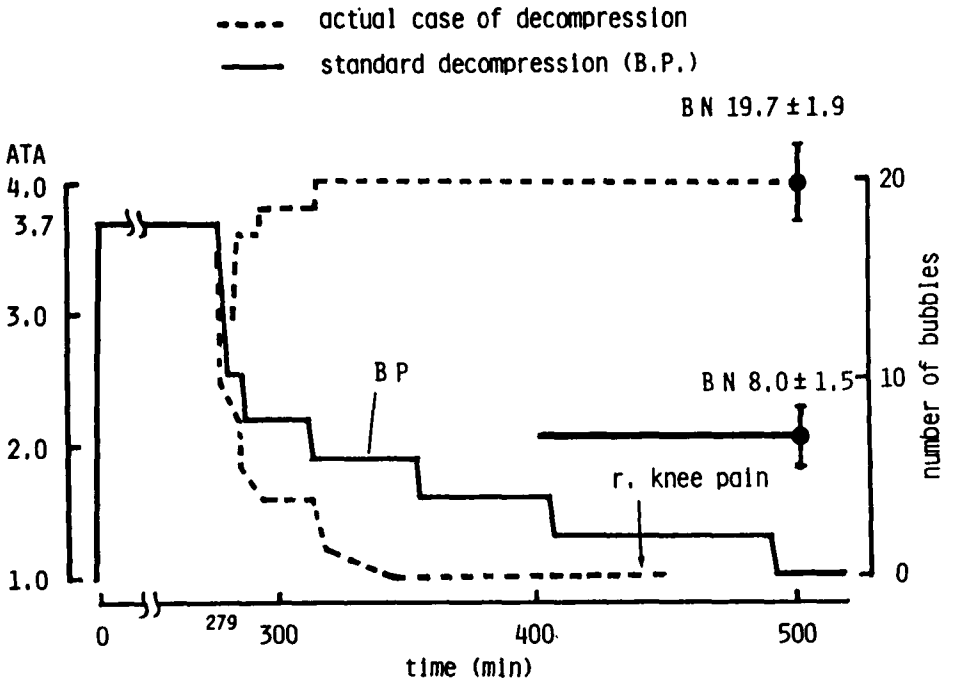



Fig. 2. Relation between decompression and number of bubbles (*Bends case #4*).

the size 10 min after decompression (Fig. 6). With this capability, we can predict the risk of decompression sickness before the onset. As an attempt to prevent bends, the workers entered the recompression chamber before onset on May 23 and 24 (*see* Fig. 4).

Many attempts to detect the "silent bubbles" have been made in the effort to predict the incidence of decompression sickness before it occurs. For example, Buckles (9,10) contrived a method using laser beams for detecting the bubbles in the blood; Smith (11) and Spencer (12) invented an apparatus harnessing the Doppler phenomenon of the ultrasonic waves, in which a receiving set transfers ultrasonic waves into sounds and the bubbles are identified by the apparatus.

Nevertheless, it is impossible to detect the number of bubbles directly either by laser beams or by ultrasonic waves. These techniques are suitable to determine the individual differences in each exposure, but workers find it difficult to use the apparatus and to become skilled to identify the difference in the sounds immediately under various conditions.

The method of this experiment was initially developed by LeMessurier et al. (2). LeMessurier also presented a report in 1972 entitled "Supersaturation

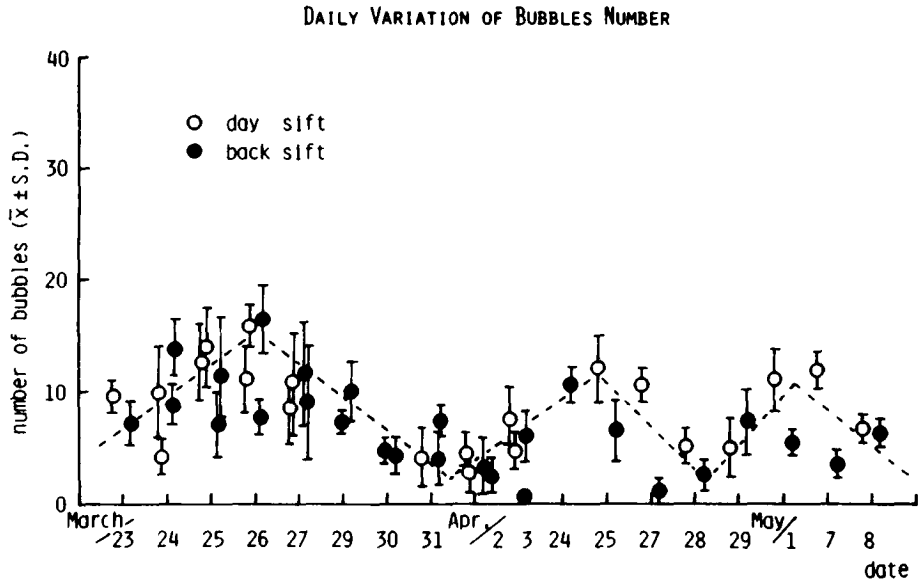



Fig. 3. Daily variation of bubble number.

and Preformed Nuclei in the Etiology of Decompression Sickness" (unpublished), emphasizing that gelatin was the most efficient for bubble formation in the study of the etiology of decompression sickness. Thereafter, Beckman, Yount, and co-workers (3,4) proved the efficiency of this method. D'Arrigo (13), one of their colleagues, reported on the improved method of bubble formation in the agarose gel. Unlike the gelatin composed of a complex mixture of charged peptide chain, agarose is an uncharged, relatively inert and homogeneous polysaccharide and is more suitable for the experiment on bubble formation in the gel (14). Mano et al. (7) suggested that the agarose gel method was useful for studying the etiology of decompression sickness. Although this agarose gel method is based merely on a physical phenomenon, it can be applied also to the clinical cases. The incidence of decompression sickness is not only influenced by the physical pressure changes in the surroundings but also by the peculiarities and the body condition of the individual workers. In other words, the risk of becoming sick is different according to the individual even when individuals are exposed to the same pressure condition. The number of bubbles was also different; nevertheless, the samples were all prepared with a homogeneous agarose solution pipetted into the counting cells with the same shape and pressurized under the same pressure condition (6,13,15). The number of bubbles was also different among the agarose gel samples of different lots, even if the lots were manufactured in the same factory by the same processes.

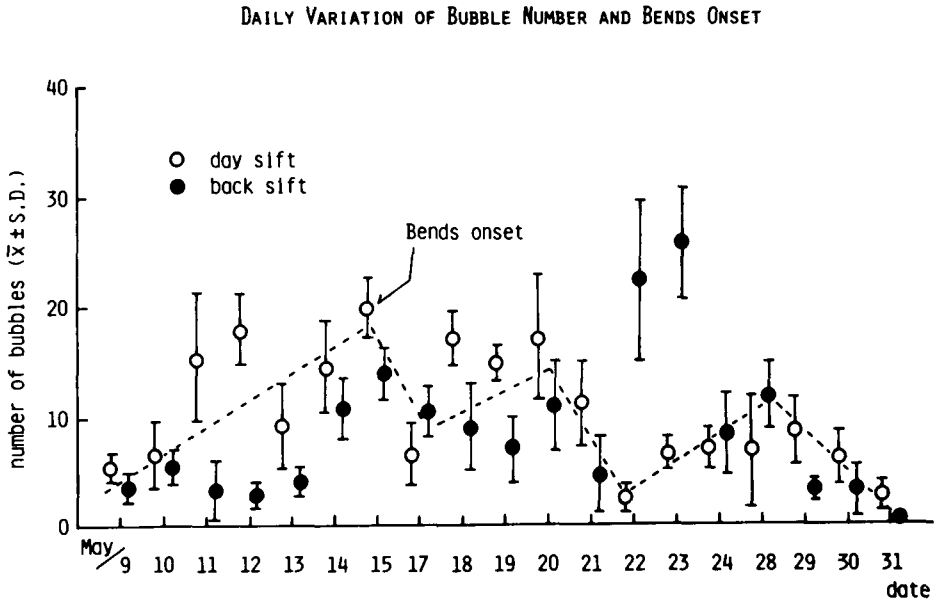



Fig. 4. Daily variation of bubble number and bends onset.

Although some skilled techniques are required for making agarose gel samples, the counting cells are small in size and portable; moreover, it is easy for the users to count the number of bubbles correctly (14). But, it is impossible to make the individual differences and daily changes of the body condition clear by this method, which covers the physical phenomenon only—the homeostasis of the organism is not taken into account.

The reliability of the Standard Decompression Schedule has been discussed only through the practical use of the table without an accurate and theoretical basis. The incidence rate of decompression sickness in the divers and compressed air workers in Japan has been reported to be 0.46% (16), 3 to 5% (17–19), and 4.5 to 28% (20,21). Such a difference in the incidence might have resulted from the misuse of the table, and it might be impossible to prevent the sickness completely even if divers consistently adhere to the table.

None of the indexes have been direct indicators of whether the decompression procedure is physically suitable to the pressure exposure; however, workers have initially used this bubble-counting technique by themselves and bends incidence decreased 0.29%. Compressed air workers were always very sensitive to the bubble formation in a model. They did not always obey the regulated decompression table but could reduce the incidence to a minimum.

Eventually, we can establish that this technique is very useful to prevent decompression sickness. The technique is not yet complete, but gives us a way

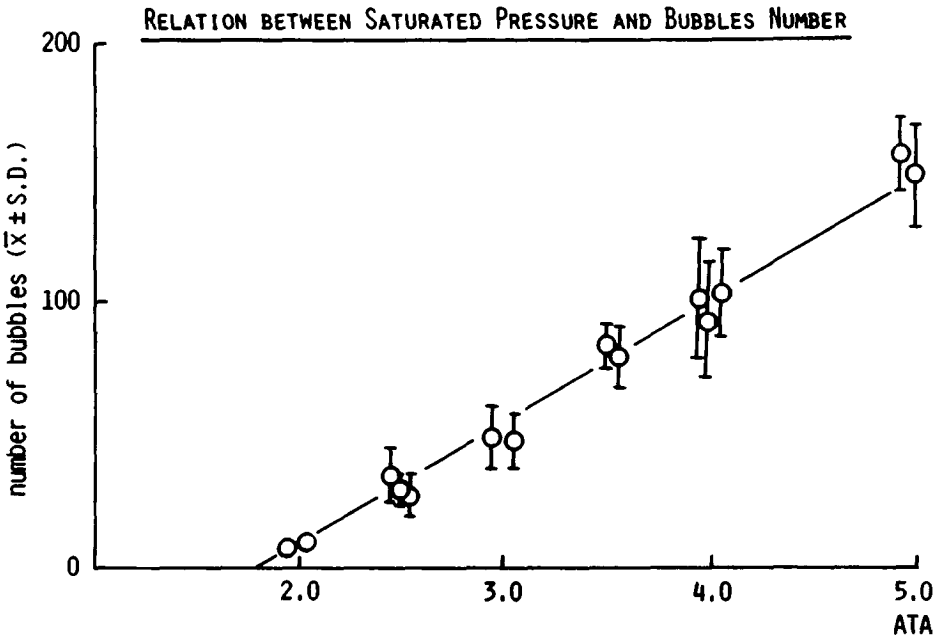



Fig. 5. Relation between saturated pressure and bubble number.

to predict from the number of bubbles whether the decompression procedure after hyperbaric exposure would physically be profitable or not. Its use could keep the bends incidence within 0.3% in actuality.

## References

1. Hempleman HV. Decompression theory: British practice. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving and compressed air work, 2nd ed. Baltimore: Williams & Wilkins, 1975:331–347.
2. LeMessurier DH, Smith TN, Wood WR. Diffusion and nucleation of gas in gel and some implications for the development of decompression. Undersea Biomed Res 1979;6:175–188.
3. Yount DE, Kunkle TD, D'Arrigo JS, Ingle FW, Yeung C, Beckman EL. Stabilization of gas cavitation nuclei by surface active compounds. Aviat Space Environ Med 1976;48:185–191.
4. Yount DE, Strauss RH. Bubble formation in gelatin: A model for decompression sickness. J Appl Phys 1976;47:5081–5089.
5. Mano Y, Shibayama M, Maeda H. Investigation on today's decompression table through formed bubbles (in Japanese). Jpn J Hyperbaric Med 1977;12:17–18.
6. D'Arrigo JS, Mano Y. Bubble production in agarose gels subjected to different decompression schedules. Undersea Biomed Res 1979;6:93–98.
7. Mano Y, Shibayama M, Ishiyama A, Maeda H. Relation between bends onset and agarose gel bubbles: Implications for the prevention of disease, In: Miller JM, ed. Proceedings of the sixth meeting of UJNR Panel on diving physiology and technology. Washington DC: U. S. Department of Commerce, 1982. (report no. 29–40)

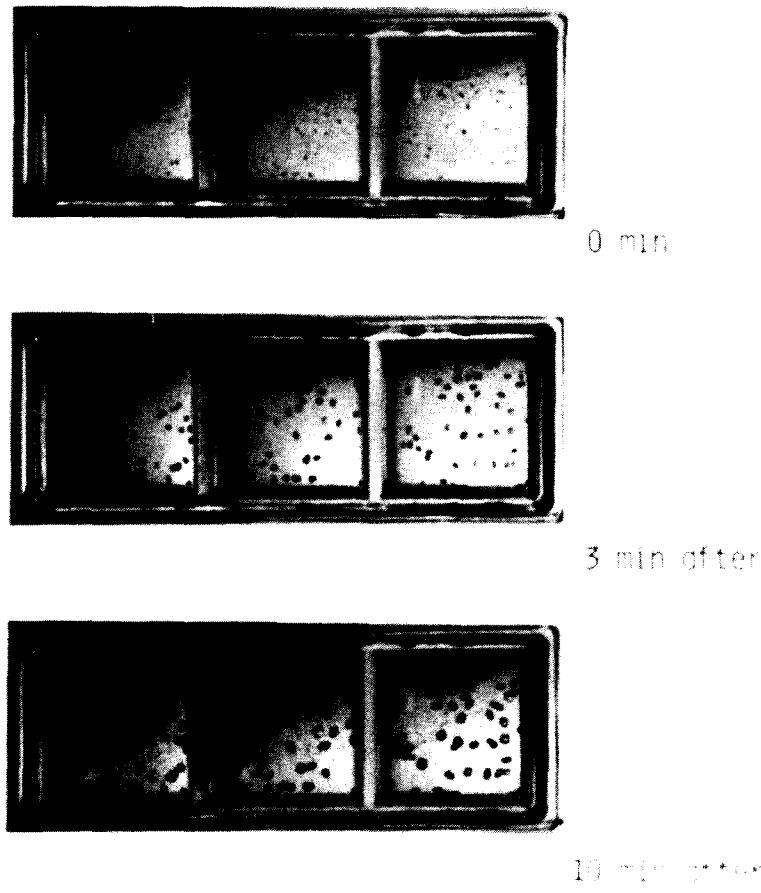



Fig. 6. Bubble formation after decompression from 3-ATA exposure for 3 h.

8. Department of Labor, Japan: Guidebook for the prevention of hyperbaric accidents during compressed air work (in Japanese). Tokyo: Kensetsugyo Saigai Boshi Kyokai (Construction Association to Prevent Labor Accidents), 1977.
9. Buckles RG. Characteristics of bubble formation in vivo. Bethesda: Naval Medical Research Institute, 1967:1–30. (NMRI report no. 1)
10. Buckles RG, Knox C. In vivo bubble detection by acoustic-optical imaging techniques. Nature 1969;222:771–772.
11. Smith KH, Johanson DS. Hyperbaric decompression by means of bubble detection. Seattle: Virginia Mason Research Center, 1969.
12. Spencer MP, Johanson DC, Clarke HF. Ultrasonic detection of intravascular gas emboli. In Reneman RR, ed. Cardiovascular applications of ultrasound. New York: American Elsevier Publishing Co., Inc., 1974:380–387.
13. D'Arrigo J.S. Improved method for studying the surface chemistry of bubble formation. Aviat Space Environ Med 1978;49:358–361.
14. Veis A. The macromolecular chemistry of gelatin. New York: Academic Press, 1960.

15. Mano Y, D'Arrigo JS. Relationship between $CO_2$ levels and decompression sickness: Implication for disease prevention. Aviat Space Environ Med 1978;49:349–355.
16. Nashimoto I, Mano Y. The effect of the Standard Decompression Schedule concerned with the prevention of bends (in Japanese). Jpn J Hyperbaric Med 1973;8:108.
17. Mano Y, et al. The actual study of divers (in Japanese). Jpn J Hyperbar Med 1972;7:B–7.
18. Mano Y, et al. The incidence of decompression sickness in caisson works (in Japanese). Jpn J Hyperbar Med 1973;8:VI–4.
19. Mano Y, Nashimoto I. Incidence of decompression sickness. In Proceedings of US-JAPAN Panel, Seattle, 1973:29–41.
20. Nashimoto I, et al. Outbreak of decompression sickness in compressed air work (in Japanese). Jpn J Hyperbar Med 1975;8:39–41.
21. Morita A, et al. Outbreak of decompression sickness in compressed air work, # 2 (in Japanese). Jpn J Hyperbar Med 1976;11:29–30.

# DECOMPRESSION SICKNESS IN SHEEP: FATAL CHOKES AFTER 24-HOUR DIVES WITH ALTITUDE PROVOCATION

*C. E. Lehner, J. A. Will, E. N. Lightfoot, and E. H. Lanphier*

*The chokes* was among the consequences of decompression described in some of the earliest reports (1), but it has always been limited to a small fraction of cases of decompression sickness (DCS) even at altitude (2). The reported incidence in divers and compressed air workers is in the neighborhood of 2% of all cases of DCS, or perhaps 11% of serious (Type II) cases (3). As described by Behnke (4) and others, symptoms include substernal pain, dyspnea, and cough aggravated by deep inspiration or cigarette smoke. Although chokes can resolve rapidly and spontaneously, the possibility of rapid progression to circulatory failure and death makes it one of the most ominous forms of DCS. Although a complex sequence of events may develop in chokes (5), the initiating factor is almost certainly embolization by venous bubbles of a critical fraction of the pulmonary vasculature.

This paper describes the unexpected production of a very high incidence of refractory chokes in sheep. The circumstances were little different from those of many uneventful exposures. Consideration of the differences in the light of earlier experiments may point to potentially important factors in chokes. It also suggests further studies and calls for greater caution in human exposure to altitude after diving.

## METHODS

Our customary procedure in investigating decompression phenomena in sheep and pygmy goats includes a) exposure of the animals to air at increased pressure in a hyperbaric chamber for a specified time, b) direct decompression

to "surface" for 20 min of observation, c) taking asymptomatic animals to 8000 ft of simulated altitude (570 mmHg) for 15 min of observation, and d) direct return to surface (Fig. 1).

The experiments that produced lethal chokes differed from the usual pattern only in keeping the animals at altitude for more than 15 min. They involved a total of 14 healthy sheep of various ages and weights exposed to 19 psig (43 fsw, 2.29 ATA) for 24 h.

A continuing study involving six sheep to date has a very similar protocol except for a longer interval at surface and the fact that one animal of each pair has implanted catheters in the pulmonary artery, left ventricle, aorta, and jugular vein. Both animals are monitored by precordial Doppler bubble detection.

## RESULTS

Findings in the first three experiments of the series are summarized in Table I. None of the animals showed any sign of DCS during the 20-min period at surface, and only 3 showed evidence of limb bends during the first 15 min at altitude.

In experiment C-158, 4 of 6 animals collapsed, and one of these died, with a 43-min exposure to altitude. Another had a recurrence of chokes and died during the decompression phase of seemingly successful treatment on U.S. Navy Table 1A.

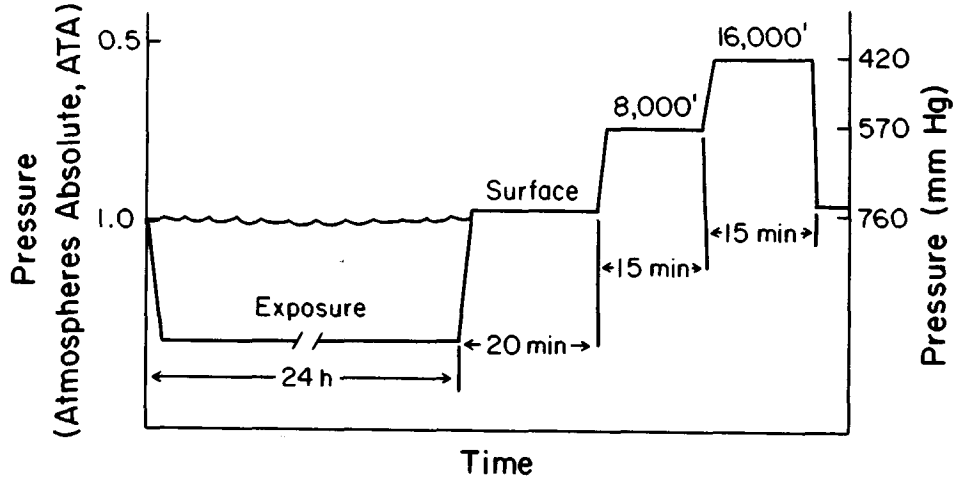



Fig. 1. Original profile of exposure for altitude provocation. (The excursion to 16 000 ft was subsequently eliminated.)

TABLE I

Response of Sheep to 24-h Dive and Reduced Pressure (570 mmHg)

| Experiment No. | Subject | Events (min from reaching altitude) | | | Outcome |
|---|---|---|---|---|---|
| | No., Sex, Age (yr), Wt (kg) | Limbs Bends | Binary Breathing | Other | |
| C–158 | 1 F 3+ 99 | 23 | late | Rx 1A 46 | Normal |
| | 2 F 3+ 94 | — | ? | Rx 1A 46 | Normal |
| | 3 F 3+ 112 | — | ? | Died 43 | Died at altitude |
| | 4 F 3+ 87 | — | 43 | Cough 43, Rx 1A 46 | Normal |
| | 5 F 2+ 85 | — | 18 | Rx 1A 46 | Died in Rx |
| | 6 M 2+ 101 | — | 23 | Rx 1A 46 | Normal |
| C–159 | 1 M 3+ 62 | 10 | 18 | Rx 2A 30 | Normal |
| | 2 N*2+ 114 | 1 | 10 | Rx 2A 30 | Died in Rx |
| | 3 F 3+ 45 | — | — | Rx 2A 30 | Normal |
| | 4 N 2+ 126 | 5 | 10 | Foam 28, Rx 2A 30 | Normal |
| C–160 | 1 F 1− 24 | 68 | — | Panting 16–90, to surface 139 | Normal |
| | 2 F 1− 42 | 25 | 29 | To surface 70 | Residual bend |
| | 3 F 1− 56 | — | 8 | To surface 12 | Normal |
| | 4 F 1− 45 | 26 | 6 | To surface 34, foam 132,d 139 | Died at surface |

*N = neutered male.

All but 1 of 4 sheep in experiment C-159 developed a characteristic breathing pattern in which 2 or 3 breaths taken in rapid succession were followed by a distinct pause. All of these animals were treated on a modified U.S. Navy Table 2A with initial success, but one suffered a recurrence of chokes with paroxysmal coughing and died abruptly during the slow return to surface.

The animals in C-160 were younger, generally smaller and leaner; but all showed unusual panting or *binary breathing*. They were taken from altitude to surface individually when they were judged to be close to the point of requiring treatment. All improved at surface, but one later developed tachypnea and died before treatment could be instituted.

Heavier animals showed a tendency toward earlier and more severe manifestations of chokes, but younger sheep showed a trend toward earlier onset of binary breathing or panting (Table I).

Overall, signs of chokes developed within 45 min at altitude in 10 of the 14 animals. Two died untreated, and two died late in treatment. Necropsy was performed in two animals, both of which showed pulmonary edema and white foam in all airways. *Animal C-158 #3* had massive edema with grossly frothy blood in the vena cava, right heart, and pulmonary arteries. *Animal C-160 #4*

had less marked edema and showed obvious vascular bubbles only in some peripheral pulmonary vessels.

Signs indicative of chokes had been observed in previous experiments; but none of the affected animals required treatment beyond a return to surface from altitude, and none of the animals died. In a series of 142 24-h dives with a 2-level altitude phase lasting up to 30 min (Fig. 1), signs of chokes were noted in 4.8% of DCS cases. Among 308 4-h dives with either a stop at surface or direct ascent to 8000 ft (Fig. 2), 3.8% of the animals that developed DCS showed signs of chokes. Modification of the 4-h profile (Fig. 2) to include 60 min at altitude for Doppler measurement produced 3 episodes of exceptional panting and one limb bend among 10 sheep in 20 dives to 20 psig (45 fsw, 2.36 ATA).

Results to date in the study in progress are summarized in Table II. (Vascular pressures, hematological changes, and other measures will be reported elsewhere when the series is complete.) In general, the instrumented animals (implanted catheters for pressure measurement and blood sampling) were more severely affected, and one of these died at altitude. Animals that appeared moribund were euthanized, and necropsy was performed. No animals in this series were treated, so the death rate of 50% is probably close to the natural course of events. With few exceptions, the more severely affected animals had higher Doppler bubble scores than the others, and signals of Grade 4 or 5 (6) were not uncommon.

## DISCUSSION

The forms of *altitude provocation* that we have used (Figs. 1 and 2) appeared to provide benign ways of multiplying the amount of information

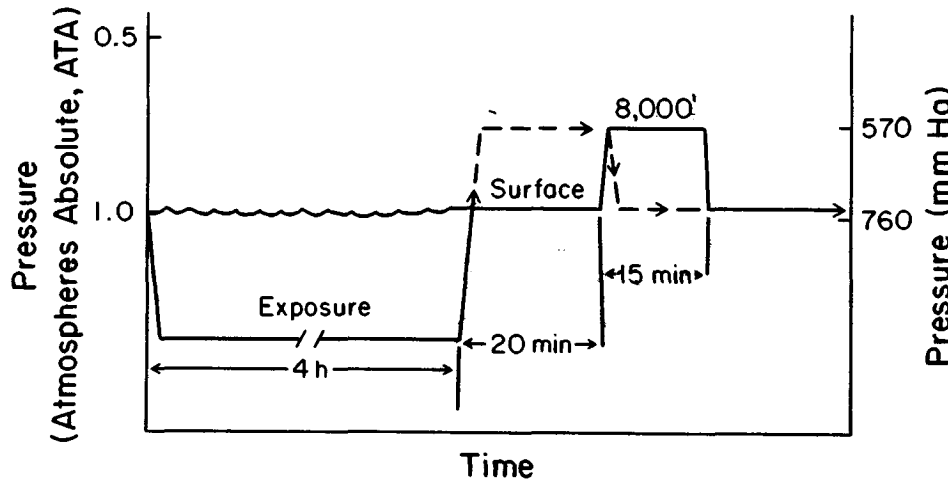



Fig. 2. Profile for 4-h exposures to depth followed by either observation at surface or direct ascent to 8000 ft. (The period at 8000 ft was later extended to 60 min for Doppler bubble detection studies.)

TABLE II

Response of Sheep to 22-h Dive and Altitude After More Than 30 min at Surface (from a study in progress)

| Experiment No. | Subject No., Sex, Age (yr), Wt (kg) | Instrumented (Vascular catheters) | Observations (Onset, min at altitude) |
|---|---|---|---|
| C–161 | 1 F 2+ 84 | Yes | Foam 19, died 36 |
| | 2 F 3+ 63 | No | Few abnormal signs |
| C–163 | 1 N* 2+ 55 | Yes | Panting 21, limb bends 89 |
| | 2 N 2+ 128 | No | Panting 17, moribund 51 |
| C–167 | 1 F 2 56 | Yes | Panting at surface, moribund 91 |
| | 2 F 2 55 | No | Few abnormal signs |

*N = neutered male.

obtained from each experiment. Signs of DCS at altitude indicated, for example, that we were approaching the critical level of exposure needed to produce signs at surface. Decompression sickness that developed at altitude almost invariably responded promptly and completely to a return to surface. Consequently, much time spent in recompression was avoided. Our experience with altitude was so encouraging, in fact, that we had planned to use 8000 ft (570 mmHg) as "home base" for 24 h following 24-h exposures to increased pressure in a study related to bone necrosis. We reasoned that if any animal developed unacceptable evidence of DCS at altitude, it would probably be treated adequately by returning it to surface.

Producing severe chokes in most of the animals early in the first run of this type was surprising not only in view of our favorable experience with altitude but because the decompression involved was not grossly inadequate by usual criteria: no DCS at surface; no central nervous system (CNS) events; limb bends at altitude in only half of the animals. Understandably, we cast about for evidence of complicating factors.

An unrelated observation had suggested the possibility that chamber ventilation might have been less abundant than usual during the altitude exposure. This in turn raised the possibility that an unanticipated degree of hypoxia had been a factor (discussed later). Experiment C-159 was conducted with superabundant chamber ventilation, but the outcome was essentially as before.

Many of the sheep in C-158 and C-159 had grown considerably larger and more obese since our earlier studies. We used younger, smaller animals in C-160 to help rule this change in or out as a significant factor. Again, signs of chokes appeared, and one animal died.

Being unable to invoke exceptional decompression stress, unexpected hypoxia, or extraordinary body mass as factors, we turned our attention to the protocol itself. The only basic difference from earlier procedures was in the amount of time spent at altitude. The procedure that involved two stages of altitude exposure and a maximum of 30 min at altitude (Fig. 1) was not strictly comparable because, in effect, only animals with the least critical degrees of gas loading were kept at altitude more than 15 min. The study in which animals had been kept at 8000 ft for 60 min differed in that the exposure to increased pressure had been only 4 h in duration. Unless proved otherwise, long exposure at depth should be considered an important factor in the production of lethal chokes.

A characteristic delay in the appearance of chokes at altitude would explain failure to observe this phenomenon in short exposures. Such a delay appears to occur, and it can be compared to the lag of events reported by Stock et al. (7) in the instrumented sheep fetus. It might be explained at least in part by time required for bubbles to form and enter the circulation or by time required for a critical volume of embolic gas to accumulate in target organs.

## Detection and Treatment

Early detection of chokes and prompt and appropriate treatment are clearly desirable. Binary breathing did not invariably occur, but it appeared to be the most clearly pathognomonic early sign. Either this or exceptional panting characteristically appeared before more ominous developments in the first three experiments, where the animals were undisturbed. In the continuing series, where the animals were being manipulated frequently for Doppler determinations or other measurements, respiratory signs were clearly less notable.

Our experience in treating chokes by recompression is limited, but a few lessons can be drawn. We were impressed by the refractory nature of the condition. Animals that were in critical condition when treatment was begun improved very slowly as pressure was increased. This is not surprising since bubble-embolization is unlikely to be the only significant problem at this stage (5), and changes like pulmonary edema would not be rapidly resolved. This also suggests that earlier treatment should be more rapidly effective.

Our situation does not permit use of oxygen in treatment, but the shorter air tables usually suffice for DCS in sheep. Although U.S. Navy Table 1A produced an adequate initial response and was sufficient in most of the animals, the late recurrence and death in *C-158 #5* indicated that it was not sufficient. The same conclusion must be reached about the longer, deeper schedule of U.S. Navy Table 2A even with some lengthened stops. At this time, we would surely use oxygen in treatment if we were able to do so. However, Pearson (8) reasons that pressure is likely to be the most important component of treatment in view of the apparent pathophysiology of chokes.

He thus recommends use of deep air tables. U.S. Navy Table 6A, with its excursion to 6 ATA, might provide a logical compromise.

## The Role of Altitude

From most standpoints, altitude provocation at 8000 ft appears to do little more than add a decompression stress equivalent to about 16 ft (4.9 m) of additional depth of exposure (9). In a well-ventilated chamber, 8000 ft (570 mmHg) would not seem to present a consequential hypoxic stress. It is, for example, an accepted cabin altitude in commercial aviation. On the other hand, Grover et al. (10) point out that ascent to 9000 ft can precipitate high altitude pulmonary edema (HAPE) in some susceptible humans. The co-existence of incipient chokes might make a significant difference, much as a limited respiratory response to hypoxia can accentuate the consequences of altitude exposure (10).

Our observations speak strongly for the importance of altitude in the etiology of lethal chokes. We have seen only one serious episode of chokes at surface, and this was in an exceptionally large, obese wether decompressed following an unusual exposure: air at 30 psig (3.04 ATA) for 24 h. In most individuals, the occurrence of limb bends or CNS signs, or both, at lower pressures would rule out exposures sufficient to produce chokes at surface. Some factor at altitude appears to favor the development of chokes without increasing the incidence of more common signs to the same extent. Many animals in related studies have presumably had degrees of supersaturation at surface at least as great as those associated with chokes at altitude. There is little reason to doubt that hypoxic pulmonary vasoconstriction, with or without frank development of HAPE, could augment effects associated with microembolization of pulmonary vessels (11).

## The Source of Gas in Chokes

Few of our animals had limb bends, and none had neurological signs during the customary periods of observation (Tables I and II). At the same time, a large volume of dissolved gas must have been liberated in the form of bubbles to overwhelm the lung's remarkable ability to dissipate bubbles. There is reason to suspect that the source of gas responsible for chokes is functionally separate from the "relevant tissues" (12) responsible for more common forms of DCS.

The apparent importance of long exposures at depth, the probable association with weight, and the evident quantity of gas speak for adipose tissue as the primary source. Yellow bone marrow, although sometimes accused and possibly involved, is unlikely to have the capacity to be responsible alone. Whatever their source, bubbles in the venous return are very unlikely to be responsible for limb bends. Whether they can be involved in spinal cord DCS in the absence of chokes is an interesting question. If it does nothing more, chokes provides a definite clinical role for Doppler-detected bubbles.

The origin of gas in chokes may be relevant to such observations as the earlier onset of binary breathing and panting in younger animals. This is reminiscent of the susceptibility of adolescents to HAPE (10). Other things being equal, younger animals may be more susceptible to chokes than older ones; but larger and more obese animals appear in general to be more at risk than leaner ones.

## Implications for Man

We could think of no situation in which human beings would be exposed to conditions closely similar to those associated with lethal chokes in our experience. At the same time, we knew that various combinations of high- and low-pressure exposures could occur, sometimes in unexpected situations. Most of all, we were impressed by the very small change in protocol that had made a benign procedure into a highly perilous one.

After many telephone calls, a letter to the editor of *Pressure* (13), and a renewed search of the literature, we could cite only one reported instance of chokes involving diving and altitude in man. This was in a case report by Balldin (14), who described chokes in an individual who had made a 100-min dive to 15 m (49.2 fsw, 22 psig, 2.5 ATA). After an interval of 24 h, he was exposed to a simulated altitude of 9000 m (29 520 ft). Vague symptoms developed after 90 min at altitude and became marked over the next 10 min. Treatment followed and was uneventful. There were no other symptoms of DCS, but a Grade 4 Doppler bubble signal had been recorded precordially for some time before chokes appeared. The individual was presumably breathing oxygen throughout the period at altitude.

Bassett (personal communication, 1982) described a study that included a 24-h exposure to air at 10.75 fsw (1.33 ATA) followed by direct ascent to 10 000 ft (3049 m, 523 mmHg) for 4 h. Precordial Doppler monitoring was conducted, and the altitude phase was aborted when bends occurred or Grade 3 signals were encountered at rest or Grade 4 signals appeared with limb movement. One of 20 "flights" was aborted because of bends; two because of Doppler signals. However, 16 of 20 subjects had no indication of bubbles or bends in the full exposure, and there were no signs of chokes. In our continuing series, which involves Doppler measurements, discontinuing altitude exposure with Grade 4 signals would probably have prevented the instances of chokes.

We remain unsure whether human subjects have ever been exposed to conditions closely comparable to those that produced chokes in our sheep. Situations that appear to involve risk include flying after diving, ascent from dives made at altitude, and decompression from caissons or tunnels at high elevations. Divers who obey current rules about flying after diving (15) seem unlikely to be at risk, but few would be aware of the possibility that lethal chokes might be the penalty for infractions.

### Research Implications

We have described a remarkably dependable method of producing consequential episodes of chokes in sheep. Having such a method should open the way toward better understanding and more confident avoidance of this rare but menacing phenomenon. Its implications may prove to be of great interest in an array of problems that includes not only DCS but also HAPE and a variety of embolic states. An approach that brings together existing knowledge of hypoxic effects (10) and microembolism (11) appears most promising.

## CONCLUSIONS

Potentially lethal episodes of chokes occur regularly in sheep under conditions that differ little from valuable protocols of altitude provocation. Crucial factors appear to include a long period under increased pressure followed by ascent to altitude and a stay of more than 30 min at reduced pressure. The relationship of this sequence to human activity is not yet clear, but considerable caution seems in order with exposure of divers and others to low pressure following significant periods under hyperbaric conditions. Ability to produce chokes in animals at will should lead to better understanding of this condition and related problems.

## Acknowledgment

This work was funded by the University of Wisconsin Sea Grant College Program under a grant from the Office of Sea Grant, National Oceanic and Atmospheric Administration, U.S. Department of Commerce and by the State of Wisconsin.

## References

1. Hill L. Caisson sickness and the physiology of work in compressed air. London: Edward Arnold, 1912.
2. Ferris EB Jr, Engel GL. The clinical nature of high altitude decompression sickness. In: Fulton JF, ed. Decompression sickness. Philadelphia: W.B. Saunders Company, 1951:4–52.
3. Elliott DH, Kindwall EP. Manifestations of the decompression disorders. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving, 3rd ed. London: Baillière Tindall; San Pedro: Best Publishing, 1982:461–472.
4. Behnke AR. Decompression sickness following exposure to high pressures. In: Fulton JF, ed. Decompression sickness. Philadelphia: W.B. Saunders Co., 1951:53–89.
5. Hallenbeck JM, Andersen JC. Pathogenesis of the decompression disorders. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving, 3rd ed. London: Baillière Tindall; San Pedro: Best Publishing, 1982:435–460.
6. Powell MR, Spencer MP, Von Ramm O. Ultrasonic surveillance of decompression. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving, 3rd ed. London: Baillière Tindall; San Pedro: Best Publishing, 1982:404–434.

7. Stock MK, Lanphier EH, Anderson DF, Anderson LC, Phernetton TM, Rankin JHG. Responses of fetal sheep to simulated no-decompression dives. J Appl Physiol: Respirat Environ Exercise Physiol 1980;48:776–780.
8. Pearson RR. A review of the therapy of decompression illnesses. In: Smith G, ed. Proceedings of the sixth international congress on hyperbaric medicine. Aberdeen: Aberdeen University Press, 1979:392–399.
9. Lehner CE, Lanphier EH, Alberti J, Lightfoot EN. Response to decompression in sheep and pygmy goats: The influence of exposure time, stops, and altitude. Undersea Biomed Res 1982; 9(1) (Suppl):26-27. (Abstr)
10. Grover RF, Hyers TM, McMurtry IF, Reeves JT. High-altitude pulmonary edema. In: Fishman AP, Renkin EM, eds. Pulmonary edema. Bethesda: American Physiological Society, 1979:229–240.
11. Staub NC. Pulmonary edema following microemboli. In: Sutton JR, Jones NL, Houston CS, eds. Hypoxia: Man at altitude. New York: Thieme-Stratton Inc., 1982:78–87.
12. Hills BA. Basic issues in prescribing preventive decompression. Undersea Biomed Res 1982;9:277–280.
13. Lanphier EH, Lehner CE. An unrecognized hazard of altitude? Pressure 1982;11(6):4 (Letter).
14. Balldin UI. Case report: Intracardial gas bubbles in relation to altitude decompression chokes. Aviat Space Environ Med 1978;49:1350–1351.
15. Preston F. Flying and diving: The dangers. Diver 1982;27:39–40.

# BUBBLE-INDUCED LOCAL HYDROSTATIC PRESSURE GRADIENTS AS A POSSIBLE CAUSE OF DYSBARIC OSTEONECROSIS

*W. D. Fraser, C. A. Ward, and W. R. Johnson*

The most commonly held cause of dysbaric osteonecrosis is the temporary disruption of the local blood supply to the bone tissue by gas emboli or gas-induced thrombi or both (1,2). Such transient disruption of the blood supply during, and following, decompression is thought to result in the death of the bone cells (osteoblasts) due to local tissue anoxia. However, no one theory appears to explain adequately the pathogenesis of aseptic bone necrosis that has been observed in human bone tissue (3). A number of arguments have been put forward suggesting that disruption of the blood supply may not be the primary cause of aseptic bone necrosis. For example, Hills (4) has pointed out that bone cells are capable of living up to 12 h without an oxygen supply. Therefore, any ischemic mechanism causing bone necrosis would have to be operative for extended periods of time.

Recent work on the histopathology of inner-ear decompression sickness (DCS) in monkeys has indicated that rapid decompression from deep heliox dives (274 metres of sea water [msw]) can result in the local traumatic fracture of the hard temporal bone that encases the semicircular canals (5,6). Histological examination of the temporal bones of monkeys that were sacrificed within 2 weeks after the dive revealed full thickness breaks across the hard canal wall, as well as damage to the softer petrous tissue (Fig. 1). The presence of necrotic bone chips in the endolymphatic space further supports the indications that the temporal bone has suffered an extensive microfracture. Interference with the blood supply to the semicircular canals is also evident since blood vessels in the bone have been severely disrupted. Examination of the semicircular canals of squirrel monkeys sacrificed several months subsequent to a DCS-provoking dive revealed the presence of necrotic bone, the

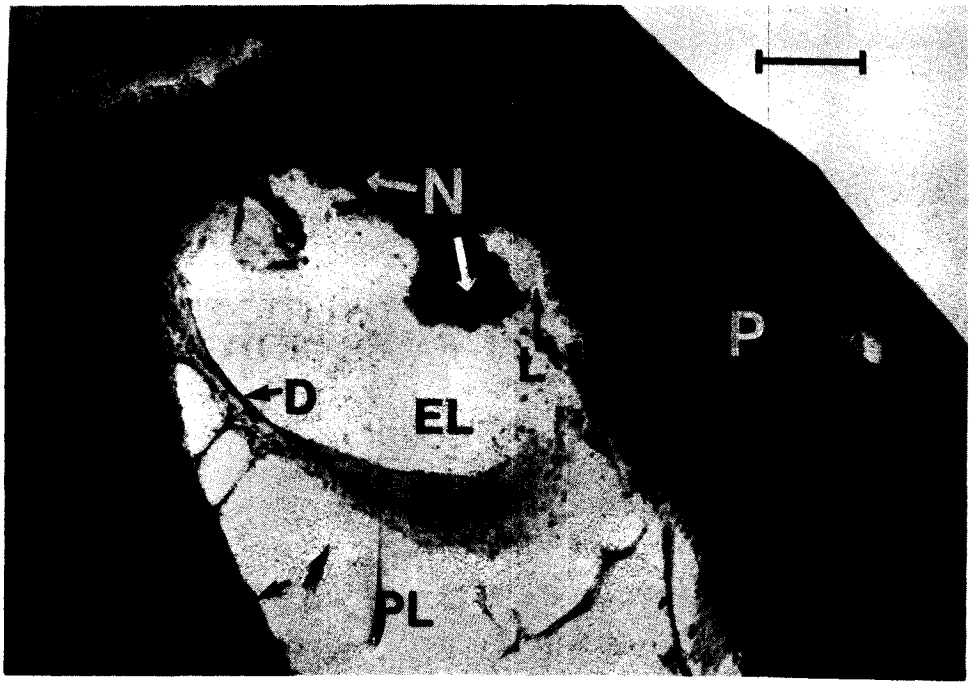



Fig. 1. Right lateral semicircular canal of *Monkey X* (8 days postdive survival) with extensive lesions of the canal wall (L) and the presence of necrotic bone (*N*) in the lumen of the canal. Abbreviations: *D* = lateral semicircular canal duct; *EL* = endolymphatic spaces; *PL* = perilymphatic space; *SC* = semicircular canal wall; *P* = petrous bone. Horizontal bar equals 0.1 mm.

growth of new bone and fibrous tissue, as well as extensive bone resorption. In many cases, new bone and tissue growth developed on the necrotic remains of the original bone (Fig. 2). The overall histological picture that is given by these specimens is almost identical to that which appears in accidental and experimentally induced temporal bone fractures (7,8).

Investigators have suggested that such localized trauma could explain the dysbaric osteonecrosis observed in human divers (9), because both gross fracture and local microfracture are known to lead to aseptic bone necrosis in nondiving populations (10). The hypothesis that a similar mechanism could be responsible for both syndromes is supported by the comparison of the histological results that are obtained after vestibular DCS incidents are detected in monkeys and aseptic bone necrosis is detected in humans and experimental animals. The presence of necrotic bone, resorption of bone by osteoclasts, new bone formation, hemorrhage, and prominent fibrosis have been observed in experimentally induced aseptic bone necrosis (11–13).

Several different mechanisms have been proposed to account for the DCS-induced extensive trauma that is found in the vestibular apparatus in the

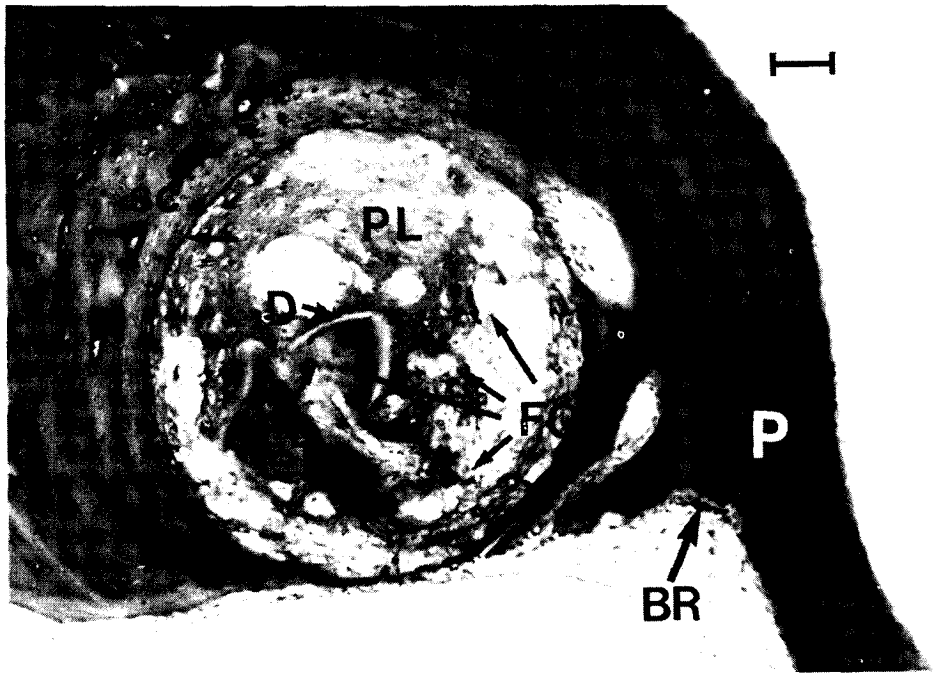



Fig. 2. Right posterior canal of *Monkey 117* (147 days postdive survival) with extensive destruction of the canal wall and surrounding petrous bone. There is resorption of petrous bone (*BR*) and the growth of fibrous and bony tissue in the endolymphatic and perilymphatic spaces (*FOL*). Abbreviations: *PL* = perilymphatic space; *EL* = endolymphatic spaces; *P* = petrous bone; *SC* = semicircular canal wall; *D* = semicircular canal duct. Horizontal bar equals 0.1 mm.

squirrel monkey (14). The most likely one appears to involve the growth of a bubble in a confined space where the boundaries surrounding the bubble would allow the development of a pressure gradient that is sufficient to produce temporal bone fracture. In this regard, it has been demonstrated that nucleation and bubble growth of hydrogen gas during the cooling of steel is capable of exerting pressures of 10 MPa or more, resulting in the formation of microcracks and the subsequent failure of the steel (14,15). The breakage of egg shells (16) and the rise in the hydrostatic pressure within the eyeball following bubble formation (17) both support the hypothesis that substantial trauma can occur this way in living tissue.

To determine if the local formation of a bubble in a rigid material, such as bone, could result in a pressure build-up sufficient to cause the fracture of bone, Ward et al. (18) developed a theoretical model of a bubble growing in a confined volume. To achieve this model, the investigators postulated that nucleation would occur in the reabsorption border of an osteoclast having a conical pit in its *brush-border* membrane in which nucleation and bubble

growth could occur. It was also assumed that the system was of constant mass (i.e., no gas diffusion in or out during bubble formation and growth) and constant volume (i.e., no flow of fluid or strain in the material to allow for relief of the pressure due to the bubble). The third assumption postulated that the hydrostatic pressure was applied to the system slowly, to allow the pressure to be transmitted to the enclosed volume as the material containing the volume relaxed under the stress.

The maximum pressure rise that could occur in the lacunae enclosing the bone cell was calculated using the conditions under which the squirrel monkeys were compressed and decompressed. The thermodynamic model was developed by determining the equilibrium values for the components of the system which maximized the Helmholtz function. The use of the Helmholtz function to describe the thermodynamic potential of the system is dictated by the constraints imposed on the model (i.e., constant mass, constant volume, and constant temperature). The model predicted that the maximum pressure rise in the lacunae of the bone would be slightly less than the pressure to which the animal had been compressed. By subjecting semicircular canal specimens of the squirrel monkeys to graded stress, investigators established that a pressure rise of this magnitude was more than sufficient to cause failure of the temporal bone (19).

It is to be emphasized that the pressure rise, as predicted by the model, would only occur if the fluid, or cell in which the bubble was growing, was contained in a fixed rigid volume (one in which the solution is unable to flow to release the stress generated by the bubble growing in the incompressible fluid). The stress developed by the growth of the bubble would also be dissipated by the elastic strain in the bone. However, traumatic failure of the bone will likely occur when the total strain is greater than 0.5% (20). Thus, unlike more elastic tissues in the body, bone is capable of only minor deformation before reaching a state of failure.

The basic premise of the model, that the bubble will generate a pressure rise, depends on the ability of the bone to contain the pressure as it develops. Bone cells are embedded in a calcium matrix that is believed capable of preventing both diffusional and volume flow transfer of water and metabolites (21). The only communication that the cells have with the blood supply is by way of bone canaliculi (less than 0.1 $\mu$m diameter) possessing narrow cellular membrane processes. Those cells farthest from the capillaries are supplied with metabolites by canaliculi that extend to cells lying closer to the blood. For rapid bubble growth, it is difficult to see how such processes could handle volume flow since the canaliculi are filled with membranous material. In other words, the bone is capable of functioning like a semipermeable membrane: it allows solute and water diffusion, but limits bulk volume flow.

A preliminary study has been performed to test the basic tenets of the model (i.e., that bubble formation will cause a local hydrostatic pressure rise). The results of a simple experiment are presented in Fig. 3. In this experiment, water was saturated with nitrogen at a pressure of 0.86 MPa in a thick-walled aluminum vessel and then decompressed to a pressure of 0.16 MPa. Following

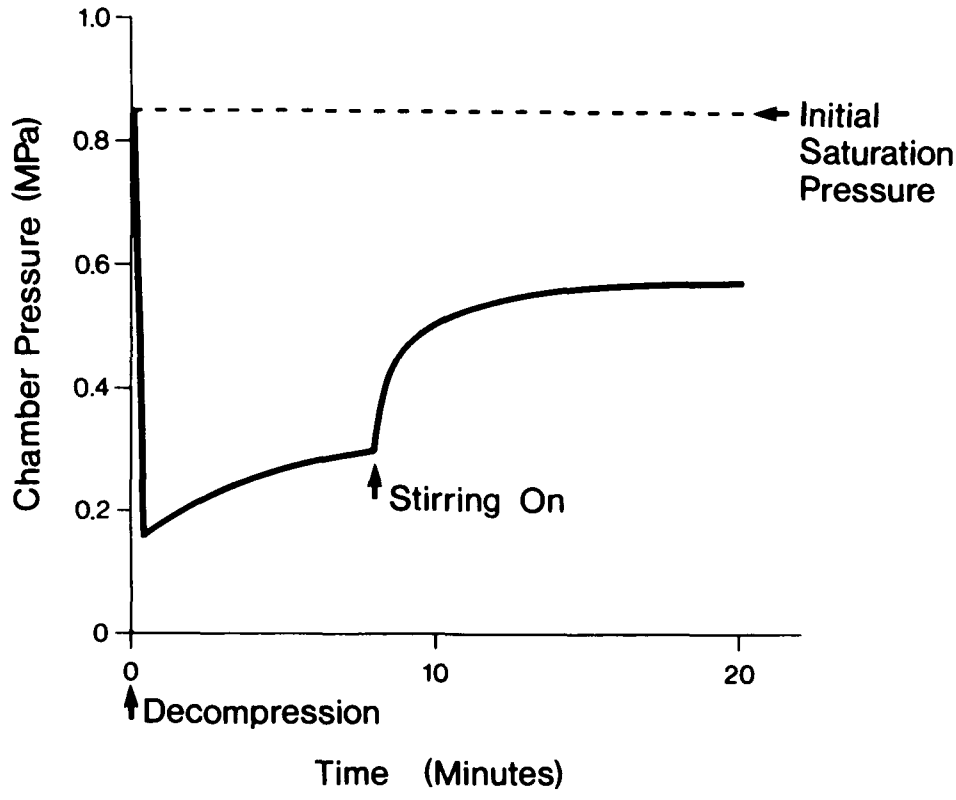



Fig. 3. The pressure rise in a thick-walled aluminum vessel filled with water that was rapidly decompressed to a pressure of 0.16 MPa after an initial saturation with nitrogen at 0.86 MPa. The increase in pressure corresponds to the growth of four small bubbles that were present in the vessel following decompression. The increase in pressure observed at 8 minutes is attributed to the renewed growth of the bubbles caused by an increased gas concentration near the bubbles that had been depleted by diffusion into the bubbles.

decompression, all valves to the system were closed and the pressure was monitored. Four small bubbles became visible and the pressure started to rise in the system along with their growth; stabilization occurred at a pressure of 0.57 MPa after about 20 min. The increase in pressure that was observed 8 min after the experiment started was attributed to the renewed growth of the bubbles caused by an increased gas concentration near the bubbles that had been depleted by diffusion into the bubbles. The rise in pressure to a value of 66% of the original saturation pressure strongly supports the validity of the theory. The pressure rise, measured experimentally, would not be expected to reach the predicted 90% of saturation pressure, because the container, the valve seals, and the plexiglass viewport will compress in response to the stress. Further experiments with a single bubble are underway to rigorously test the quantitative predictions of the theory.

In a similar experiment Kalser et al. (22) inserted a brass cannula that was filled with saline into the cortex of a freshly dissected dog femur. When the bone was decompressed in a hypobaric chamber, the pressure within the cannula rose to 8.0 kPa with respect to the chamber pressure. At the final altitude of 12 000 m the pressure in the bone fell as the interior of the bone approached equilibrium with the chamber pressure. After 15 min, the pressure within the bone increased again to reach a value of 16 kPa with respect to the chamber pressure; at that point, the experiment was terminated. Kalser et al. (22) concluded that the pressure rise was due to bubble formation within the interior of the femur. In this case, the pressure inside the bone reached 37% of the value at which it was originally saturated. This experiment indicates that the bone is capable of containing an elevated ambient pressure. The proposed theory accounts for the observed pressure increase.

In addition to the similarity in the histopathology of aseptic bone necrosis and vestibular DCS, there are other indicators which support the hypothesis that traumatic damage is occurring in the bone. Cockett et al. (23) and Mitano and Hayashi (24) have observed the presence of marrow-derived megakaryocytes in the lung after fatal DCS. This type of fat embolism usually occurs after fractures of large bones (25). Brickley-Parsons and Bradley (26) have shown that repetitive necrosis-inducing dives in mice resulted in the synthesis of an embryonic, hyperhydroxylated collagen of a type similar to the collagen that is formed during the healing of bone fractures.

Mechanical trauma, as a cause of aseptic bone necrosis, is not a new concept. Both Frost (27) and Lagier (28) have proposed that localized microfracture is the initial triggering mechanism for aseptic bone necrosis. The growth of bubbles within the bone provides a mechanism for this microfracture during decompression.

The epidemiological patterns associated with aseptic bone necrosis also support the concept of traumatic failure due to bubble growth within a confined volume. Because the local hydrostatic gradient associated with the bubble growth is a function of the saturation depth, deep saturation diving would tend to favor the development of severe trauma in the bones. This is in accord with the dramatic increase in the incidence of bone necrosis in diving as depth increases beyond 100 msw (29). Shallow saturation dives (e.g., in caisson work) would also tend to favor bone necrosis, since saturation of the poorly perfused bone would result. Though the theoretical pressure rise due to bubble growth is limited, bubble formation could still occur within the bone cells in the lacunae. Even if sufficient pressure to break the bone did not develop, cell death could still occur. If a sufficient number of cells were destroyed, then bone necrosis could result without either the traumatic failure or the disruption of the blood supply. This aspect of the model and our overall hypothesis is supported by the studies of Smith et al. (12) who observed the compression of an osteoblast cell against the walls of the bone lacunae by a bubble. Such a mechanism would also explain the development of aseptic bone necrosis after a single hyperbaric exposure (30). In this case, a signifi-

cant portion of the bone cells in critical sites may be damaged by localized bubble growth.

The low incidence of aseptic bone necrosis in the aviator can also be explained from this theory. The maximum pressure rise that can be generated by the growth of a bubble after hypobaric exposures is less than the original saturation pressure. Therefore, the maximum pressure rise will be less than 100 kPa (1 atm). The rate of change of pressure during decompression is also limited, therefore less stress develops.

One problem that the above model does not address pertains to the observation that aseptic bone necrosis appears to be linked to the compression phase of the dive, as well as the decompression portion of the dive profile (26,31). However, the rate of compression will affect the hydrostatic balance between the lacunae of the bone and the ambient pressure. If the rate of compression is sufficiently fast, then there will be large stresses that are set up at the boundaries between the fluid-filled cavities of the bone and the portion containing the calcium matrix. This stress occurs because of the difference in the elastic properties of the two media. The stresses and the resultant damage may explain the link between compression and aseptic bone necrosis, as well as account for the increase in serum ferritin levels that are observed during the compression phase of a dive, since such levels are believed to indicate damaged bone marrow cells (32).

Physical damage through growth of bubbles may be expected to occur in the more elastic tissues. If the properties of these tissues are such that the stress-strain curve has a small slope, then large deformations will be possible without permanent damage occurring. However, in a relatively inelastic tissue, such as the blood vascular wall, substantial bubble growth within the structure may cause extensive trauma, similar to that observed in bone. In this regard, Smith et al. (12) have detected several instances in which the epithelial lining of the arterioles was ripped away from the basement membrane of the vessel as a result of decompression.

By comparing the histology of vestibular DCS with that observed in experimental DCS, it may be concluded that a similar physical traumatic experience provides a likely explanation for the initial triggering of aseptic bone necrosis. The growth of a bubble in the enclosed lacunae of the bone has been shown to provide a probable mechanism to cause such damage. Experiments using isolated perfused tissues could be performed to test the theory. As well, a rigorous thermodynamic and mechanical analysis of the growth of bubbles in softer tissue is required. Such an analysis would have to include the mechanical properties of the tissues. This type of approach may provide useful information on the degree of damage that could occur in elastic and viscoelastic tissues such as the spinal cord and tendon during hyperbaric exposures.

## Acknowledgments

Figures 1 and 2 have been provided by courtesy of Drs. J. P. Landolt and K. E. Money of DCIEM. The histological sections were prepared by Mr. J. Laufer of DCIEM. DCIEM Publication No. 83–P–24.

## References


1. Gersh, I. Gas bubbles in bone and associated structures of guinea pigs decompressed from high pressure atmospheres. J Cell Comp Physiol 1945;26:101–117.
2. Jones PJ, Sakovich L. Fat embolism of bone. J Bone Joint Surg 1966;48–A:149–164.
3. McCallum, RI, Harrison, JAB. Dysbaric osteonecrosis: Aseptic necrosis of bone. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving. San Pedro: Best, 1982:488–506.
4. Hills BA. Decompression sickness. The biophysical basis of prevention and treatment. Chichester: John Wiley, 1977.
5. Fraser WD, Landolt JP, Money KE. Semicircular canal fracture in squirrel monkeys resulting from rapid decompression. Acta Otolaryngol 1983;95:95–100.
6. Landolt JP, Money KE, Topliff EDL, Nicholas AD, Laufer J, Johnson WH. Pathophysiology of inner ear dysfunction in the squirrel monkey in rapid decompression. J Appl Physiol: Respirat Environ Exercise Physiol 1980;49:1070–1082.
7. Altmann F. Healing of fistulas of the human labyrinth. Histopathological studies. Arch Otolaryngol 1946;43:409–415.
8. Perlman HB. Process of healing in injuries to the capsule of the labyrinth. Arch Otolaryngol 1939;48:48–49.
9. Fraser WD, Landolt JP, Money KE, Laufer J. Decompression-induced semicircular canal fractures in squirrel monkeys. In: Proceedings 7th Annual congress and symposium, European Undersea Biomedical Society, Cambridge, U.K. (July 21–24, 1981).
10. Manhin, HJ, Brower, TD. Bilateral idiopathic aseptic necrosis of the femur in adults: *Chandler's disease*. Bull Hosp Joint Diseases 1962;23:42–57.
11. Smith, KH, Stegall, P. Experimentally induced osteonecrosis in miniature swine. In: Beckman EL, Elliott DH, eds. Dysbarium-related osteonecrosis. Washington DC: U.S. Dept. of Public Health, 1974:105–112.
12. Smith KH, Stegall PJ, Harker LA, Slichter SJ, Richmond VL, Hall MH, Huang TW. Investigation of hematologic and pathologic response to decompression. Seattle, Wash: Virginia Mason Research Center, 1973. (Office of Naval Research Contract N00014–71–C–0273)
13. Catto M. Pathology of aseptic bone necrosis. In: Davidson JK, ed. Amsterdam: Excerpta Medica 1976:3–88.
14. Choo WY, Lee LY. Hydrogen trapping phenomenon in carbon steel. J Mater Sci 1982;17:1930–1935.
15. Hasnain, S. When the bubble bursts. Sci Dimension 1981;13(4):2.
16. Paganelli CV, Strauss RH, Yount DE. Bubble formation within hen's eggs. Aviat Space Environ Med 1977;48:48–50.
17. Cooke JP. Effects of helium and nitrogen upon intraocular pressure during near vacuum exposure. J Appl Physiol 1971;30:318–324.
18. Ward CA, Johnson WR, Venter RD, Ho BN, Forest TW, Fraser WD. Heterogeneous bubble nucleation and conditions for growth in a liquid-gas system of constant mass and volume. J Appl Physics 1983;1833–1843.
19. Venter RD, Ward CA, Ho S. Fracture studies on a mammalian semicircular canal. Undersea Biomed Res 1983;10:225–240.
20. Fung YC. Biomechanics. New York: Springer-Verlag, 1981.
21. Ham AW, Cormach DH. Histology. Philadelphia: J.P. Lippincott, 1979.
22. Kalser MH, Ivy HK, Pevsner JP, Marbarger JP, Ivy AC. Changes in bone marrow pressure during exposure to simulated altitude. Aviat Med (August) 1951:286–293.
23. Cockett ATK, Kado RT, Nakamura RM. The pathophysiology of decompression sickness. Revue de Physiologie Subaquatique 1969;1:199–202.
24. Mitano M, Hayashi K. Acute decompression sickness: An autopsy case with wide spread fat embolism. Acta Pathol (Japan) 1981;31:269–276.
25. Mohr JP, Fisher CM, Adams RD. Cerebrovascular disease. In: Petersdorf RG, ed. Principles of internal medicine. New York: McGraw Hill, 1976:1832–1868.
26. Brickley-Parsons D, Bradley ME. Abnormal bone and cartilage collagen metabolism in experimentally induced dysbaric osteonecrosis. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII:

Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Biomedical Society, Inc., 1981:837–846.
27. Frost HM. The etiodynamics of aseptic necrosis of the femoral head. In: Proceedings conference of aseptic necrosis of the femoral head. St. Louis: National Institutes of Health, 1964:393–414.
28. Lagier R. Idiopathic aseptic necrosis of the femoral head. In: Zinn WM, ed. Idiopathic ischemic necrosis of the femoral head in adults. Stuttgart: Georg Thieme, 1963:49–65.
29. Underwater Engineering Group. Aseptic bone necrosis in commercial divers. UEG Technical Note 25, 1981.
30. James CCM. Late bone lesions in caisson disease. Lancet 1945;2:6–8.
31. Hills BA, Straley R. Aseptic osteonecrosis: A study of tibial blood flow under various environmental conditions. Aerosp Med 1972;43:724–728.
32. Gilman SC, Biersner RJ, Piantadosi C. Serum ferritin increases during deep saturation dives. Aviat Space Environ Med 1982;53:1014–1016.

# INNER EAR DECOMPRESSION SICKNESS IN THE SQUIRREL MONKEY: OBSERVATIONS, INTERPRETATIONS, AND MECHANISMS

*J. P. Landolt, K. E. Money, M. W. Radomski, R. G. Riusech, P. C. Odell, and W. J. Watson*

The insidious nature of inner ear decompression sickness (DCS) during deep simulated dives is now well documented in the squirrel monkey (*Saimiri sciureus*) (1–3). In provocative ascents from 274 metres sea water (msw) while breathing a heliox gas mixture, the signs of DCS appear suddenly, mainly in the form of a vigorous head nystagmus, which occurs between 62 msw and the surface. If these monkeys are sacrificed a few days after their dive, the inner ear spaces are usually congested with blood and blood proteins. In those monkeys sacrificed 20 or more days after sustaining an inner ear *hit*, the most common pattern is that of ectopic new bone growth or the presence of fibrous material or both in the fluid spaces of the vestibular apparatus, particularly in the semicircular canal spaces. Damage to the central nervous system (CNS) in the centers subserving vestibular and auditory functions was found only when there was brain damage elsewhere as a result of a severe, generalized DCS.

The purpose of the present report is to summarize the main findings of our research in the area of inner ear DCS in deep diving environments. From these observations, a plausible theory has been developed which explains many of the results that were obtained. Furthermore, interpretation of the results suggests what procedures constitute acceptable therapeutic treatment for the successful management of inner ear decompression sickness.

## MATERIALS AND METHODS

### Animal Model

Male squirrel monkeys, free from ear infections and other disorders, were used in the study. On the day before the dive, both ear drums were surgically

perforated under anesthesia so that the animal could equilibrate the middle ear with ambient pressure. Functional testing of the vestibular system was performed before and at selected intervals after the dives until the animal was sacrificed for histological study (*see* Ref. 3 for further details). We processed the temporal bones (horizontal sections, 20 μm thick) using the method of Igarashi (4); brain sections were similarly prepared (coronal plane, 20 μm thick).

### Decompression Procedures

All experiments were performed in a small animal chamber (Bethlehem Corp., 0.173 $m^3$ capacity). The dive commenced with air to 13.9 msw; then with helium at a rate of 32.6 msw/min to a depth of 274 msw. After 1 min of bottom time, decompression began at a rate of 18.3 msw/min to 61 msw; then in steps of 6 msw every 4 min to the surface. Throughout the dive, the oxygen partial pressure was maintained at 50.7 kPa (= 0.5 atm).

### Hearing Tests and Training

To assess for possible cochlear damage, we tested the hearing in a number of animals, both before and after the dive. The animals were trained by a shock-avoidance procedure to respond to tones. Predive hearing thresholds were determined for a range of frequencies in each animal. The presentation of tones was controlled and delivered by a computer-based system (PDP 11/04 computer; Digital Equipment Corp.). During training and testing, the animals were isolated in a sound-proof booth (Industrial Acoustics Corp.).

After the dive, when the animals had recovered sufficiently, we again tested their hearing to obtain postdive hearing thresholds. These tests were conducted on a regular basis until the animal's audiogram showed no further change, whereupon the animal was sacrificed for histology.

### Hyperbaric Oxygen Therapy

With some animals, a regimen of hyperbaric oxygen therapy was instituted immediately after the dive. These animals were treated for three successive days with the U.S. Air Force modification of the U.S. Navy Table 6 Treatment (5) for DCS. Initial trials with control animals had shown that the monkeys could withstand the Table 6 treatment without showing visible signs of oxygen toxicity.

## RESULTS AND DISCUSSION

At the time of this report, some 250 monkeys have received inner ear hits in experiments using this diving profile at the Defence and Civil Institute of Environmental Medicine (DCIEM). Table I lists the distribution of new bone

TABLE I

Distribution of New Bone Growth and Fibrous Material in Squirrel-Monkey Semicircular Canals*

| Monkey | Depth of Initial Hit on Ascent (msw) | Postdive Survival Time (days) | New Bone Growth and Fibrous Material | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Left Labyrinth | | | Right Labyrinth | | |
| *8* | *12.2* | *38* | | | | *ASC(p)* | | |
| 24 | 61.9 | 379 | | | | *ASC(p),* | *LSC(p),* | *PSC(p)* |
| 32 | 18.3 | 211 | | | | *ASC(p),* | *LSC(p),* | *PSC(p)* |
| 33 | 6.1 | 93 | | | | *ASC(p)* | | |
| 36 | 6.1 | 184 | *ASC(e,p)†,* | *LSC(p),* | *PSC(e,p)†* | | | |
| 51 | 12.2 | 56 | | *LSC(p),* | *PSC(p)* | *ASC(p),* | *LSC(p),* | *PSC(e,p)* |
| 52 | 36.6 | 126 | | *LSC(p),* | | | *LSC(p)* | |
| 62 | 24.4 | 383 | | | | | | *PSC(p)* |
| 68 | 30.5 | 290 | *ASC(p)†,* | *LSC(p),* | | | | |
| 103 | 6.1 | 637 | | *LSC(p),* | | | | |
| 117 | 18.3 | 147 | *ASC(e,p)†,* | *LSC(e,p)†,* | *PSC(e,p)†* | *ASC(e,p)†* | | *PSC(e,p)†* |
| 119 | 18.3 | 133 | *ASC(e,p),* | *LSC(p),* | *PSC(e,p)†* | | | |
| 135 | 18.3 | 388 | *ASC(p),* | *LSC(p),* | *PSC(p)* | *ASC(e,p),* | *LSC(e,p)†,* | *PSC(e,p)* |
| 136 | 30.5 | 213 | *ASC(p)* | | *PSC(p)* | *ASC(p),* | *LSC(p),* | *PSC(p)* |
| 145 | 7.6 | 161 | *ASC(e,p)†,* | *LSC(e,p)†,* | *PSC(p)* | | | |
| 147 | 30.5 | 188 | | | *PSC(p)* | | | |
| 160 | 30.5 | 65 | *ASC(p),* | *LSC(p),* | *PSC(p)* | | | |
| 166 | 18.3 | 70 | | | | *ASC(p)* | | |
| 169 | 12.2 | 20 | *ASC(p)†,* | *LSC(p)†,* | *PSC(p)* | | | |
| 183 | 11.3 | 490 | *ASC(p),* | *LSC(p),* | *PSC(p),* | *ASC(p)* | | |
| 185 | 6.1 | 353 | *ASC(e,p)†* | *LSC(p),* | *PSC(p)* | | | |
| 187 | 12.2 | 21 | | | | *ASC(p),* | *LSC(p),* | *PSC(p)* |
| 201 | 18.3 | 197 | *ASC(p),* | *LSC(p),* | *PSC(p)* | *ASC(p),* | *LSC(p)* | |
| 207 | 30.5 | 185 | *ASC(e,p),* | *LSC(p),* | *PSC(e,p)* | *ASC(p),* | *LSC(p),* | *PSC(p)* |
| 211 | 18.3 | 661 | *ASC(p),* | *LSC(p)†,* | *PSC(p)* | | | |
| *Pancho* | 6.1 | 239 | *ASC(e,p)†,* | *LSC(p)†,* | *PSC(e,p)†* | *ASC(p),* | *LSC(p),* | *PSC(e,p)* |
| *Manuel* | 30.5 | 333 | | *LSC(p)†,* | *PSC(p)* | *ASC(p),* | *LSC(p)†,* | *PSC(p)* |
| *Juan* | 30.5 | 178 | *ASC(p),* | *LSC(p)†,* | *PSC(p)* | | | |
| *Enrico* | 6.1 | 332 | *ASC(p)* | | | | | |

*ASC*, *LSC*, and *PSC* signify the anterior, lateral, and posterior semicircular canals; *e* and *p*, the endolymphatic and perilymphatic spaces, respectively. *Information for *Monkeys 8–68* inclusive has appeared previously in Tables 4 and 5 of Ref. 3 (by permission of the American Physiological Society). †New bone growth and fibrous material were also found near ampulla and utricle in perilymphatic space.

growth and fibrous material that has been observed in the semicircular canal spaces in 29 of these monkeys, which were sacrificed between 20 days and 661 days after inner ear DCS. (The complete absence of ectopic new bone growth or fibrosis, or both, in the vestibular spaces has been observed in only seven hit monkeys with these long-term survival times.) *Monkeys 119* and *145* were not recompressed after receiving a vestibular hit, but were brought to the surface slowly. *Monkeys 135, 160, 166*, and *169* were recompressed to depths that eliminated all behavioral signs of vestibular dysfunction before being brought to the surface slowly, at the rate of 0.3 msw/min. The remaining 23 monkeys listed in Table I were compressed to twice the depth at which the hit occurred and then brought to the surface at the slow rate indicated before. Starting immediately after receiving their vestibular hit, *Monkeys 201* and *207* were subjected to a Table 6 treatment for three successive days.

*Monkeys Pancho, Manuel, Juan,* and *Enrico*, who had been conditioned to discriminate between tones and no-tones, were tested for hearing thresholds before, and at selected intervals after, their dives.

Of the 34 monkeys with long-term survival times that received some form of the recompression treatment, 29 had no apparent behavioral signs of inner ear DCS (such as nystagmus and/or unsteadiness) upon completion of the recompression schedule (including 6 of those in which there was no evidence of bone growth in the canal spaces). However, about one-third of these monkeys developed a positional nystagmus the following day, a clear indication of continuing vestibular dysfunction.

Regardless of whether or not the animal received the recompression or hyperbaric oxygen treatment(s), or both, the entries in Table I indicate the same outcome, i.e., the gross infiltration of fibrotic tissue or new bone, or both, in the canal spaces, as a consequence of inner ear DCS (*cf.* Figs. 1 and 2). (In animals sacrificed shortly after their dive, the manifestations of vestibular-apparatus damage appear in the form of severe hemorrhage in the semicircular-canal perilymphatic spaces, and as blood-protein exudates, mainly, in the endolymphatic spaces; in particular, as an agglutinate to the cupula of the crista ampullaris [3].) This new bone continues to grow slowly until it also occludes the endolymphatic space of the semicircular canal in some cases (*see* (*e,p*) entries in Table I); in others, it continues until it encroaches on the perilymphatic space of the ampulla (*see* * entries in Table I). In either case, the likely result is the same: a progressive lessening of vestibular function, until total malfunction of the involved duct(s) can occur. The table entries also illustrate that this bone growth occurs 1.5 times more often in the left labyrinth than in the right. Furthermore, it may occur in one or more canals on the same side of the head, or it may occur in one or more canals on both sides of the head. Any and all combinations are possible.

It appears that this new bone growth is caused, partly, by a ripping or irritation of the endosteum, which lines the inside of the bony semicircular canals (3). There is also convincing evidence that rapid decompression can generate forces of a magnitude sufficient to fracture the hard temporal bone

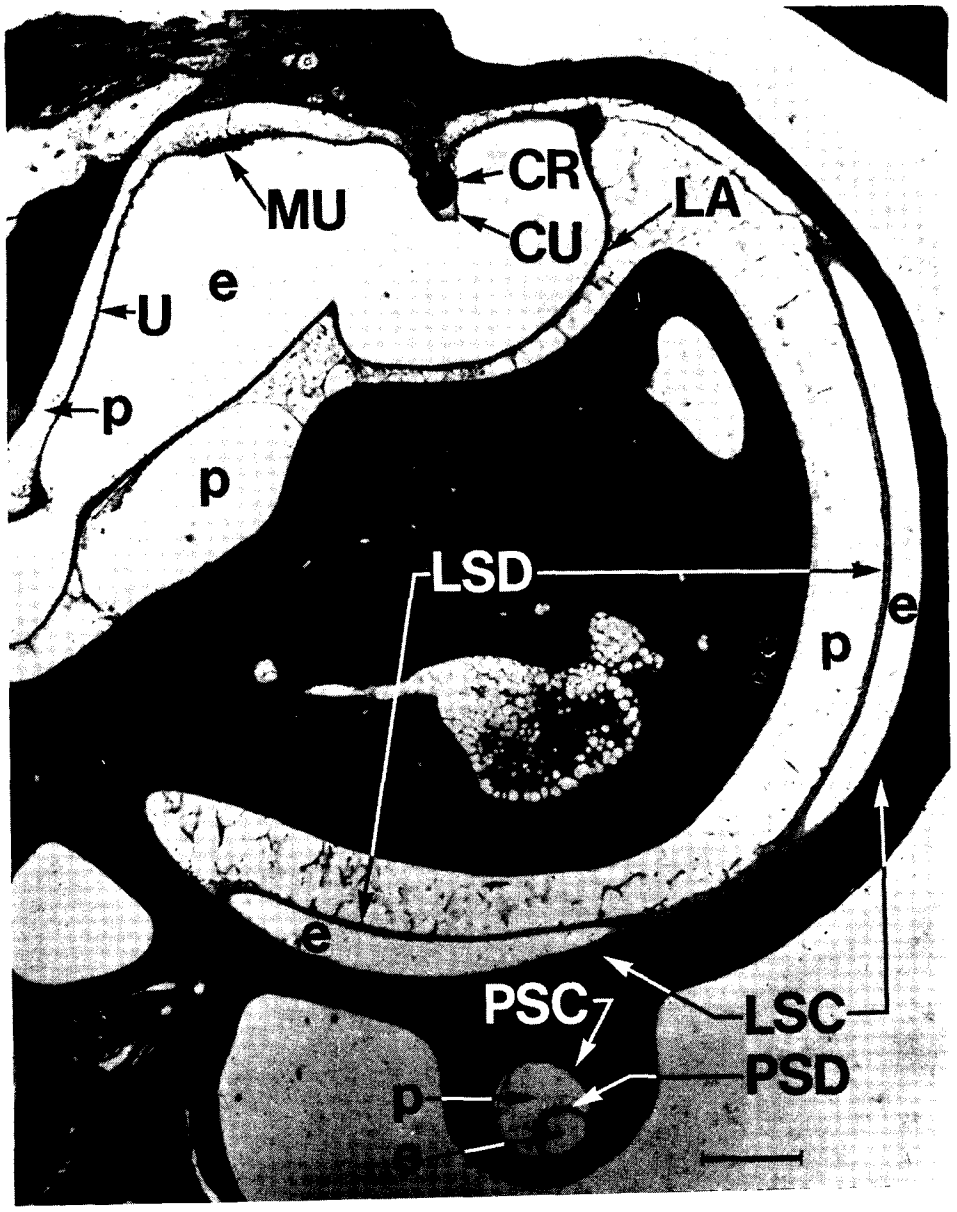



Fig. 1. Normal posterior (*PSC*) and lateral (*LSC*) semicircular canals and associated structures in a horizontal section from the right labryinth of *Monkey 166*. The membranous posterior (*PSD*) and lateral (*LSD*) semicircular ducts lie within the respective canals. *Abbreviations*: *MU*: macular utriculi, the sensory end organ for the detection of linear accelerations and gravity, which is located in the utricle (U); *CR*: crista ampullaris, the sensory end organ for detecting angular accelerations; *CU*: cupula, which interfaces with *CR* and normally fills the ampullary space in its plane of projection, but has shrunk as a result of tissue fixation; *LA*: ampulla of *LSC*; *e* and *p*: endolymphatic and perilymphatic fluid spaces, respectively. The dark half-circular band on the distal surface of the crista is its sensory epithelium. *Bar* = 50 μm.

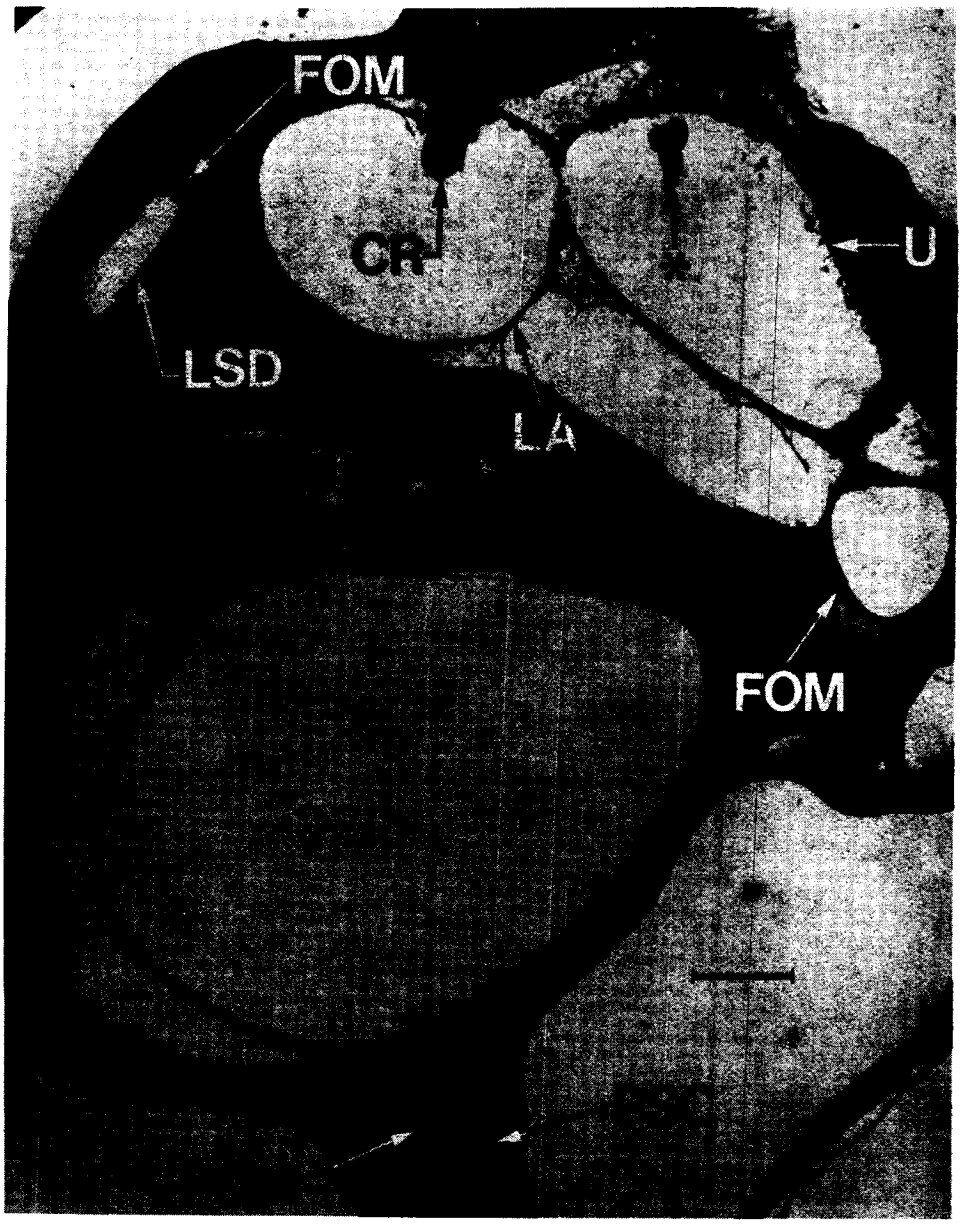



Fig. 2. Horizontal histological section from the left labyrinth of *Monkey Pancho*, illustrating extensive bone and fibrotic growth (*FOM*) in the otic fluid spaces in the posterior (*PSC*) and lateral semicircular canal (*LSD, LA*) systems. Note that the cupula is missing and that the "bald" crista ampullaris (*CR*) has only a very thin band of sensory epithelium. This clearly indicates a nonfunctioning sensory end organ. Both otic fluid spaces in the *PSC* have been blocked by FOM, rendering that canal nonfunctional also. *Symbols*: *U*: utricle; *: designates otoconial (ear-stone) mass that has been displaced from the macula utriculi (which is not clearly defined in this photo). *Bar* = 50 μm.

that is contiguous to the bone comprising the semicircular canal, as well as the canal wall itself (6).

In an investigation of the biophysical mechanisms responsible for causing this type of damage in monkeys during rapid decompression, Ward and his colleagues (7) have produced a model which theorizes that, for certain conditions during decompression, large stresses can be produced by bubble nucleation and growth within osteoclast cell cavities in the bone, such as are found in temporal and canal bone. Bubble nucleation is thought to occur within the conical processes of the "ruffled" border—a specialized membrane involved in bone resorption—of an osteoclast cell. This theory predicts that the essentially incompressible cytoplasm in the bone cell (which forms a constant-volume, basically closed cavity of liquid-gas solution) experiences a pressure rise with bubble growth sufficient to produce a force capable of fracturing the bone in which it is contained. Near the surface during ascent, the fluid spaces of the semicircular canals would be at ambient pressure compared to the very high pressure that would be experienced by the cytoplasm within the osteoclast cavity during bubble growth, after exposure to 274 msw. Therefore, once a critical state is achieved, the bubble would grow, causing a large transient increase in pressure relative to that of ambient pressure. Accordingly, this would result in a sudden implosive fracture of the (presumably) rigid temporal and canal bone into the semicircular canal spaces during the final phases of decompression. This theory is consistent with some of the types of semicircular canal damage that have been observed histologically after rapid decompression (6). Venter and his colleagues (8) have found that a mean pressure of 1.6 $\pm$ 0.4 MPa is required to fracture the full thickness of the semicircular canals in squirrel monkeys; this pressure is consistent with that predicted by Ward's theory.

The implosive force also causes a bolus of pressure wave energy to move rapidly along the canal; this energy could tear the endosteum and loosen the membranous semicircular ducts from their anchorage to the canal wall as well as provide a substantial transient stimulus to the vestibular end organs. This transient stimulus likely explains why a vestibular hit appears suddenly during decompression in most of these cases. (Vestibular hits resulting from blood supply changes to the vestibular end organs and from central vestibular dysfunction can also appear suddenly.)

The theory of Ward and his colleagues (7) also predicts that, under similar conditions, for less severe pressure, bubble nucleation and growth in bone cells will not produce the pressure required to break the bone. However, this pressure difference, which could be of the order of 0.5 MPa, would still damage or kill the bone cells by mechanical distortion. This mechanism could explain the empty bone cell cavities found in the long bones of divers suffering from aseptic bone necrosis, a debilitating condition that becomes progressively more evident several years after undergoing decompression procedures.

It is instructive to compare the manifestations and symptoms of inner ear DCS in the squirrel monkey with those observed in the commercial diver. In

particular, the clinical observations on eight divers by Komordin (9) are pertinent and characteristic. The sickness appeared suddenly during decompression, at depths of 40–45 msw, 1 ½ to 3 h from the time the dive began. In all cases, the bottom depths exceeded 150 msw, and the breathing gas mixture was oxy-helium. Severe vertigo, nystagmus, nausea, emesis, tinnitus, a loss of spatial orientation, and a decrease in hearing were evident in most cases. Symptoms associated with the decompression syndrome, such as joint pain or itching of the skin, were usually absent. Immediate recompression was successful in only about a third of these instances, and then only under the conditions of high pressure for long periods.

We have recently completed the histological study of the temporal bones of a professional diver who died (of unrelated causes) 56 days after sustaining a severe inner ear hit to his left labyrinth after a dive to about 100 msw for 19 min on trimix (10% $N_2$). Some 6 hours after the dive, during sleep, this diver experienced dizziness and knee pain and was, therefore, promptly recompressed at least twice (one for 52 h) in an attempt to provide relief (unsuccessfully). Clinical tests indicated a total loss of vestibular function and a partial hearing loss in the left ear. The histological study revealed ectopic new bone growth and fibrosis in one of the semicircular canals of the left ear (report in preparation, 1983). Clearly, many of the manifestations and symptoms of inner ear DCS in man and monkey are of a similar nature.

The insidious nature of the vestibular lesions makes it imperative that any diver who has experienced inner ear DCS should obtain follow-up clinical evaluation over a period of several years before a clean bill of health is given. The fact that many divers experience vertigo during decompression suggests that pathological bone growth may, perhaps, be quite common in older divers. (It would be interesting to know whether or not divers hear a click, snap, or bang preceding a vestibular hit, as might be expected on the basis of Ward's model [7].) The slow growth rate of this ectopic bone would be expected to give CNS compensatory mechanisms time to develop and restore normal balance during ambulatory situations when there is good visibility. However, exposure to conditions of neutral buoyancy and poor visibility, such as can occur while diving, could lead to disorientation and may threaten the life of a diver who had previously received such a hit.

Accordingly, it may be prudent for every diver who has sustained such a hit to obtain either a temporal-bone computerized tomographic scan (10), or, better yet, one that utilizes nuclear-magnetic-resonance imaging techniques (since this does not require exposure to radiation but provides similar information) to assess the true nature of the damage before returning to diving. Moreover, older divers, even if they have not experienced a direct vestibular hit, should routinely obtain a thorough otoneurological evaluation (complete with scan) because there is some evidence that a series of subthreshold vestibular assaults could produce a similar pathology.

The clinical practices of the French specialists in the treatment of decompression-caused ear injuries in divers are of interest to this study (11–14). Regardless of the dive, whether on compressed air or helium, shallow or deep

saturation, the recommended practices appear to be the same: the treatment consists of hyperbaric oxygen therapy in combination with vasodilators, corticosteroids, and heparin in small doses. Experience has shown that hyperbaric oxygenation with this type of adjuvant drug therapy is very effective in reversing peripheral cochlear dysfunction. However, the French have noted that, in spite of immediate treatment, peripheral vestibular lesions from dives to great depths can be severe from the onset and, furthermore, may be permanent (15) and worsen with time (16). McCormick et al. (17) have recommended the prophylactic use of heparin and Novotny (18) the use of nicotinic acid, a reputed vasodilator, for ameliorating decompression-induced hearing loss.

Given the nature of the damage sustained by the peripheral vestibular apparatus during inner ear DCS, it is not surprising that neither hyperbaric oxygen therapy (*Monkeys 201* and *207* listed in Table I) nor prompt recompression were found to be beneficial forms of therapy. Similarly, the beneficial effects of adjuvant drug therapy with vasodilators, anti-inflammatory agents, and anticoagulants would likely be extremely limited. Diazepam, a tranquilizer and anticonvulsant agent, which has been recommended as adjuvant therapy for labyrinthine DCS (19), would also seem to be powerless to either reverse or prevent this kind of damage, though it may provide some much-needed relief from vertigo.

Because the nature of the damage to the peripheral hearing organ in squirrel monkeys is quite different from that to the vestibular apparatus, it may be that hyperbaric oxygen therapy as used by the French (11–14) and others (9,18) could be a beneficial form of treatment. Indeed, studies to date in our laboratory have shown that the cochlear damage following a successful hit first appears histologically only in the form of a blood-protein exudate and an occasional hemorrhage of the cochlea. Therefore, it is important that the highly specialized receptor hair cells, which consume large amounts of oxygen, do not become anoxic as a result of blood interruption to the organ of Corti. (It bears mention that the organ of Corti always appears intact and functional in celloidin histological sections.) In animals sacrificed several months after receiving an inner ear hit, the cochlear fluid spaces have become quite clear, containing much less exudate than is observed shortly after the dive (Fig. 3). In this regard, the amount of exudate is similar to that observed in control animals. Moreover, bone or fibrotic growth, or both, have never appeared in the cochlea. Notwithstanding the cochlear deficits, gradual recovery of some hearing function was evident in monkeys that were tested behaviorally for hearing loss (Fig. 4), even though there was strong evidence that vestibular function remained severely interrupted (e.g., *Pancho, Manuel, Juan*, and *Enrico* listed in Table I). Of course, the fluid spaces in the inner ear are shared by both the vestibular apparatus and the cochlea. Thus, injury to one organ often results in a decrement of sensory function in the other organ. In this regard, the hemorrhage or blood vessel blockage that may result from bubble nucleation and growth in the ear vessels during decompression can be significant. The effect on the fluids in one organ may cause changes in the

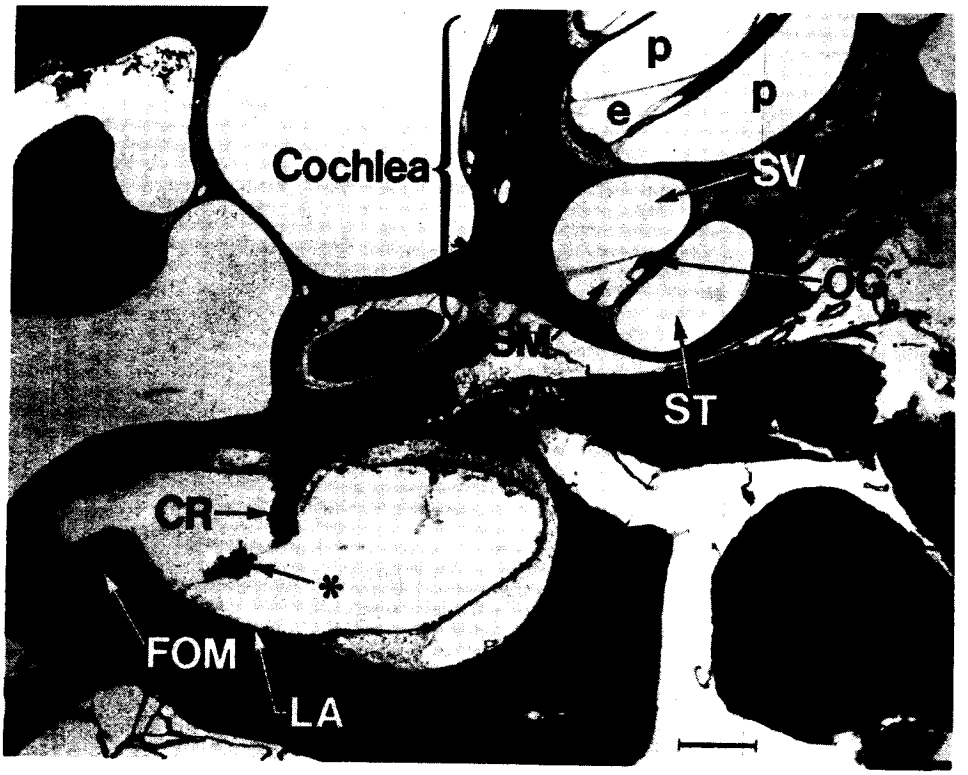



Fig. 3. Horizontal histological section illustrating the presence of a normal-appearing cochlea next to a pathological lateral ampulla (*LA*) from the left labyrinth of *Monkey 169*. Both the endolymphatic (*e*) and perilymphatic (*p*) spaces in the cochlea are clear, and the organ of Corti (*OC*), the sensory end organ of hearing, appears normal. The presence of fibrotic material (*FOM*) in the perilymphatic space of the *LA* is evident. *Abbreviations*: *SV*, *ST*, *SM*: scalae vestibuli, tympani, and media, respectively (otic fluid spaces in the cochlea); *CR*: crista ampullaris. The * identifies cupular remnants that have become detached from the *CR*. *Bar* = 50 μm.

electrolytic and protein compositions in the other organ, resulting in an interference with the physiological mechanisms involved in normal sensory function. Presumably, as the initial disorder subsides and clears, the mechanisms which control sensory function are re-established. This might explain why, sometimes, there is sensori-neural hearing loss without any apparent concomitant pathological basis.

All of the hits that are recorded in Table I occurred at various depths during ascent and never at the surface. There are, however, many instances of human divers whose symptoms appear suddenly, only after decompression has been completed. This could signify that there are gas bubbles that are trapped in the conical processes, but whose further growth has been inhibited by the rigid surrounding bone. It is possible that such bubbles could remain quiescent

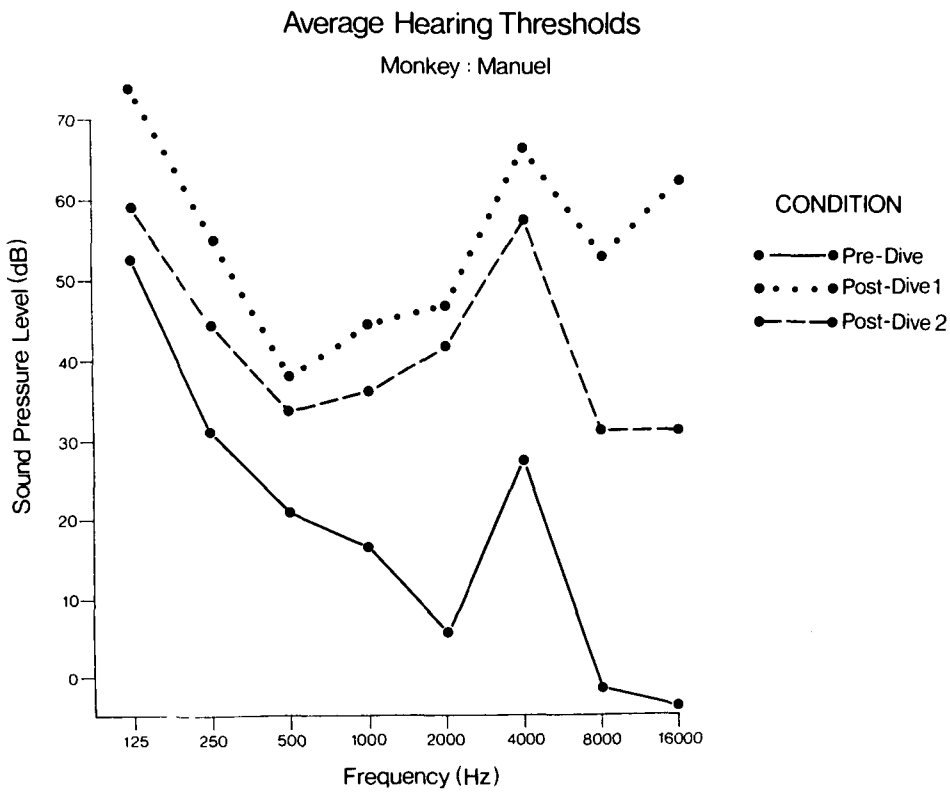



Fig. 4. Curves of average hearing thresholds, in decibels (dB), as a function of the stimulus frequency for *Monkey Manuel*. The *conditions* are: *Predive*, averaged over 3 tests; *Postdive 1*, 92–125 days after the dive, averaged over 4 tests; and *Postdive 2*, 287–301 days after the dive averaged over 4 tests. As indicated, there was some recovery of hearing between the *Postdive 1* tests (when the hearing threshold was at its worst) and the *Postdive 2* tests (the final tests before the animal was sacrificed 32 days later). The *curves* indicate that a (possibly permanent) residual hearing deficit of 10 dB (at the lower frequencies) to 30 dB (at the higher frequencies) remains at the time of sacrifice.

and asymptomatic, until some unknown, cataclysmic "jolt" causes them to continue to grow and, thereby, fracture the bone containing them. Accordingly, a prudent method for preventing this type of subsequent bubble growth (and a treatment that probably would benefit the cochlea) might be the mandatory use of the U.S. Air Force modification of the U.S. Navy Table 6A Treatment (5) for gas embolism whenever DCS is suspected. It bears mention here that the only effort at this laboratory (DCIEM) with the modified Table 6A Treatment was equivocal. *Monkey WI3* (not mentioned previously), upon receiving a CNS hit at 30.5 msw on ascent from a 274-msw dive, was recompressed to 50 msw. This treatment removed the CNS hit but left the monkey with an apparent vestibular hit (possibly in the vestibular central

pathways), which persisted until the animal was (slowly) decompressed to 19.5 msw, whereupon all signs of the hit disappeared. On reaching surface, *Monkey W13* was given a single modified Table 6A Treatment. This monkey was sacrificed 86 days after the dive; subsequent temporal bone histology indicated that the vestibular organs were intact and in good condition.

Farmer (19) has indicated that a drug treatment which relies upon increasing inner ear blood flow, as has been recommended by the French and others (11–14,17,18), may result in additional bleeding; or, it might cause blood flow to be shunted to more peripheral regions, thereby counteracting its intended purposes. Such agents are considered by Farmer to be potentially harmful and are not recommended. The results from this study would tend to support Farmer's reasoning.

## SUMMARY AND CONCLUSIONS

As a result of our research on the nature of the inner ear DCS resulting from deep dives, the following statements can be made:

1) The sickness occurs very suddenly during the ascent phase of the dive (between 62 msw and the surface for squirrel monkeys on a 274-msw dive).

2) Prompt recompression appears to lessen (or even eliminate) the acute behavioral signs and symptoms of inner ear DCS; it does not, however, reverse the pathological damage to the vestibular apparatus that is provoked by the dive.

3) Serial histological sections of the brains and temporal bones of monkeys which had received inner ear hits show that, if the only symptoms are of a vestibular nature, then the problem is likely to be only in the ear and not in the brain.

4) Cochlear damage first appears in the form of blood and blood-protein exudates in the otic fluid spaces; much later, these spaces appear clear, similar to those observed in control animals. Hearing tests show that some of the monkeys gradually recover some of their hearing deficit (without benefit of adjuvant drug therapy); however, a residual loss remains permanently.

5) Vestibular-apparatus damage first appears in the form of severe hemorrhage and blood-protein exudates in the otic fluid spaces; later, these spaces become invaded by ectopic new bone growth and fibrous material.

6) Histologically, temporal- and canal-bone breaks are prevalent in some of the hit monkey ears. A theory was developed (7) which predicts that such breaks could occur from "imploding" forces that are caused by the large pressures (1.6 MPa or more [8]) that are generated as a result of bubble nucleation and growth within osteoclast (bone) cells during decompression.

7) Cochlear lesions likely occur as a result of bubble formation and growth within microvessels, and their consequent blockage or rupture, or both, causes hemorrhage or blood-protein exudatation, or both. Because of the damage of anoxia to the highly specialized receptor hair cells of the cochlea under such conditions, immediate hyperbaric oxygen treatment would appear to be a necessary requirement.

8) Vestibular lesions of the type described in this report would not benefit from (or be aggravated by) hyperbaric oxygen therapy, whether the treatment used is for DCS (Table 6 treatment) or for gas embolism (Table 6A treatment). However, since cochlear lesions often appear with vestibular lesions, such treatment should be a recommended practice whenever a cochleovestibular insult is indicated.

9) Adjuvant drug therapy that increases inner ear blood flow is contraindicated in the treatment of inner ear DCS.

10) Any diver who has experienced inner ear DCS should obtain extensive follow-up clinical evaluations before returning to diving. Older divers, whether or not they have received a direct inner ear insult during decompression, should routinely obtain a thorough otoneurological examination.

## Acknowledgments

This research was supported, in part, by the USN Office of Naval Research, Contract N00014–77–G–0077. DCIEM Publication Number 83–P–23. The authors thank Mr. Jerry Laufer for the histological preparations, Mr. Alcide Nicholas for general technical help, and Mr. Ron Cardin for editorial assistance with the manuscript.

This work is dedicated to the memory of Dr. Gosta F. Dohlman, whose association with DCIEM enriched us all.

## References

1. Landolt JP, Money KE, Topliff EDL, Powers KD, Johnson WH. Vestibulocochlear dysfunction in squirrel monkeys during rapid decompression. Med Aeronaut Spat, Med Subaquat Hyperbar 1977;16:377–381.
2. Landolt JP, Money KE, Topliff EDL, Ackles KN, Johnson WH. Induced vestibular dysfunction in squirrel monkeys during rapid decompression. Acta Oto-Laryngol, 1980;90:125–129.
3. Landolt JP, Money KE, Topliff EDL, Nicholas AD, Laufer J, Johnson WH. Pathophysiology of inner ear dysfunction in the squirrel monkey in rapid decompression. J Appl Physiol: Respirat Environ Exercise 1980;49:1070–1082.
4. Igarashi MA. A standard technique for temporal bone preparation. Pensacola, FL: Naval Aerospace Medical Institute, 1966. (Monograph 13)
5. Kindwall EP. Decompression sickness. In: Davis JC, Hunt TK, eds. Hyperbaric oxygen therapy. Bethesda: Undersea Medical Society, Inc., 1977;125–140.
6. Fraser WD, Landolt JP, Money KE. Semicircular canal fractures in squirrel monkeys resulting from rapid decompression: Interpretation and significance. Acta Oto-Laryngol 1983;95:95–100.
7. Ward CA, Johnson WR, Venter RD, Ho BN, Forest TW, Fraser WD. Heterogenous bubble nucleation and conditions for growth in a liquid-gas system of constant mass and volume. J Appl Phys 1983;54:1833–1843.
8. Venter RD, Ho BN, Johnson WR, Ward CA, Fraser WD, Landolt JP. Fracture studies on semicircular canals of the inner ear. Undersea Biomed Res 1983;10:225–240.
9. Komordin IP. Some peculiarities of Meniere's syndrome in deep sea divers (in Russian). Voennomed Z 1973;5:66–67.
10. Valvassori GE, Mafee MF, Dobben GD. Computerized tomography of the temporal bone. Laryngoscope 1982;562–565.

11. Le Mouel C, Fretillere H, Oliva A, Renon P, Angot A. L'oxygénothérapie hyperbare dans le treatment des accidents cochléo-vestibulaires de la plongée. Med Aeronaut Spat, Med Subaquat Hyperbar 1977;64:385–388.
12. Renon P, Angot A, Suc B. Conceptions pratiques des accidents de l'oreille interne chez les plongeurs. Med Aeronaut Spat, Med Subaquat Hyperbar 1980;74:95–101.
13. Demard F. Variations de pression et oreille interne en plongée. Med Aeronaut Spat, Med Subaquat Hyperbar 1980;74:89–94.
14. Le Mouel C, Suc B, Asperge A. Traitement hyperbare des accidents de l'oreille interne liés à la plongée. Med Aeronaut Spat, Med Subaquat Hyperbar 1981;79:242–246.
15. Demard F, Olivier JC, Paolino M. Evolution et séquelles tardives des accidents cochléo-vestibulaires de la plongée profonde. Med Aeronaut Spat, Med Subaquat Hyperbar 1977;64:389–393.
16. Bertoin R. Evolution clinique des accidents labyrinthiques survenant chez les ouvriers travaillant en air comprime. Arch Mal Prof 1953;14:221–224.
17. McCormick JG, Philbrick T, Holland W, Harrill JA. Diving induced sensori-neural deafness: Prophylactic use of heparin and preliminary histopathology results. Laryngoscope 1973;83:1483–1501.
18. Novotny, GM. "Cochlear bends." J Otolaryngol 1980;9:395–398.
19. Farmer JC. Inner ear injuries in diving—Differential diagnosis of inner ear decompression sickness and inner ear barotrauma. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981;805–810.

# A RETROSPECTIVE EVALUATION OF OXYGEN RECOMPRESSION PROCEDURES WITHIN THE U.S. NAVY

*C. G. Gray*

Procedures for treatment of decompression sickness (DCS) and arterial gas embolism (AE) have undergone a considerable evolution since their inception. In spite of substantial research, the pathophysiology of DCS remains in question, and much of the progress in recompression therapy has been based on empirical data. Adoption of minimal recompression procedures using oxygen (1-3) and the use of adjunctive therapy with recompression more recently have claimed improvements in treatment results (4,5). Previous surveys of treatments (6-10) have demonstrated the advantages of the oxygen treatment tables over most of the air treatment tables. These data and more recent reports (11-14) have emphasized that severity of symptoms and treatment delays are considered primary determinants affecting therapeutic success, although reported departures from established treatment criteria (13,15) could be responsible for some of the poor results observed. Refinements in treatment procedures may be partially negated by inadequate patient evaluation, incorrect diagnosis, inappropriate treatment-table selection, improper delivery of the prescribed therapy, and confusion on criteria regarding endpoints of therapy.

Within the jurisdiction of the U.S. Navy, a firm and logical usage of existing treatment tables has been prescribed in the *U.S. Navy Diving Manual* (16). The treatment flow diagram contained in Chapter 8 used in conjunction with the recompression treatment tables is intended to provide adequate guidelines to treat most cases properly. However, the changes implemented during the last decade have not been adequately evaluated and an evaluation of treatments delivered by U.S. Navy facilities has not been completed since 1970. At the Naval Safety Center recompression treatments including a computer-tabulated questionnaire and a narrative summary have been recorded.

This survey retrospectively reviews all treatments of DCS and AE reported from 1971 to 1981. The cases are separated according to the treatment table used with an assessment of therapeutic results, appropriateness of table selection, and adequacy of treatment when possible. This summary should provide some additional insight on the effectiveness of the published U.S. Navy standard treatment recommendations.

## METHODS

Diving ACCIDENT/INJURY Reports provided by the Naval Safety Center for DCS and AE cases occurring from 1971 to 1981 were reviewed. However, at least through 1978, many treatments were unrecorded. The cases were subdivided according to treatment table used, and the narrative descriptions provided with each case were evaluated and compared with the tabular data. The narrative, when adequate, provided a descriptive background of the incident, the treatment, and the results, with the results reported as Complete Relief, Substantial Relief, Partial Relief, or Fatal. Based on the guidelines for treatment (16) at the time the treatments were delivered and a clinical interpretation of the situations based on the narratives, the results of the treatments were assessed. These evaluations were based on the author's qualification and experience as a Navy-qualified Diving Medical Officer. Where inadequate information for a judgment on the criteria for treatment, or where inadequate treatment results were provided, the cases were assumed to be appropriately treated with relief as indicated. A number of missed decompression cases were included, but poor narrative and tabular data prohibited adequate separation of asymptomatic from symptomatic cases. These cases may serve to elevate the treatment success rates. Cases attributable to non-DCS causes and undergoing full or partial treatments are not included. It was not possible in all cases to differentiate who was responsible for the treatment decisions (Diving Medical Officer or Master Diver) and no summary was attempted. All patients were treated in Navy recompression facilities using U.S. Navy procedures.

The *U.S. Navy Diving Manual* (16) has given authority to the Diving Medical Officer to alter the recompression tables at his discretion. The treatment logic provided in the diving manual indicates that if "relief" is not achieved on a shorter table, then a longer table should be employed. Treatment Table 5 (TT-5) was adopted for a specific use (pain-only symptoms, relieved within 10 minutes at 60 fsw). If those criteria are not met, then TT-6 or 6A are to be employed with extensions as necessary to achieve relief.

This conservative treatment scheme has been used as a basis for delineating standard from nonstandard use of the recompression therapy tables; *for this analysis* nonstandard uses of the tables are:

1) Any modification or extension to TT-5;

2) Cases treated on TT-5 where the narrative indicates pain was relieved *after* 10 min at 60 fsw or cases with Type 2 symptoms;

3) Cases treated on TT-6 or TT-6A, with or without extensions that yielded incomplete relief and *may* have benefited from further extensions; or

4) Nonstandard use of TT-5A between 1971 and 1977 or any use after 1977 (TT-5A was eliminated in 1976).

All of these criteria for the selection of nonstandard treatments resulted in shortening of the treatment schedules and are considered as nonstandard regardless of the results. One-treatment success is derived by adding complete relief and substantial relief cases and subtracting retreatments and fatalities.

*Change 2 to the U.S. Navy Diving Manual* (16) was implemented in June 1978. This change was intended to improve treatment results by clarifying the treatment criteria and decision processes. The effectiveness of this change was assessed by a separate evaluation of the years 1979–1981 which allowed comparison with the 1971–1978 period. Additionally, for 1979–1981, the cases treated on TT-6 or TT-6 EX were further subdivided into treatments administered for Type 1 (pain only) and Type 2 (serious symptoms) DCS for assessment of relative use and results for these types of cases.

The data for complete relief, substantial relief, fatalities, overall success, and nonstandard treatments are expressed as a percent of the total number of cases (% of TOTAL). The cases are designated as Complete Relief or Substantial Relief as the outcome of the *initial* treatment, and the retreatment rate is expressed as a percentage of the group from which they came (% of Complete Relief or % of Substantial Relief).

For statistical comparisons it is assumed that the population from which diving injuries occur is no different for each treatment group. For this analysis a $z$-test for independent samples drawn from populations of equal proportions was used (17). The probability of a difference is stated for each sample comparison that fulfills the required criteria for sample size and composition.

## RESULTS

Table I shows for 1971–1978 there were a total of 477 cases treated on the minimal recompression oxygen tables, with 33 retreatments and 3 fatalities. Of the cases experiencing Complete Relief, there was a 3.6% retreatment rate. The one-treatment success rate of the standard $O_2$ treatments is significantly better than the success rate for the nonstandard treatment cases ($P < 0.001$). The success rate for the Air or Other treatments is not significantly different from the total of the $O_2$ treatments, but the number is relatively small ($n = 47$).

For the 1979–1981 period, one-treatment success for standard ($n = 247$) treatment cases (95.6%) is greater than for the nonstandard ($n = 26$) treatment cases (92.6%), at a significance of $P < 0.05$. The nonstandard utilization of the treatment tables was 12.2% and 10% for the 1971–1978 and 1979–1981 periods, respectively, and was not significantly different. The one-treatment success when using nonstandard treatment schedules was significantly ($P < 0.01$) improved for the 1979–1981 period over the 1971–1978 period.

Success for a single recompression treatment using TT-5 is summarized in Figs. 1 and 2 for the years 1971–1978 and 1979–1981, respectively. Referring to Fig. 1, one sees there was a total of 181 cases treated on TT-5 for the

## TABLE I

### Summary of Success from a Single Recompression Treatment

| | No. of Cases | No. of Complete Reliefs (% of Total) | No. of Retreatments (% of Comp. Relief) | No. of Substantial Reliefs (% of Total) | No. of Retreatments (% of Subst. Relief) | One-Treatment* Success (% of Total) | Fatal |
|---|---|---|---|---|---|---|---|
| *1971–1978* | | | | | | | |
| $0_2$ Table Standard TX† | 418 | 369 (88.3) | 13 (3.5) | 47 (11.2) | 10 (21.3) | 94.0 | 2 |
| $0_2$ Table Nonstandard TX | 59 | 17 (28.1) | 1 (5.9) | 41 (69.5) | 9 (22.0) | 81.4 | 1 |
| Total $0_2$ TX | 477 | 386 (80.9) | 14 (3.6) | 88 (18.5) | 19 (21.6) | 92.5 | 3 |
| Air or Other TX | 53 | 34 (72.3) | 3 (8.8) | 13 (27.7) | 0 | 93.6 | 6 |
| *1979–1981* | | | | | | | |
| $0_2$ Table Standard TX | 247 | 230 (93.1) | 5 (2.2) | 13 (5.3) | 2 (15.4) | 95.6 | 4 |
| $0_2$ Table Nonstandard | 26 | 4 (14.8) | 0 | 23 (85.2) | 2 (8.7) | 92.6 | — |
| Total $0_2$ TX | 274 | 234 (85.4) | 5 (2.1) | 36 (13.1) | 4 (11.1) | 95.3 | 4 |
| Air or Other TX | 8 | 8 (100) | 0 (0.0) | — | — | 100 | 0 |

TX is treatment. *One-treatment success $= \frac{\#\text{Complete Relief} + \#\text{Substantial Relief} - \#\text{Retreatments} - \#\text{Fatal}}{\text{Total } \#\text{ Cases}} \times 100.$

†Recompression treatment cases reported to the Naval Safety Center and summarized for the 1971–1978 and 1979–1981 periods. $0_2$ Table Standard TX cases were treated on Treatment Tables 5, 5A, 6, 6A, 6 EX, or 6A EX according to guidelines published in the *U.S. Navy Diving Manual*. $0_2$ Table Nonstandard TX cases were any cases at variance with guidelines.

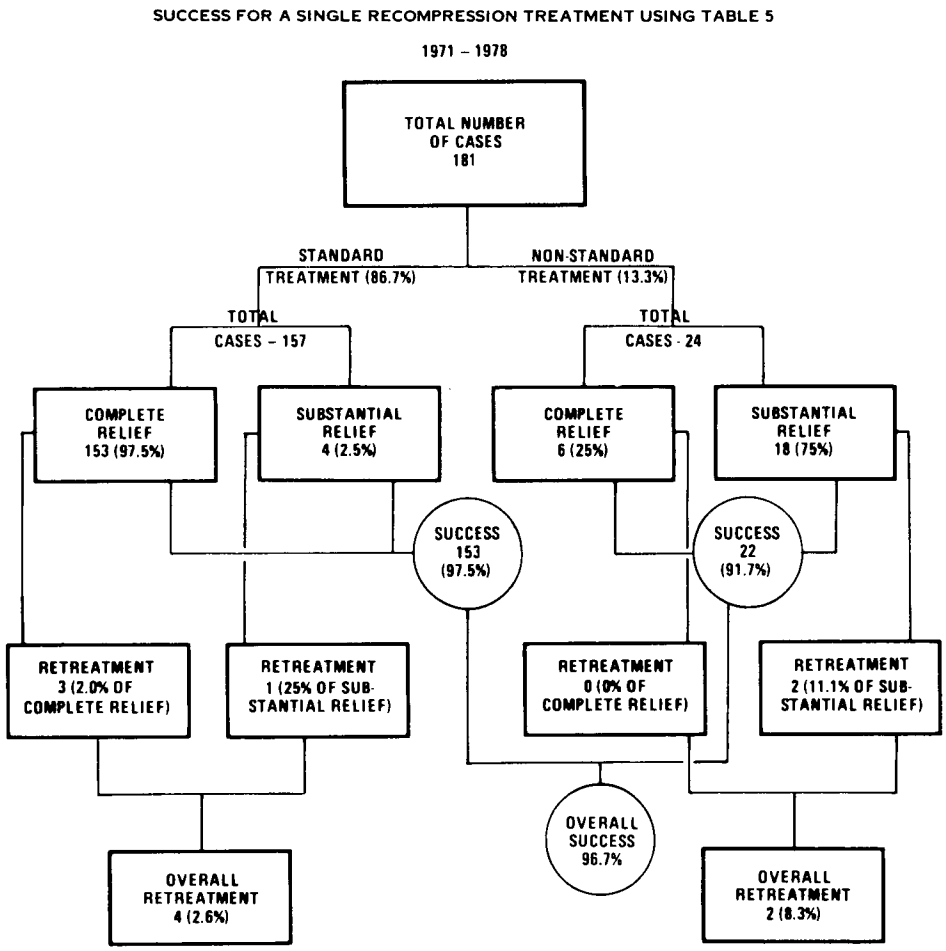



Fig. 1: All reported cases treated with TT–5 from 1971 to 1978. Standard treatment indicates diagnosis was pain-only DCS resolved within 10 min at 60 fsw. Adding all Complete Relief cases yields 159 with 3 retreatments (2.0% retreatment rate). Adding all Substantial Relief cases yields 22 with a 3 retreatments (13.6% retreatment rate).

1971–78 period. Of those cases, 157 were treated according to standard treatment criteria and 24 cases used TT-5 when the standard treatment criteria were not met (pain-only DCS, relieved within 10 min at 60 fsw). Of the 157 standard treatment cases, there were 4 retreatments, yielding a 97.5% one-treatment success (153/157). Of the 24 nonstandard treatments, there were 2 retreatments, yielding only a 91.7% one-treatment success (22/24). The overall retreatment rate for nonstandard treatment cases (8.3%) is more than 3 times the standard treatment-retreatment rate (2.6%). Additionally, adding the Complete Relief cases for both standard and nonstandard treatments yields 159 cases and adding the Substantial Relief cases yields 22 cases. Adding the

Retreatments-following-Complete Relief therapy yields 3 cases, and adding Substantial-Relief-Retreatments yields 3 cases. Thus, the retreatment rate for Substantial Relief cases (13.6%) is more than 6 times the Complete Relief retreatment rate (2.0%). Referring to Fig. 2, one sees the incidence of nonstandard treatments using TT-5 during the 1979–1981 period (6.6%) is lower than for the 1971–1978 period (13.3%). There is a marked improvement in treatment success for the 1979–1981 period for unknown reasons possibly related to the effects of other changes, such as adjunctive therapies and decreased treatment delays, which could not be evaluated.

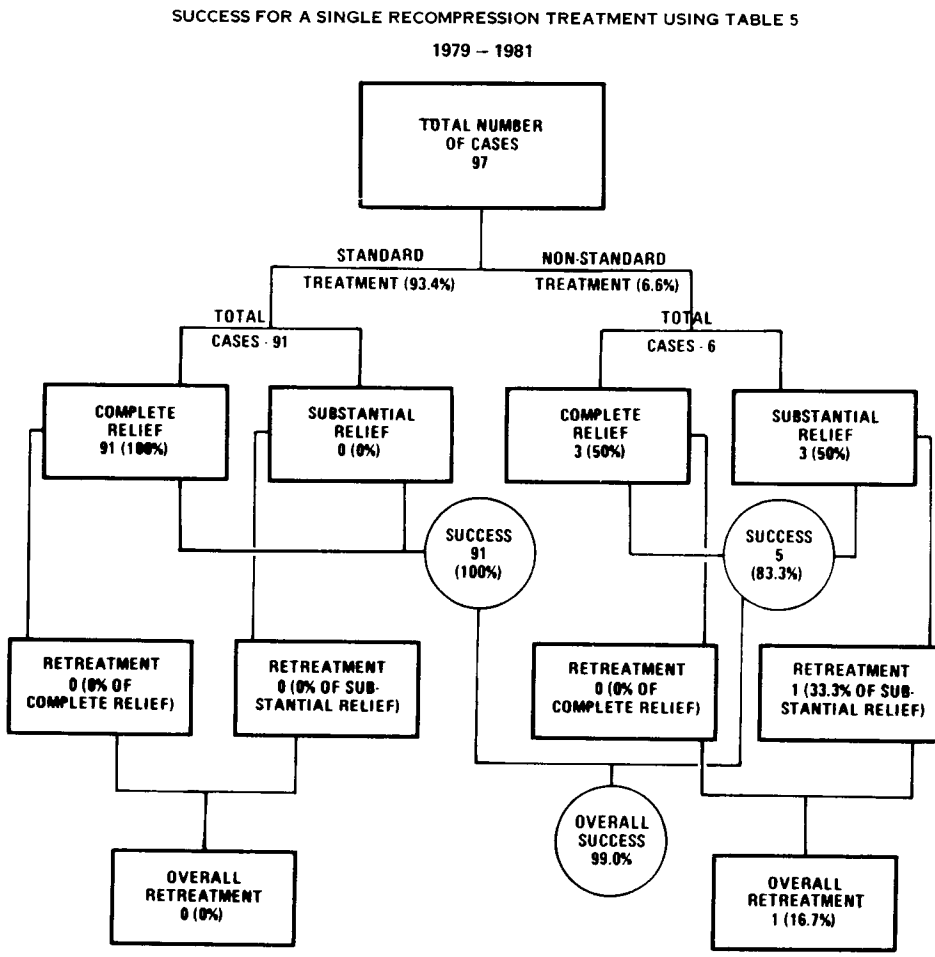



Fig. 2: All reported cases treated with TT–5 from 1979 to 1981. Standard treatment indicates diagnosis was pain-only DCS resolved within 10 min at 60 fsw. Overall one-treatment success (99%) is improved over the 1971–1978 period.

From 1971 to 1978 there were 21 cases treated on TT-5A. The 16 cases treated according to prescribed criteria had a 100% one-treatment success (no retreatments); whereas, 5 cases not treated according to standard criteria experienced two retreatments (40% retreatment rate). Although the number is small, this finding suggests that when appropriately used, TT-5A was an effective table. There were no recorded uses of TT-5A after 1978.

Modifications to Treatment Table 5 (TT-5 EX) are at variance with established criteria. For the 1971–1981 period there were 13 cases recorded which involved modifications (considered nonstandard for this analysis). Those with reported Complete Relief (9 cases) had no retreatments, whereas those with Substantial Relief (4 cases) had 3 retreatments (75% retreatment rate). This yields an overall retreatment rate of 23.0% for the 13 reported cases, which is approximately 7 times greater than the retreatment rate experienced with either TT-5 or TT-6.

The use of TT-6 for 1971–1978 is shown in Fig. 3. Treatments administered in accordance with standard guidelines yielded a 97.8% one-treatment success. The nonstandard usage of TT-6 produced a retreatment rate 12 times greater (25%) than the standard use of the table (2.2%). The retreatment rate of 11.1% for the combined Substantial Relief categories is substantially greater than for the combined Complete Relief categories (2.4%).

Table II summarizes the subdivided results for the use of TT-6 and TT-6 EX from 1979 to 1981 for a single recompression. Treatment Table 6 was used to treat Type 2 (serious symptoms) complaints ($n=57$) twice as frequently as for Type 1 (pain only) complaints ($n=27$). The overall TT-6 retreatment rates for Type 1 and Type 2 cases for 1979–1981 are not significantly different from the retreatment rate for the 1971–1978 standard treatment cases or from each other. Treatments using TT-6 EX were predominantly for Type 2 symptoms with an overall retreatment rate of 8.9%. No inferences are made from these more difficult cases.

Figures 4 and 5 recount the use of TT-6A or TT-6A EX for the 1971–1978 and 1979–1981 periods, respectively. From 1971 to 1978 for these difficult and serious cases, there was a 91.7% one-treatment success when used according to the standard guidelines, whereas nonstandard usage produced only a 77.8% one-treatment success (a 4 times greater retreatment rate). There is an improving trend for the 1979–1981 period with no retreatments in the nonstandard category. For the 1971–1978 period the retreatment rate for the combined Substantial Relief categories (18.7%) is more than 5 times greater than for the combined Complete Relief categories (3.7%). This may not be surprising if the Substantial Relief category is intended to imply that retreatments may follow as needed.

Cases treated on a TT-6 EX with additional $0_2$ breathing periods at 60 fsw or 30 fsw, or returned from 30 fsw to 60 fsw after a recurrence of symptoms, are illustrated in Fig. 6 and Table II for the 1971–1978 and the 1979–1981 periods, respectively. These cases constitute more difficult treatment decisions and are more difficult to evaluate critically. However, of the combined 120 cases for 1971–1981 there were 8 cases (6.7%) that were improving but

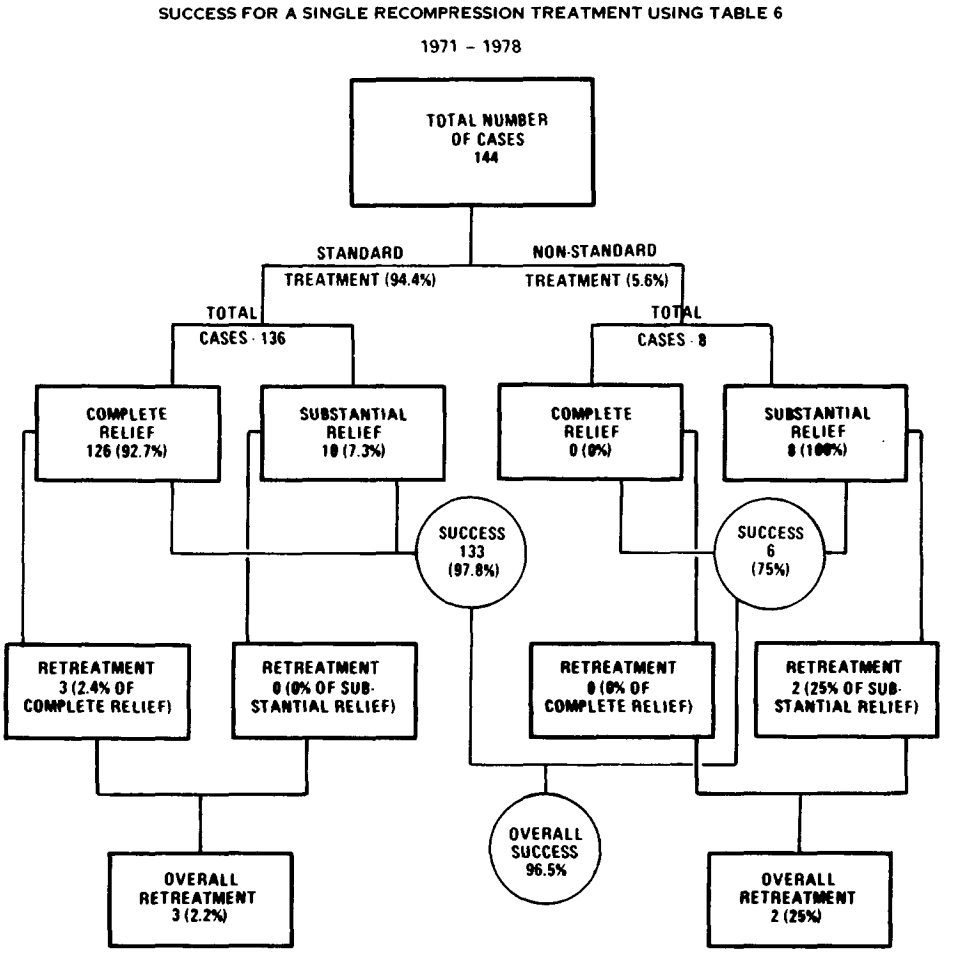



Fig. 3: All reported cases treated with TT–6 from 1971 to 1978. Standard treatment indicates complete resolution of primary symptoms or stable minor residual (i.e., musculoskeletal soreness) symptoms unchanged for at least one $0_2$ period and not worsening when decompression proceeded. All nonstandard cases exhibited significant residual symptoms when decompression proceeded with no extensions used.

unresolved when decompression began or continued. These cases were *not* extended to the authorized limit (without Medical Officer recommendation) provided in the *Diving Manual* (16). Although the retreatment rates are significantly higher than for other tables, the serious nature of these cases does not allow appropriate comparisons. Combining the TT-6 EX totals for 1971–1981 yields 73 cases with Complete Relief and 44 cases with Substantial or Partial Relief. Of those cases, the retreatment rates of 6.8% and 22.7%, respectively, are markedly different, but the significance for these serious cases is uncertain and may relate to intentions to retreat as necessary.

TABLE II

Success for a Single Recompression Treatment Using *Table 6 and Table 6 Extended* from 1979 to 1981

| Cases | No. of Complete Reliefs (% of Total) | No. of Retreatments (% of Comp. Relief) | No. of Substantial Reliefs (% of Total) | No. of Retreatments (% of Sub. Relief) | Overall Retreatments (% of Total) |
|---|---|---|---|---|---|
| *Table 6* | | | | | |
| Type 1: 32 | 27 (84.4) | 1 (3.7) | 5 (15.6) | 0 | 3.1 |
| Type 2: 57 | 54 (94.7) | 1 (1.9) | 3 (5.3) | 0 | 1.8 |
| Total*: 89 | 81 (91.0) | 2 (2.5) | 8 (9.0) | 0 | 2.5 |
| *Table 6 Extended* | | | | | |
| Type 1: 10 | 6 (60.0) | 1 (16.7) | 4 (40.0) | 0 | 10 |
| Type 2†: 35 | 20 (57.1) | 1 (5.0) | 14 (40.0) | 2 (14.3) | 8.6 |
| Total‡: 45 | 26 (57.8) | 2 (7.7) | 18 (40.0) | 2 (11.1) | 8.9 |

Reported treatments subdivided according to type of presenting symptoms. Type 1 is pain only, and Type 2 is serious symptoms DCS. *For *Table 6* treatments there were 8 cases of apparent incomplete treatment and no retreatments within those cases yielding a nonstandard treatment incidence of 9.0 (8/89). †For *Table 6 Extended* treatments the Type 2 cases include 1 fatality. ‡The total for this period includes 7 cases of apparent incomplete treatment and no retreatments within those cases yielding a nonstandard treatment incidence of 15.9% (7/44).

All other recompression treatments occurring during saturation dives or those using air tables and retreatments are listed in Table III. The results of retreatment are listed according to the table used for the repeat treatment. These cases are included for completeness with no conclusions drawn from these small numbers.

## DISCUSSION

The data presented herein suffer from several weaknesses. It is a retrospective secondary evaluation, all conditions are not known, cases with insufficient data could not be fully scrutinized, some semantic problems are evident, and the evaluations are based on one Medical Officer's intepretation. Additionally, asymptomatic cases, included under the Missed Decompression category of causes, are incorporated in the treatment results. There were also a significant number of seemingly partially treated cases that were lost to follow-up and may have benefitted from extended or repeated treatments. These cases could not be included, but would probably have elevated the retreatment rates for TT-6 or longer tables in the Substantial Relief category. *Diving Manual* (16) criteria for treatments are disputed by some, but they are defined as *standard* for this evaluation. Many cases before 1978 were not recorded at the

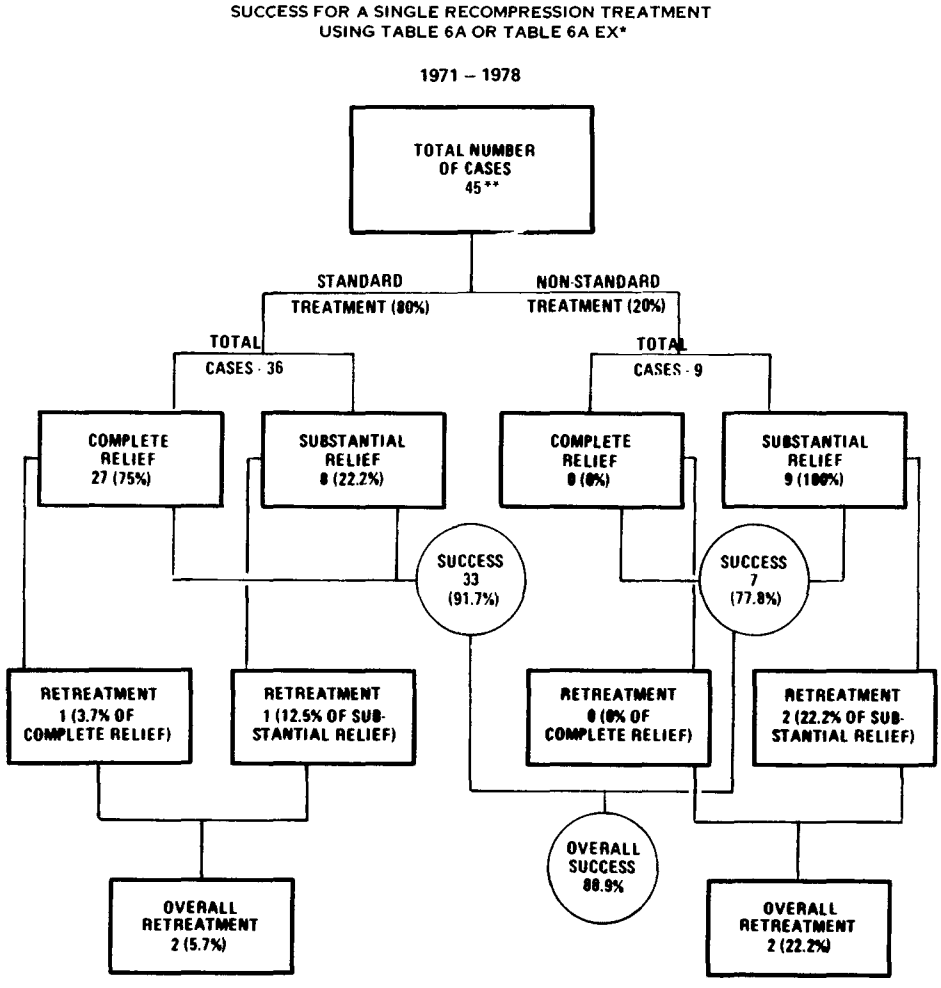



Fig. 4: All reported cases treated with either TT–6A or 6A EX (*) from 1971 to 1978. Standard and nonstandard treatment criteria are the same as for Fig. 3. Treatment failures for standard treatments include 2 retreatments and 1 fatality (**) yielding a 91.7% one-treatment success (33/36). Adding all Complete Relief cases yields 27 with 1 retreatment (3.7% retreatment rate). Adding all Substantial Relief cases yields 17 with 3 retreatments (18.7% retreatment rate).

Naval Safety Center and some of the selected subgroups for this analysis are relatively small samples. If one accepts these shortcomings, the data provide some interesting information.

The *U.S. Navy Diving Manual* (16) presents a well-constructed treatment criteria and treatment logic system in Volume 1, Chapter 8. Delivery of nonstandard treatments generally may not be from lack of evaluation, although inadequate evaluation by diving corpsmen and Medical Officers is considered a

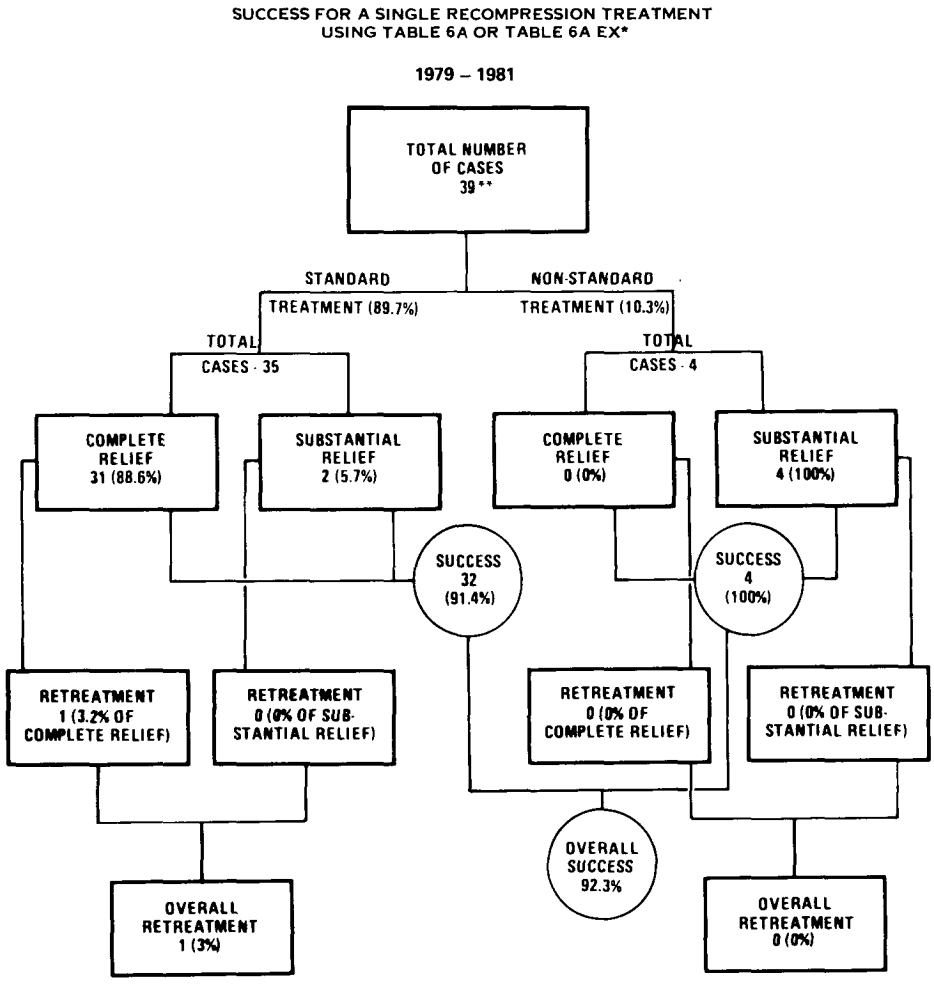



Fig. 5: All reported cases treated with TT–6A or 6A EX (*) from 1979 to 1981. Standard and nonstandard treatment criteria are the same as for Fig. 3. Treatment failures for standard treatments include 1 retreatment and 2 fatalities (**) yielding a 91.4% one-treatment success (32/35).

significant problem by some knowledgeable Diving Medical Officers. The criteria for this review required that adequate information was presented to allow judgment of the appropriateness of treatment. This fact means that some evaluation was performed, but generally in these selected nonstandard cases, treatment criteria provided in the *U.S. Navy Diving Manual* (16) were apparently either misunderstood or disregarded. Some of these nonstandard treatments probably represented real or perceived difficult cases that needed an alteration in the standard tables, or a well-rationalized decision of the individual delivering the treatment. But, for whatever reason these abbreviated treat-

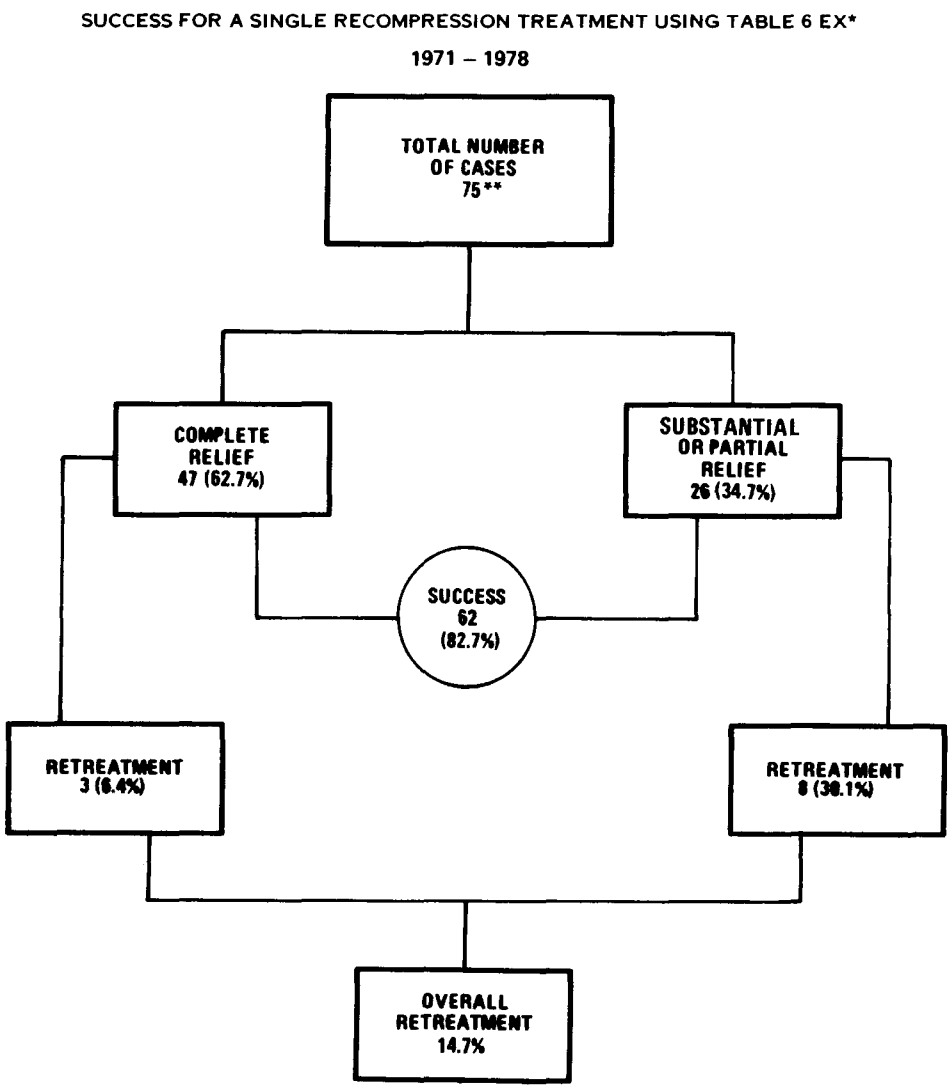



Fig. 6: All reported cases treated with TT–6 EX (*) from 1971 to 1978. Difficult treatment decisions preclude division into standard and nonstandard treatments. These cases include 2 fatalities (**).

ments were administered, the overall results indicate that their one-treatment success rates were significantly lower than for standard treatments. For the 1979–1981 period, there may be a better understanding of treatment rationale revealed in improvements in the results of nonstandard treatments, but improvement in treatment delays and usage of adjunctive therapy may have contributed to this improvement. However, there remain many cases that are

TABLE III

Success for Recompression Treatment Using *Air or Saturation Treatments* and all *Retreatments* 1971 to 1981

| Treatment Table Used | No. of Cases | No. of Complete Reliefs | No. of Retreatments | No. of Substantial Reliefs | No. of Retreatments |
|---|---|---|---|---|---|
| *Primary Treatments* | | | | | |
| 1,1A | 10 | 10 | 0 | 0 | 0 |
| 3 | 6 | 3 | 2 | 3 | 0 |
| 4,4 EX | 16* | 4 | 0 | 7 | 0 |
| Saturation† | 24 | 22 | 1 | 2 | 0 |
| Other | 5‡ | 3 | 0 | 1 | 0 |
| TOTAL§ | 61 | 42 | 3 | 13 | 0 |
| *Retreatments* | | | | | |
| 5,5A,5 EX | 4 | — | | 4 | |
| 4,4 EX | 4 | 2 | | 2 | |
| 6,6 EX | 29‖ | 17 | | 11 | |
| 6A | 1 | — | | 1 | |
| Saturation | 1 | 1 | | — | |
| Other | 2 | — | | 2 | |

Reported treatments using treatment tables other than the Minimal Recompression tables (5, 5A, 6, 6A, 6 EX, 6A EX) (EX means extended) for primary treatments and all retreatments regardless of the treatment used. Total cases for individual tables are too small to draw valid conclusions. *Includes 5 fatalities. †Cases occurred during saturation dives, treated with saturation procedures. ‡Includes 1 fatality. §Overall retreatment rate 4.9%. ‖Includes 1 fatality.

apparently undertreated due to misunderstandings of treatment logic or treatment endpoints.

From this evaluation, the term *substantial relief* apparently means different things to different people, ranging from only minor musculoskeletal soreness following a pain-only treatment to a significant neurological deficit in a serious case. This semantic vagary may provide a haven for treatment shortcuts or incomplete evaluations. Although Substantial Relief with an intention to administer follow-up recompression treatments may be an acceptable result for TT-6A EX and 6 EX, or even 6 and 6A (with extenuating circumstances), this should not be an acceptable logic for use of TT-5. It is not possible from this evaluation to separate a) the cases in which the treating authority selected abbreviated treatment schedules based on full knowledge of the compromise employed, from b) those who misunderstood or incompletely evaluated the situation. There were many cases where the term *substantial relief* was apparently used to describe "relief" as stated in the *Diving Manual*. In many cases the use of "substantial relief" was considered to be equivalent to the implied,

but not stated, "complete relief" criteria used in the *Diving Manual*. This semantic ambiguity was apparently the basis for misunderstanding the appropriate endpoint for treatment tables (and extensions). This is particularly significant in the substantially higher retreatment rate for TT-5 in those cases with reported "substantial relief."

This problem may be avoided if the term *substantial relief* is prohibited in report phraseology. Any result other than Complete Relief should be specified as Partial Relief, with specific deficits (i.e., residual soreness; two-point discrimination 2 inches; deficit cold sensation; parasthesia; 3+ strength in a specific muscle group, etc.) identified as a percentage of normal or a zero to 5+ grading scale. This change in reporting format may prompt more complete evaluations and reduce incomplete therapies, which should serve to improve the one-treatment results.

The incidence of nonstandard treatments was no different for the 1979–1981 period than for the 1971–1978 period, although there was improved success for the 1979–1981 period. It is evident from these results that publication of guidelines and criteria does not completely eliminate problems in treatment inadequacies. Some yearly update of treatment selection procedures for all responsible personnel may be considered via a self-administered course, test, or other means. People responsible for the medical evaluations may additionally have periodic updates in diagnostic procedures, i.e., differentiation of pain syndromes and neurologic exams and their interpretation.

Bayne (11) has demonstrated that prompt, aggressive treatment provides a 100% success rate. With delays in treatment and physiologic variability we may be willing to accept a 1 to 5% retreatment rate—but should we accept a 10% divergence from standards in treatment delivery with decreased success rates or increased retreatment rates for initially undertreated cases? Minimal recompression tables provide excellent results, with an overall success of 95.5% for one treatment when used according to published standards, and exhibit an improvement over most of the air tables, with a reported overall success of 88% (6). Removal of ambiguity in reporting results and periodic educational updates seem to be the next step in improving treatment results.

## SUMMARY

1) Use of the minimal recompression oxygen tables according to published criteria produces a 95.5% overall positive one-treatment success. This result provides an advantage over the standard air treatment tables in a better success rate and shorter treatment times.

2) The incidence of recompression therapy departing from the *U.S. Navy Diving Manual* (16) standards exceeds 10%, and these cases exhibit a substantially greater retreatment rate than cases treated on standard tables.

3) The descriptive term *substantial relief* is ambiguous, apparently promotes misinterpretation of the logical treatment scheme, and should not be used to describe treatment results.

4) There is a general retreatment rate of 1 to 3% even with properly treated cases (probably due to delays and physiological variability).

5) The retreatment rate for the cases resulting in Substantial Relief is significantly higher than those cases resulting in Complete Relief; this finding indicates that incomplete treatments may be disguised within this treatment outcome classification (particularly for TT-5).

6) Based on the data presented here, the only significant improvement in treatment results since the changes to the *U.S. Navy Diving Manual* in 1978 is an improvement in nonstandard (cases treated with less than recommended recompression schedules) treatment results. The incidence of nonstandard treatments has remained unchanged.

## Acknowledgments

Supported by Naval Medical Research and Development Command, under research Work Unit M009—PN.001–1157. The views presented in this paper are those of the author. No endorsement by the Department of the Navy has been given or should be inferred.

I wish to express my thanks to CDR T. E. Berghage for the stimulation he provided to accomplish this analysis. His assistance throughout the process has proven invaluable in the production of this Report.

## References

1. Bornmann RC. Experience with minimal recompression, oxygen breathing treatment of decompression sickness and air embolism. Washington, DC: U.S. Navy Experimental Diving Unit, 1967. (memorandum report, 10 Feb 1967)
2. Goodman MW. Decompression sickness treated with compression to 2–6 atmospheres absolute. Aerosp Med 1964;35:1204–1212.
3. Goodman MW, Workman RD. Minimal-recompression, oxygen-breathing approach to treatment of decompression sickness in divers and aviators. Washington, DC: Navy Experimental Diving Unit, 1965. (report no. 5–65, November 1965)
4. Broussolle Dr. Drugs for the treatment of decompression sickness. In: Mines and safety health commission congress on medical aspects of diving accidents, 1978:77–94.
5. Kindwall EP. Role of adjunctive drug and fluid therapy. In: Treatment of serious decompression sickness and arterial gas embolism. Bethesda: Undersea Medical Society, Inc., 1979. (UMS pub. no. 34 WS [SDS] 11–30–79)
6. Rivera JC. Decompression sickness among divers: An analysis of 935 cases. Washington, DC: Navy Experimental Diving Unit, 1963. (NEDU report no. 1–63, Feb 1963)
7. Doll RE, Berghage TE. Interrelationships of several parameters of decompression sickness. Washington, DC: Navy Experimental Diving Unit, 1967. (NEDU report no. 7–65)
8. Kelly JS, Berghage TE, Summitt JK. Review of diving accident reports 1967. Washington, DC: Navy Experimental Diving Unit, 1968. (NEDU report no. 10–68)
9. Summitt JK, Berghage TE. Review of diving accident reports 1968. Washington, DC: Navy Experimental Diving Unit, 1970. (NEDU report no. 11–70)
10. Summitt JK, Berghage TE. Review of diving accident reports 1969. Washington, DC: Navy Experimental Diving Unit, 1970. (NEDU report no. 12–70)
11. Bayne CG. Acute decompression sickness: A curable disease. JACEP 1978;7:351–354.
12. Davis JC, Sheffield PJ, Schuknecht L, et al. Altitude decompression sickness: Hyperbaric therapy results in 145 cases. Aviat Space Environ Med 1977;48:722–730.

13. Davis JC. Treatment of decompression accidents among sport scuba divers with delay between onset and compression. In: Treatment of serious decompression sickness and arterial gas embolism. Bethesda: Undersea Medical Society, Inc., 1979. (UMS pub. no. 34 WS [SDS] 11–30–79)
14. Pelosi G, Proietti R, Dellamorte F, et al. Decompression sickness: A medical emergency. Resuscitation (Netherlands) 1981;9:201–209.
15. Leitch DR. Treatment of air decompression illness in the Royal Navy. In: Treatment of serious decompression sickness and arterial gas embolism. Bethesda: Undersea Medical Society, Inc., 1979. (UMS pub. no. 34 WS [SDS] 11–30–79)
16. U.S. Navy. U.S. Navy Diving Manual. Vol. 1 (NAVSEA 0994–LP–001–9010), Change 2, 1978. Washington, DC: U.S. Navy Department.
17. Mendenhall W. Introduction to linear models and the design and analysis of experiments. Belmont, CA: Wadsworth Publ. Co., Inc., 1968.

# THE EFFECT OF PRESATURATION ON THE MAXIMUM SUBMARINE ESCAPE DEPTH OF GOATS AND THE IMPLICATIONS FOR HUMAN RESEARCH

*P. Y. Bell, D. W. Burgess, M. Summerfield, and E. J. Towse*

The technique of hooded escape has been proven both in sea tests to depths as great as 180 m (600 ft) (1) and in laboratory tests to a simulated depth of 191 m (625ft) (2).

Nevertheless, depths of this magnitude will only be achievable, in comparative safety, if the submariner is escaping from a compartment with an ambient pressure of 1 bar. The possibility of the compartment pressure being above this level by flooding or escape of high pressure gas has been of concern to those investigating the safety of hooded ascent for many years. It is possible, therefore, that the submariner could be exposed to pressure above normal for an unknown period of time before commencing his escape. To define the effect that this increased pressure could have on the depth of safe escape, we believed it necessary to investigate the "worst possible case" of a prolonged pre-exposure to pressure of sufficient magnitude to saturate fully the tissues of the subject with the inert gases in the compartment.

The nature of these experiments precluded the use of human volunteers and, consequently, demanded the use of a suitable experimental animal. For our investigation the goat was chosen as the experimental subject for three reasons:

1) The body of data concerning the performance of goats during decompression has been growing since they were first used in decompression sickness studies (3).

2) Goats have been used extensively within the Admiralty Marine Technology Establishment, Physiological Laboratory (AMTE[PL]) for many years (4–12) and continue to be used during initial stages of decompression table development.

3) A recent publication (13) has examined the accumulated data on six commonly used laboratory animals (goats, dogs, guinea pigs, rats, hamsters, and mice). The findings indicate that the goat is similar to man, both in its susceptibility to decompression sickness and in the signs presented.

Nevertheless, because of the blood characteristics and anatomy of goats and men, the rates of both the inert gas equilibration and the exchange of dissolved gas between the tissues and the blood are different. Previous data (8) indicate this equilibration or saturation time is 6 h for the goat, and this value has been used in preliminary studies. The present investigation is an extension of an original study, but utilizes a more realistic escape profile involving an exponential compression, instead of a linear compression, and an overnight presaturation to ensure full saturation of the tissues.

## METHODS

A total of 37 adult goats were used. To remove any effects of previous experiments on the subsequent results, we chose goats that had no previous exposure to pressure and used each goat only once.

Three goats were placed in the end-lock of the facility at approximately 1600 h and compressed in air to the required pre-exposure depth. They were left at this pressure overnight to ensure that the tissues of the animals were fully saturated with inert gas; the escape profile was carried out at 0900 h the following morning.

The escape profile consisted of a rapid exponential compression, a doubling of pressure every 4 s, a 4-s hold at the required depth, and a linear decompression at 2.75 m/s back to atmospheric pressure. This profile reproduces the pressure conditions during an ideal hooded ascent escape. A typical pressure profile is shown in Fig. 1.

It was our intention to define the point where 50% of the animals would experience decompression sickness. Thus, the pre-exposure depths and the escape depths were chosen in an attempt to "bracket" the critical depth, which would allow more accurate calculation of this variable. There were, at the commencement of these experiments, significant gaps in our knowledge of the effect on escape depth of certain pre-exposure pressures, i.e., 1.5 and 2 bars. These pressures were therefore investigated, and to relate these experiments to earlier work, we repeated the effect of an escape profile without pre-exposure to raised pressure.

The experimental protocol dictated that if there was no incidence of decompression sickness (the indicator chosen to be measured), the depth of the simulated escape would be increased by 25 m and the experiment repeated with three more goats. If decompression sickness was elicited, the next experiment depended on the number of animals exhibiting symptoms. If two or more goats experienced decompression sickness, the escape depth was decreased by 25 m, the experiment was repeated, and the 50% bends point was calculated. If only one animal experienced decompression sickness, the

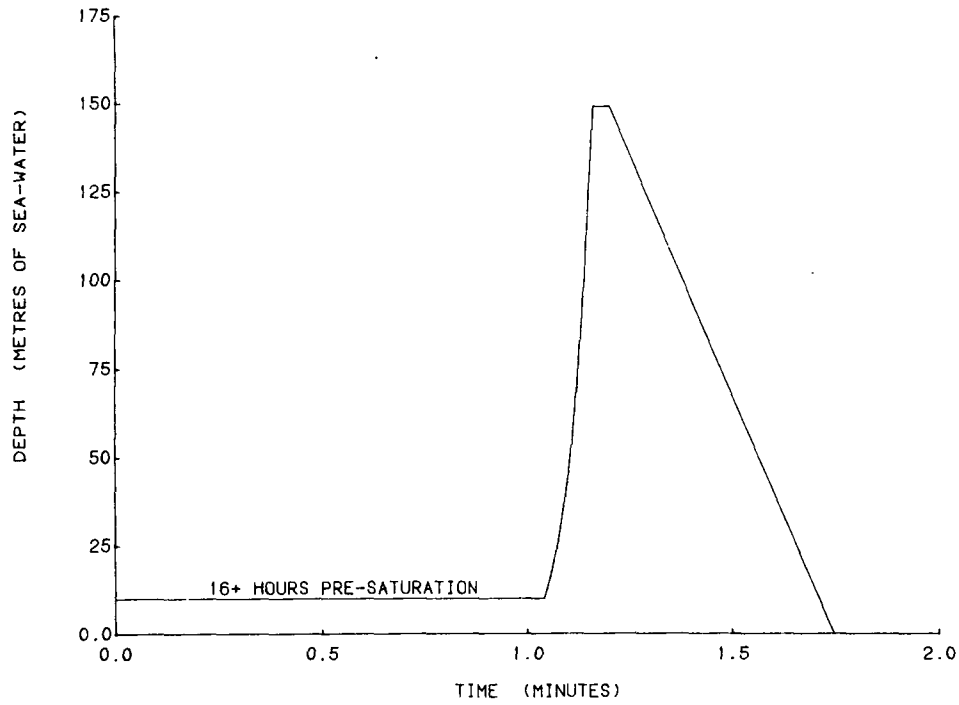



Fig. 1. Pressure profile followed during a 150-m submarine escape following 16+ h presaturation at 10 m.

experiment was repeated at the same depth and the 50% bends point was extrapolated from available data.

## RESULTS

Previous studies (14) had shown that without pre-exposure to pressure, goats have a 50% probability of experiencing decompression sickness from a depth of 287 m (942 ft). The results of the present study show remarkable agreement with this figure, predicting a 50% bends probability from a depth of 287.5 m. This finding indicates that the exponential compression is likely to produce similar results to the linear compressions used in earlier studies.

The goats that were pre-exposed to 1.5 and 2.0 bars showed a 50% bends probability from depths of 275 m and 212.5 m, respectively. These data points are shown in Fig. 2 together with previously unpublished data. The previous experimenters had not adopted the approach of our investigation: in only subjecting each goat to one experiment and before continuing with their experimental series they had attempted to find the 50% bends point for individual animals decompressed to the surface from a 6-h pre-exposure. To

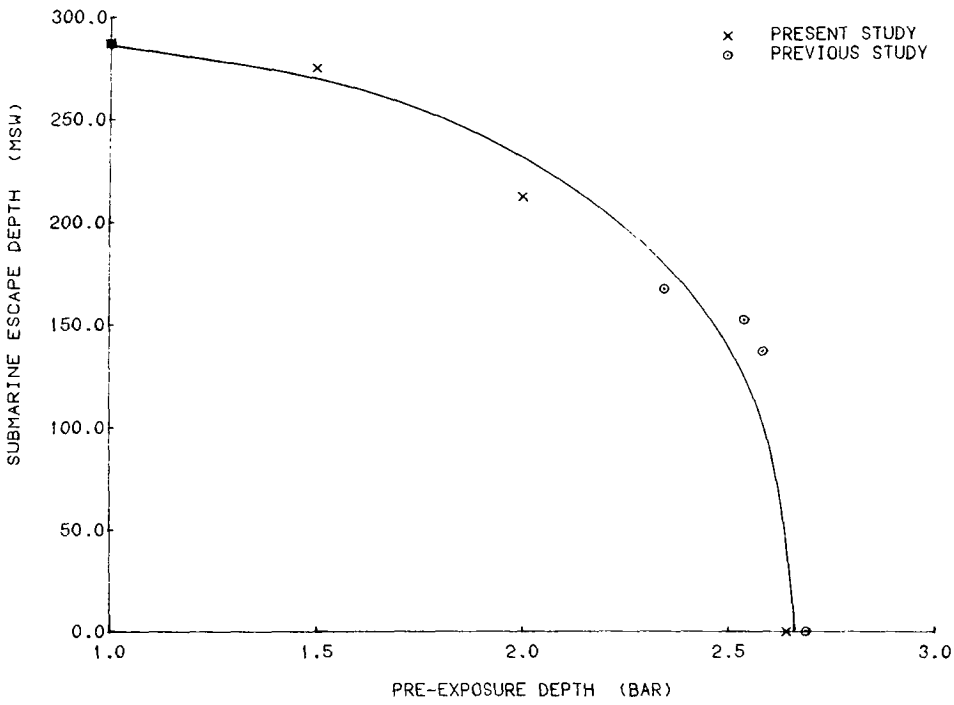



Fig. 2. Graph showing the 50% bends point of goats exposed to simulated submarine escape profile following prolonged presaturation. Curve is eye-fitted to the data points. Each point is the 50% bends point of at least 6 animals.

make the results compatible with our study, we re-examined the original results, and the pressure at which the first sign of decompression sickness appeared in each individual animal was used to calculate the 50% bends point of the group. The value obtained of 16.4 m is very similar to the value of 16.87 m obtained from the goat population curve produced by a decompression study carried out at AMTE (PL) in 1962 (8). Both points are plotted on Fig. 2.

As the experimental series continued, it became apparent that the character of the decompression sickness being provoked was changing from predominantly Type I limb "bends" following deeper presaturation, shallower submarine escape, to the more serious Type II central nervous system (CNS) bends following shallower presaturation, deeper submarine escape. This change can be seen in Fig. 3, where the percentage of CNS bends in the total bends recorded is plotted.

The extrapolation of the goat results to that of men is made more difficult by the lack of available data on which to make any prediction. The decompression performance of man is based on studies which, in the main, attempt to define the 0% bends point not the 50% point; therefore, we will

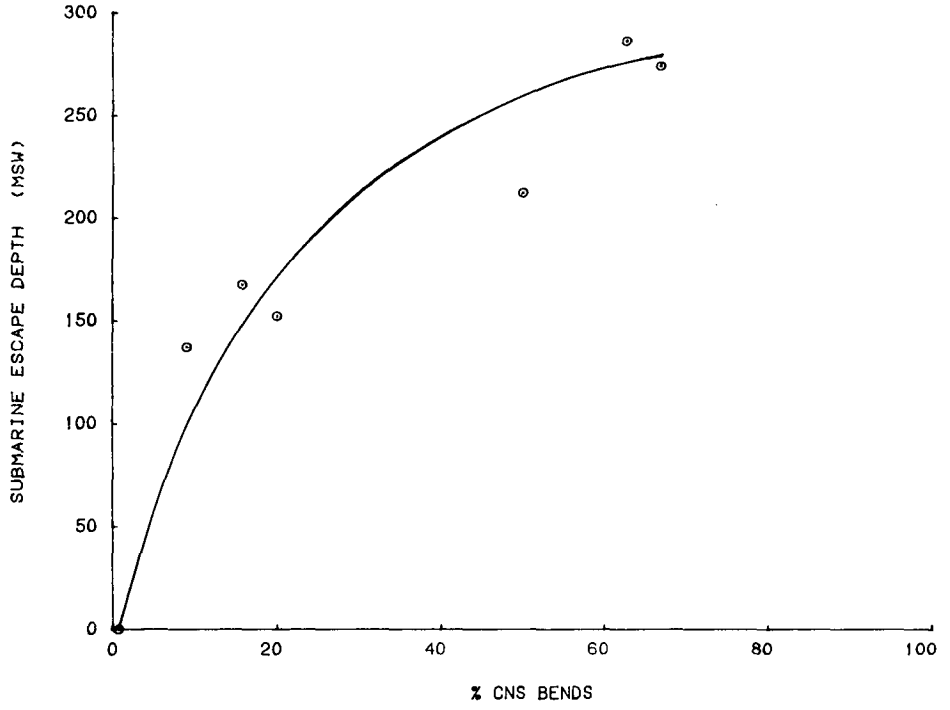



Fig. 3. Percentage of CNS bends in the total bends following submarine escape from prolonged presaturation.

attempt to predict the depths that will provoke 0–5 % bends. The maximum tested depth for a simulated hooded ascent without pre-exposure to increased pressure is 191 m (625 ft) (2). These investigators recorded no incidence of decompression sickness, only mild cases of itching (pruritus) which, on subsequent analysis, showed no relation to the escape depth. Their results indicate that, based on the nonoccurrence of decompression sickness, the maximum depth has not yet been reached. Therefore, the inferred maximum safe depth for hooded ascent is thought to be deeper at 200 m (656 ft). This point is indicated on Fig. 4.

The other end of the curve is equally deficient in data concerning the maximum safe decompression to the surface from an exposure of sufficient duration to fully saturate the tissues. This is principally because the shallow depth involved is unlikely to be used as a saturation storage depth; thus, the research has been directed to staged decompression from deeper depths. However, early experimenters attempted to define this point and predicted a value of 10 m (2). Subsequent analysis of their data has revealed certain experimental conditions that may have influenced their results. Firstly, they used the same subjects repeatedly, thus introducing the phenomenon of "habituation"; secondly, the pre-exposure time to saturate was 9 h instead of the

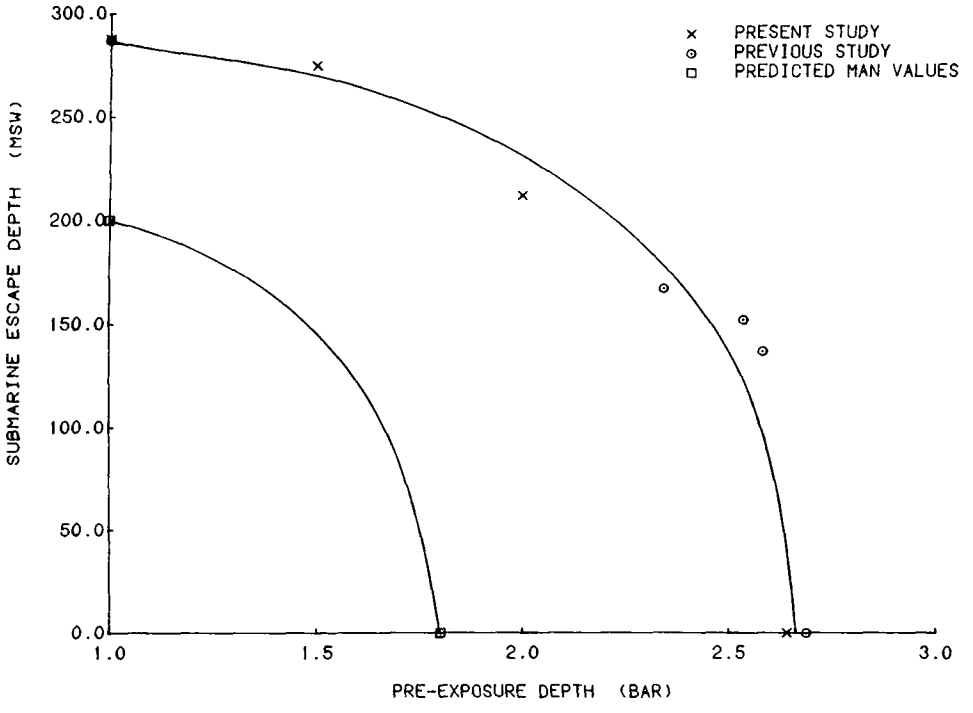



Fig. 4. Predicted 0% bends curve for men based on the 50% bends curve of goats. Both are shown for comparison.

now accepted minimum value of 12 h. The result of both of these conditions is to increase artificially the "max-pull" value, which would indicate that the figure of 10 m may be an overestimate. More recently, the French GALATHEE experimenters (15) carried out an investigation using Doppler equipment; they found that decompression from a depth of 6 m produced no Doppler-detected bubbles and no signs of decompression sickness. The use of the appearance or nonappearance of bubbles as criteria gives a lower value than would be expected from a study using decompression sickness as the endpoint. It would therefore appear that these two studies have given the upper and lower bounds of the area of interest, and any choice of particular value is merely an estimate. The value estimated for the purposes of Fig. 4 is 8 m (1.8 bars).

[Other works pertinent to the overall subject are also referenced (16, 17).]

## DISCUSSION

We have made several assumptions to relate the results obtained using goats to those expected to occur with men.

1) The shape of the curve obtained using the 50% bends point for goats is similar to that which would be expected to occur in men.

2) The shape of the 0% bends curve is similar to the shape of the 50% bends curve for both men and goats.

3) The progression of signs of Type I limb bends that increase in severity to Type II CNS bends observed in goats is also present in men.

The initial assumption of similarity of curve between the two species is believed justifiable based on the knowledge of the performance of goats and men in a wide variety of circumstances. The second assumption that the 0% bends curve is the same shape as the 50% curve is a reasonable one, although the experiments indicate that the difference between the 0% bends point and the 50% bends point may vary with the depth of the hooded ascent and is narrower at deeper depths. The nature of the end point used in the goat study of 50% bends to some extent dictates that the above assumption be made as the number of experiments to define the lower limit of the occurrence of decompression sickness in goats would be very large.

The third assumption that the progression of symptoms from Type I to Type II in goats is reflected in humans is an important one because a small incidence of Type I limb bends could be considered, in an emergency situation, to be acceptable; however, even a small incidence of potentially fatal Type II CNS bends would be looked upon as serious. The mass of data relating the progression of the signs of decompression sickness in the goat to that of men would suggest that they are substantially similar.

The observation recorded in Fig. 3 of the change in character of the decompression sickness recorded at deeper depths is indicative of a change in the "critical" tissue from the slower tissues which give rise to Type I limb bends to the more rapid tissues in the CNS. We believe this change indicates that there is a narrowing of the difference between the depths that provoke a Type I bend and those that provoke a Type II bend, and it emphasises the care with which future experiments involving human volunteers must be designed to avoid such serious incidents.

Indeed, the results of this investigation would indicate that any investigation of the effect of a submarine escape on a human would be safer if commenced from an existing presaturation because, although the depth capability is reduced, the decompression sickness provoked is likely to be less serious than that which occurs during submarine escape to a simulated depth near to the maximum safe value from ambient pressure.

## Acknowledgments

The authors gratefully acknowledge the permission of Dr. H. V. Hempleman and Mr. W. J. Eaton to use their unpublished results.

The skill, judgment, and dedication of the chamber house staff in carrying out the experiments has proved, once again, to be invaluable.

## References


1. Donald KW. A review of submarine escape trials from 1945 to 1970 with particular emphasis on decompression sickness. Alverstoke, England: Royal Naval Physiological Laboratory, 1970. (Underwater Physiology Subcommittee report no. 290/70)
2. Barnard EEP, Eaton WJ, Snow RE. Experiments in submarine escape. Rapid compression of men to 625 feet (191 metres). Alverstoke, England:Royal Naval Physiological Laboratory, 1971. (RNPL report no. 10/71)
3. Boycott AE, Damant GCC, Haldane JS. The prevention of compressed-air illness. J. Hyg Camb 1908;8:342–443.
4. Duggan E. Taylor HJ. The risk of bends or vascular embolism in goats during simulated submarine escapes from 300 ft after exposure to pressure for 1 ½ min and longer. Alverstoke, England: Royal Naval Physiological Laboratory, 1948. (RNPL report no. 1/48)
5. Eaton W. Survey of one hundred and ten cases of compressed air illness in goats which required therapeutic recompression. Alverstoke, England: Royal Naval Physiological Laboratory, 1959. (RNPL report no. 1/59)
6. Eaton WJ. Experiments in submarine escape: I. Goat experiments on fast decompression from pressures equivalent to 300–500 ft of sea water. II. Extension of the maximum safe depth of escape for goats by faster rates of ascent. Alverstoke, England: Royal Naval Physiological Laboratory, 1967. (RNPL report no. 1/67)
7. Eaton WJ. Experiments in submarine escape: Brief exposures of goats to air at high pressures equivalent to 550-800 ft of sea water. Alverstoke, England: Royal Naval Physiological Laboratory, 1967. (RNPL report no. 4/67)
8. Eaton WJ, Hempleman HV. The incidence of bends in goats after direct surfacing from raised pressures of air. Alverstoke, England: Royal Naval Physiological Laboratory, 1962. (RNPL report no. 5/62)
9. Hempleman HV. Decompression ratios for goats after long exposures to various air pressures. Alverstoke, England: Royal Naval Physiological Laboratory, 1962. (RNPL report no. 6/62)
10. Sutton B, Davidson WM, Taylor HJ. Decompression ratio for goats following long exposure and return to atmospheric pressure without stoppages. Alverstoke, England: Royal Naval Physiological Laboratory, 1950. (RNPL report no. 1/50)
11. Taylor HJ. Simulated unaided ascents of goats in water from depths of a) 220 ft, b) 300 ft. Alverstoke, England: Royal Naval Physiological Laboratory, 1947. (RNPL report no. 1/47)
12. Taylor HJ. Simulated unaided ascents of anaesthetised animals in water from depths up to 300 ft. Alverstoke, England: Royal Naval Physiological Laboratory, 1948. (RNPL report no. 2/48)
13. Berghage TE, David TD, Dyson CV. Species differences in decompression. Undersea Biomed Res 1979;6:1–13.
14. Anon. Minutes of the 9th meeting of the underwater physiology subcommittee. Alverstoke, England: Royal Naval Physiological Laboratory, 1973. (RNPRC UPS report no. 7/73)
15. LeFur M, Gardette B, Masurel G, Ohresser P. Detection Doppler de boules apres plongees a l'air avec remontee sans palier. In: Elliott DH, ed. Proceedings of the European Undersea Biomedical Society, Seventh Annual Congress, 1981:218–232.
16. Eaton WJ. Depth limitations of the buoyant (hooded) ascent submarine escape procedure. Alverstoke, England: Royal Naval Physiological Laboratory, 1971. (RNPL report. no. 9/71)
17. Hempleman HV. Investigation into decompression tables. Further basic facts on decompression sickness. Alverstoke, England: Royal Naval Physiological Laboratory, 1957. (RNPL report no. 8/57)

# RECENT EXPERIMENTS USING ULTRASONIC IMAGING TO MONITOR BUBBLE FORMATION IN DIVERS

*S. Daniels, J. M. Davies, W. D. M. Paton, and E. B. Smith*

Ultrasound can be used in one of two ways for the study of bubble formation after decompression. It can be employed in fundamental studies on the factors controlling bubble formation, or it can be used to monitor bubble formation during a decompression with the aim of using the information about the extent of bubble formation to predict the likelihood of symptoms. This paper is concerned with the second of these approaches.

The most widely investigated means of studying bubble formation during a decompression has been the Doppler method (1–5). This method has normally been employed to monitor bubble formation in the total venous return, the so-called "Pre-Cordial" method (6). Despite the undoubted success of this technique in revealing bubble formation after a wide variety of decompressions that are conventionally accepted as safe, a number of recent studies have cast doubt on the link between the number of bubbles in the pulmonary artery and the likelihood of decompression sickness (5,7–9). These studies, involving a total of 624 man decompressions from widely differing exposure pressures and durations and gas mixtures, show that in approximately 10% of the cases significant numbers of bubbles are found without symptoms appearing, but that in up to 77% of the cases severe bubble scores, normally taken to indicate the probability of decompression sickness, were in fact asymptomatic.

Our pulse-echo ultrasound imaging studies of bubble formation after decompression in small animals led us to believe that before symptoms of decompression sickness appear an accumulation of stationary bubbles occurs (10–11). This type of bubble formation would not, of course, be detected by the Doppler techniques. We have developed, therefore, a time-integrated version of pulse-echo ultrasound imaging, which presents a simple output

related to the extent of bubble formation whether it is moving or stationary (12). Preliminary results with small animal and human experiments proved encouraging, with positive prediction of decompression sickness possible with the animal experiments (11). The preliminary human trials were inconclusive, although no case of symptoms in the absence of bubble formation occurred. This finding has led to a more extensive human trial involving some 56 man decompressions.

## EXPERIMENTAL METHOD

The ultrasound system uses a 64-element co-phased linear transducer array, which, with the appropriate electronics, produces 20 images per second. The echoes comprising each image are electronically counted by the integrating unit. A fixed subtraction is applied to each count, which is set to correspond to the number of tissue interfaces in the area scanned by the ultrasound. Based on previous experience, two signal-processing facilities were incorporated into the integrating unit. The first allows an average count of over 1–20 images to be output. The second allows a running average of over 1–8 outputs to be selected. In practice it has been found that a combination of averaging the count over 2 images and outputing a running average over the maximum 8 totals gives the greatest reduction in the output variation as a result of movement. The final output is available as a digital reading and as a trace on a chart recorder.

## MONITORING CONVENTIONAL DECOMPRESSIONS

A total of 35 decompressions have been monitored, 16 following the Royal Naval Table 11 (BR2806) and 19 following the U.S. Navy Standard Air Dive Table. These decompressions have ranged from 15-min exposures at 30 msw to 180-min exposures at 30.5 msw. The results of monitoring these decompressions are given in Tables I and II.

A portion of the integrator output from one of the dives to 30 msw for 60 min using the RN Table 11 decompression schedule is shown in Fig.1. This output was recorded from the back of the right knee. The decompression involved four stages, with stops at 9, 6, and 3 msw. The portion of trace shown in Fig.1 was recorded during the reduction in pressure from 6 to 3 msw. A small transient increase in the output can be seen, which lasted 7 min and was associated with the appearance in the images of discrete echoes in the region of the popliteal vein. No other changes in the integrator output were seen during the 45 min of decompression. Overall, 10 of the decompressions using RN Table 11 gave rise to bubbles detected by the pulse-echo ultrasound apparatus. In two cases the subjects complained of minor skin itching. In no case was the rise in the integrator output large; the output shown in Fig.1 was typical. Although a high proportion of these decompressions (62%), which are

TABLE I

Analysis of Bubble Formation Detected by Integrating Pulse-Echo Ultrasound Imaging following Air Bounce Dives Using RN Table 11 (BR2806)

| No. Men | Depth & Duration (msw) (min) | No. with Bubbles | No. with DCS | No. Requiring Therapy |
|---|---|---|---|---|
| 1 | 30 15 | 0 | 0 | 0 |
| 10 | 30 20 | 6 | 1 | 0 |
| 4 | 30 60 | 3 | 1 | 0 |
| 1 | 50 60 | 1 | 0 | 0 |
| 16 | | 10 | 2 | 0 |

considered among the safest in use, showed bubble formation, the lack of overt symptoms of decompression sickness prevented an assessment of the relationship between bubble formation and symptoms.

The decompressions following the USN Standard Air Dive Table were chosen to include both wet dives and repeat dives, which were thought likely to provoke more extensive bubble formation. For the wet dives the subjects swam using normal scuba equipment in the wet pot attached to the main pressure chamber. Immediately before their decompression the subjects entered the dry chamber and attached the ultrasound transducer; they were then monitored as usual. An example of a portion of the integrator output after one of these wet dives is shown in Fig.2. A small rise in the output was recorded 40 min after the start of the decompression. No symptoms were reported after this dive. Only one of these decompressions required a therapy—after decompression from a 180-min exposure to 30.5 msw. After this decompression

TABLE II

Analysis of Bubble Formation Detected by Integrating Pulse-Echo Ultrasound Imaging following Air Bounce Dives Using the USN Standard Air Dive Table

| No. men | Depth & Duration (msw) (min) | No. with Bubbles | No. with DCS | No. Requiring Therapy |
|---|---|---|---|---|
| 6 | 30 25 | 3 | 0 | 0 |
| 3 | 30.5 25 | 3 | 1 | 0 |
| 2 | 30 25+10 | 1 | 0 | 0 |
| 6 | 30.5 25(wet) | 6 | 2 | 0 |
| 2 | 30.5 180 | 1 | 1 | 1 |
| 19 | | 14 | 4 | 1 |

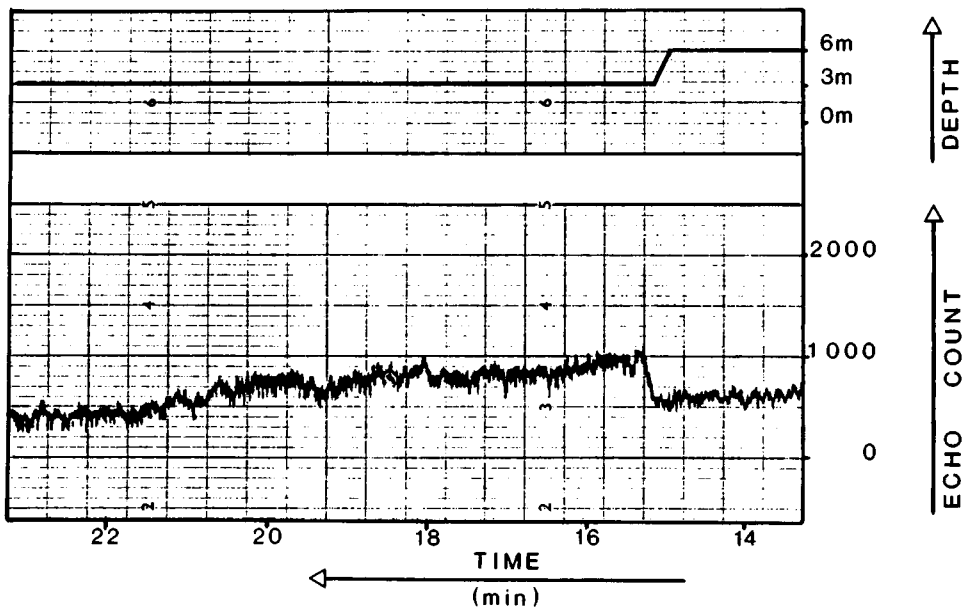



Fig. 1. Integrator output recorded from the back of the right knee of a man after decompression from a 60-min exposure to 30 msw. The decompression lasted a total of 45 min and required stages at 9, 6, and 3 msw. The portion of trace shown was recorded as the pressure was reduced from 6 to 3 msw. Time is shown increasing from *right to left* from 14 min after the start of the decompression.

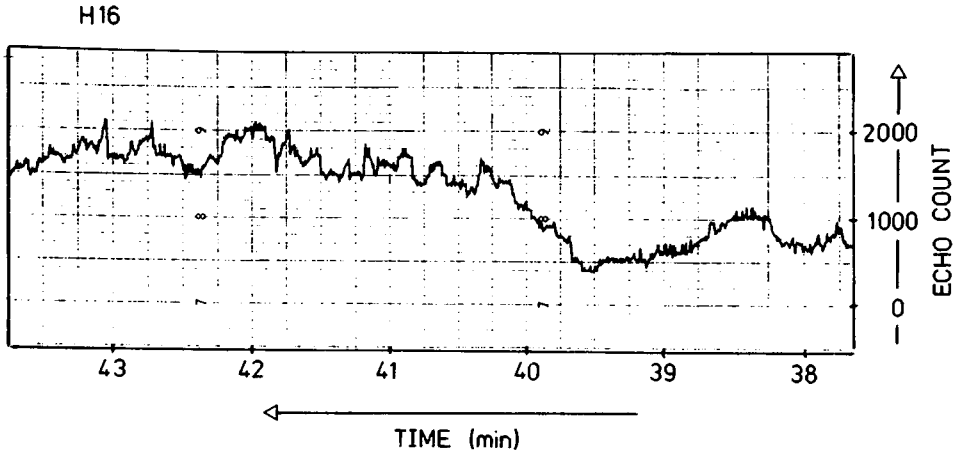



Fig. 2. Integrator output from the right thigh of a man after decompression from a wet dive of 25-min duration to 30.5 msw. Time is shown increasing from *right to left* beginning 38 min after the decompression.

a rise in the integrator output began after 32 min and the output remained elevated for 33 min. The output had not returned to normal by the end of the monitoring session. After surfacing, the subject reported that he had experienced itching in his forearms, which had coincided with the rise in the integrator output. The following morning the subject reported stiffness in his middle finger; he was recompressed to 18 msw for 25 min and breathed oxygen intermittently. During this therapy we monitored the subject using the ultrasound apparatus and found that the integrator output fell during the stay at 18 msw and did not rise again after the final decompression. The therapy did not relieve the stiffness.

As expected, a higher proportion of these decompressions (75%) showed bubble formation than did the RN Table 11 decompressions. However, the inclusion of the repeat and wet dives prevents any comparison of the efficacy of these tables. Again, it should be noted that bubble formation was routinely observed after decompressions in world-wide use and symptoms were only reported by subjects in whom bubble formation had been detected. As regards the appearance of symptoms, the only conclusion that can be drawn from these two sets of experiments is that a prolonged elevation in the integrator output may be followed by symptoms requiring treatment.

## EXPERIMENTAL DECOMPRESSIONS

The next series of experiments involved monitoring a number of experimental decompressions. These experiments were expected to provide a greater opportunity to relate symptoms and bubble formation. The results from seven experiments are summarized in Table III.

A wide variety of decompressions have been monitored, ranging from decompression after a saturation exposure to 540 msw heliox to decompression after a 15-min bounce dive to 80 msw. The most dramatic increase in the integrator output was seen after decompression to 10 msw from a 24-h exposure at 23 msw nitrox ($Po_2$ 0.5 bar) (*see* Fig.3). The rapid two-stage increase in the output occurred 30 min after the decompression. The elevated level was maintained for 100 min, with little reduction. Approximately 8 h later, the subject reported pain in his right knee, which was not being monitored; the accumulation of stationary bubble observed in his left thigh did not give rise to symptoms. Recompression relieved the symptoms.

Monitoring bubble formation during the long decompressions following deep saturation dives introduces additional problems. Clearly, the entire decompression cannot be monitored. However, it has been established experimentally that with sufficient training in the use of the equipment and by establishing a regular monitoring routine subjects can position the transducer reproducibly for daily scanning sessions. A portion of the integrator trace recorded from one of the subjects during decompression from 540 msw heliox is shown in Fig.4. This trace was recorded at 92 msw and shows a transient increase in the output. No symptoms were reported at this time. Later in the

TABLE III

Analysis of Bubble Formation Detected by Integrating Pulse-Echo Ultrasound Imaging following Experimental Decompressions

| No. men | Depth & Duration (msw) | | Gas Mixture | No. with Bubbles | No. with DCS | No. Requiring Therapy |
|---|---|---|---|---|---|---|
| 1 | 80 | 15 min | Trimix 20/40/40% $0_2/N_2/He$ | 1 | 0 | 0 |
| 2 | 540 | 6 days | Heliox $Po_2 = 0.4$ bar | 2 | 1 | 1 |
| 2 | 61 | 6 days | Nitrox $Po_2 = 0.4$ bar | 2 | 0 | 0 |
| 2 | 23 | 24 h | Nitrox $Po_2 = 0.5$ bar | 2 | 2 | 2 |
| 7 | | | | 7 | 3 | 3 |

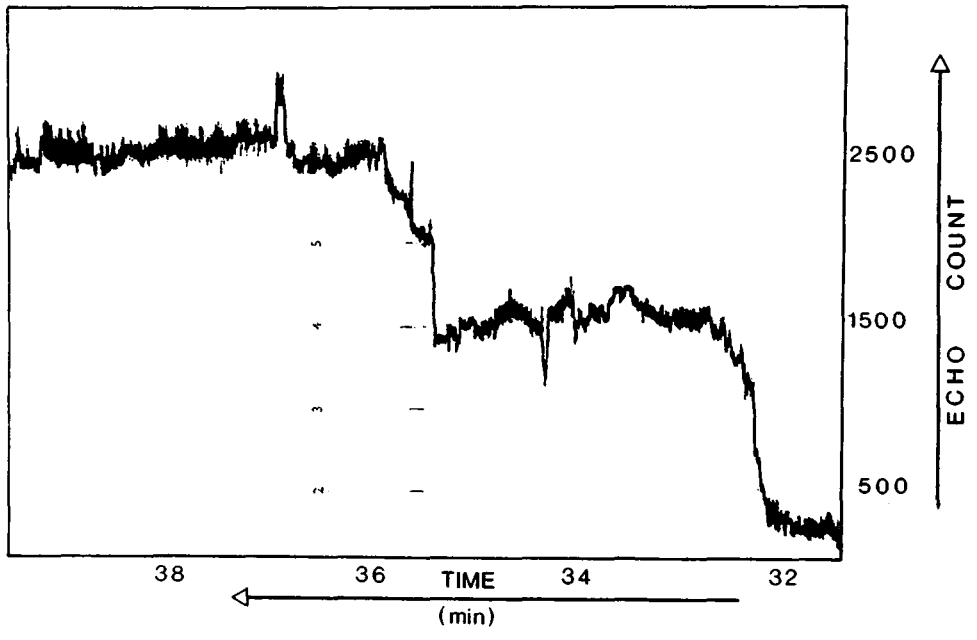



Fig. 3. Integrator output from the left thigh of a man after decompression to 10 msw following a 24-h saturation exposure to 23 msw nitrox ($Po_2 = 0.5$ bar). This portion of the trace begins 30 min after the decompression.

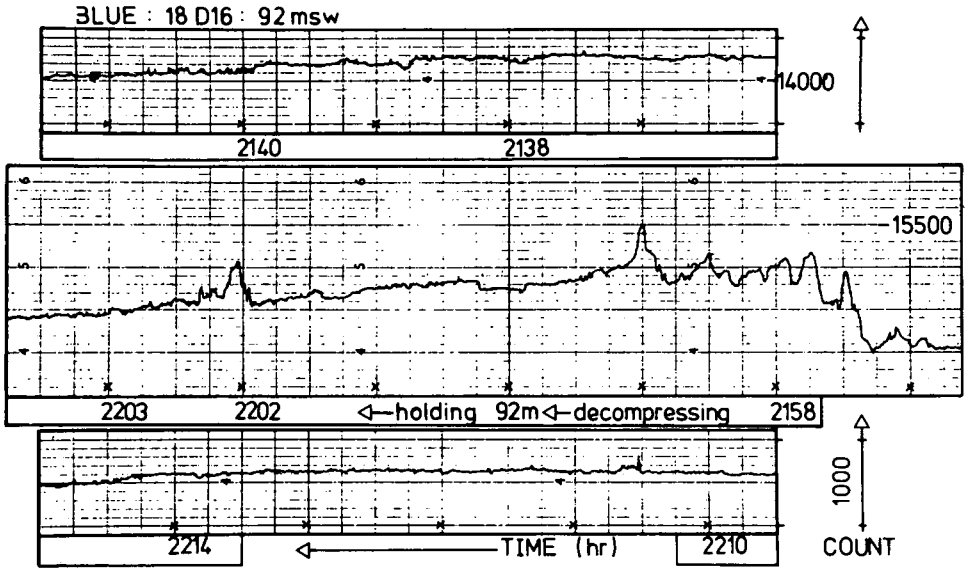



Fig. 4. Integrator output from the left thigh of *Subject A* during decompression from a saturation exposure to 540 msw heliox ($Po_2 = 0.4$ bar). This portion of the trace was recorded at 92 msw.

decompression, at 10.7 msw, the subject reported difficulties with his vision, which lasted 4 min and left him with a severe headache. Recompression to 25 msw was begun and during this recompression his partner was monitored with the ultrasound apparatus (Fig.5). A fall in the level of the output was seen during the recompression. The final decompression to surface produced further rises in the integraor output, but no further symptoms were reported. Postdive, the clinical assessment of the decompression incident was that the subject had suffered an arterial embolism. However, before the incident it had been noted that both subjects showed a substantial accumulation of stationary bubbles.

## DISCUSSION

Applying the technique of integrating pulse-echo imaging, we have shown that bubble formation occurs routinely after decompressions that are widely accepted as safe on the basis of the incidence of symptoms. The occurrence of symptoms appears to be related to the accumulation of stationary bubbles, and the rapidity with which such bubbles accumulate would seem to indicate the rapidity with which symptoms appear. This is in agreement with the more extensive tests performed with small animals. The most dramatic accumulations of stationary bubbles were seen after nitrox (or air) exposures, and the relationship between the extent of bubble formation and the appearance of

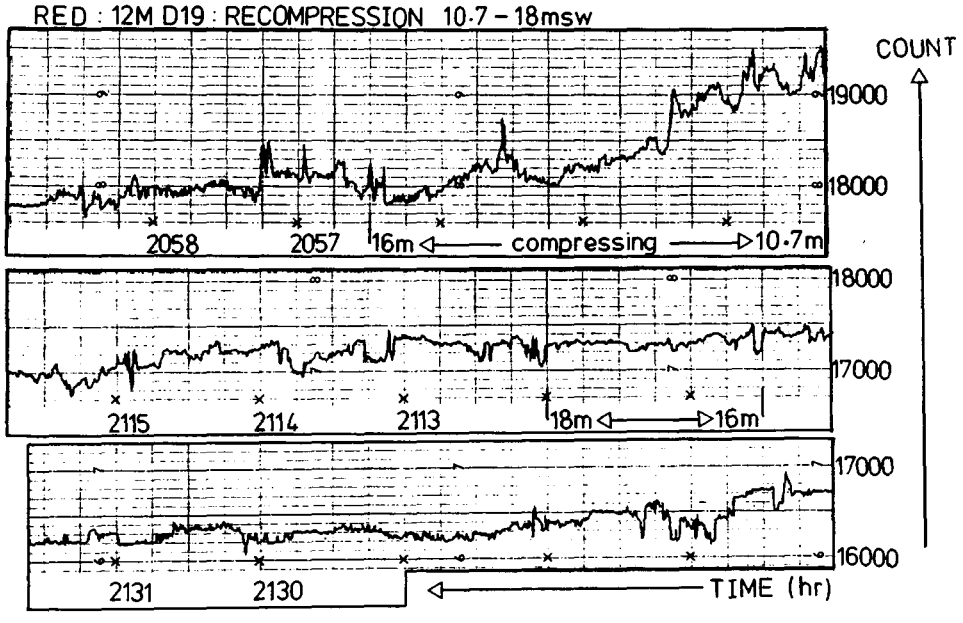



Fig. 5. Integrator output from the right thigh of *Subject B* recorded during the decompression from saturation at 540 msw heliox, beginning at 10.7 msw and showing the effect of recompression to 25 msw. The recompression had been required to alleviate symptoms of decompression sickness experienced by *Subject A*.

symptoms seems at present to depend on the breathing gas used. A greater number of bubbles without the appearance of symptoms seems to be possible when air or nitrox is the breathing gas than is the case when breathing heliox. A further point emerging from these experiments was that when at depth the acceptable extent of bubble formation was less than when at, or close to, the surface.

It has proved possible to monitor bubble formation in man and to relate the extent of the bubble formation to the likelihood of decompression sickness. This method may provide a more satisfactory basis for the assessment of the safety of decompression tables than the subjective reporting of symptoms currently in use.

## Acknowledgments

We thank the Admiralty Marine Technology Establishment Physiological Laboratory for their help in conducting these trials and the members of staff who volunteered as subjects. Research support was provided by the MRC. JMD was supported by the SRC via a Case award in co-operation with AMTE/PL.

## References

1. Gillis MF, Peterson PL, Karagianes MT. In vivo detection of circulating gas emboli associated with decompression sickness using the Doppler flowmeter. Nature 1968;217:965–967.

2. Spencer MP, Campbell SD, Sealey JL, Henry FC, Lindbergh J. Experiments on decompression bubbles in the circulation using ultrasonic and electromagnetic flowmeters. J Occup Med 1969;11:238–244.
3. Smith KH, Spencer MP. Doppler indices of decompression sickness: their evaluation and use. Aerospace Med 1970;41:1396–1400.
4. Powell MR. Leg pain and gas bubbles in the rat following decompression from pressure: monitoring by ultrasound. Aerospace Med 1972;43:168–172.
5. Nashimoto I, Gotoh Y. Ultrasonic Doppler detection of blood bubbles in caisson work. In: Pearson R, ed. Early diagnosis of decompression sickness. Twelfth Workshop, Undersea Medical Society, Inc., Bethesda. 1977:171–183.
6. Spencer MP, Clarke HF. (1972) Precordial monitoring of pulmonary gas embolism and decompression bubbles. Aerospace Med 1972;43:762–767.
7. Bayne CG, Hunt WS, Bray PG, Johanson DC. A prospective clinical trial in human decompression sickness. Undersea Biomed Res 1979;6(Suppl 1):17 (Abstr.)
8. Gardette B. Correlation between decompression sickness and circulating bubbles in 232 divers. Undersea Biomed Res 1979;6:99–107.
9. Nishi RY, Kisman KE, Eatock BC, Buckingham IP, Masurel G. Assessment of decompression profiles and divers by Doppler ultrasonic monitoring. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:717–727.
10. Daniels S, Davies JM, Paton WDM, Smith EB. The detection of gas bubbles in guinea-pigs after decompression from air saturation dives, using ultrasonic imaging. J Physiol 1980;308:369–385.
11. Daniels S, Davies JM, Eastaugh KC, Paton WDM, Smith EB. Monitoring bubble formation with an integrating pulse-echo ultrasonic method. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:729–740.
12. Daniels S. An integrating pulse-echo ultrasonic bubble detector for use during decompression procedures. Patent Appn. No. 40103/73 (1978) superceded by No. 7934423 (1979).

# EXPERIMENTAL ATTEMPTS TO INFLUENCE THE BUBBLE THRESHOLD FROM SATURATION DIVES IN ANIMALS

*Y. C. Lin, G. W. Mack, D. K. Watanabe, and K. K. Shida*

Decompression sickness follows excessive pressure reduction ($\Delta P$) from a prolonged stay at depth and is a result of bubble formation in vivo. Though venous bubbles often can be detected without any indication of decompression sickness during so-called "safe" decompression procedures, the detection of subsymptomatic bubbles remains the meaningful and only objective criterion indicating an in vivo condition of supersaturation. The threshold for bubble detection, the largest $\Delta P$ from a given saturation pressure that just produces detectable venous bubbles, has been shown to be free of symptoms indicating decompression sickness (1). More importantly, the threshold for bubble detection has been shown to be the same in rat, cat, and dog (2). This species-independent phenomenon is useful for obtaining the threshold data from an animal of experimental convenience and is applicable to another species, an obvious economical advantage. Whether this threshold can be experimentally altered remains to be tested and is the objective of this study.

In this study, we examined the effect of over-pressure spike prior to decompression and the effect of ambient temperatures on the bubble threshold. The rationales for these studies are based on a) current belief that pre-existing gas nuclei grow, upon inappropriate decompression, to form bubbles in the blood and tissues, and that crushing of these nuclei with brief over-pressure spike prior to decompression reduces the number of bubbles formed during the subsequent decompression (3); b) the process of inert gas elimination can be altered by changes in cardiopulmonary functions and blood flow distribution (4,5). These physiological alterations can be produced appropriately by changes in ambient temperatures, as well as by application of drugs, in the rat

(6,7). Effect of changes in ambient temperatures on bubble threshold is examined in this study.

## METHOD

Male Wistar rats were anesthetized with sodium pentobarbital (40 mg/kg) and surgically prepared by implanting a 3-mm diameter perivascular Doppler flow probe (Parks Electronics, Beaverton, OR) on the posterior vena cava caudad to the renal veins (1). This chronic preparation was chosen over an external probe to eliminate the necessity of anesthetizing the animal during compression and subsequent decompression. The wire leads from the probe were run subcutaneously to the top of the head between the two ears (Fig. 1). The flowmeter probe with frequencies of 8.0–10.0 MHz was tested in vitro prior to implantation for its bubble-detecting ability by introducing bubbles into water flowing through a polyethylene tubing. The ability to detect bubbles in vivo was again confirmed following surgical preparation by injecting small

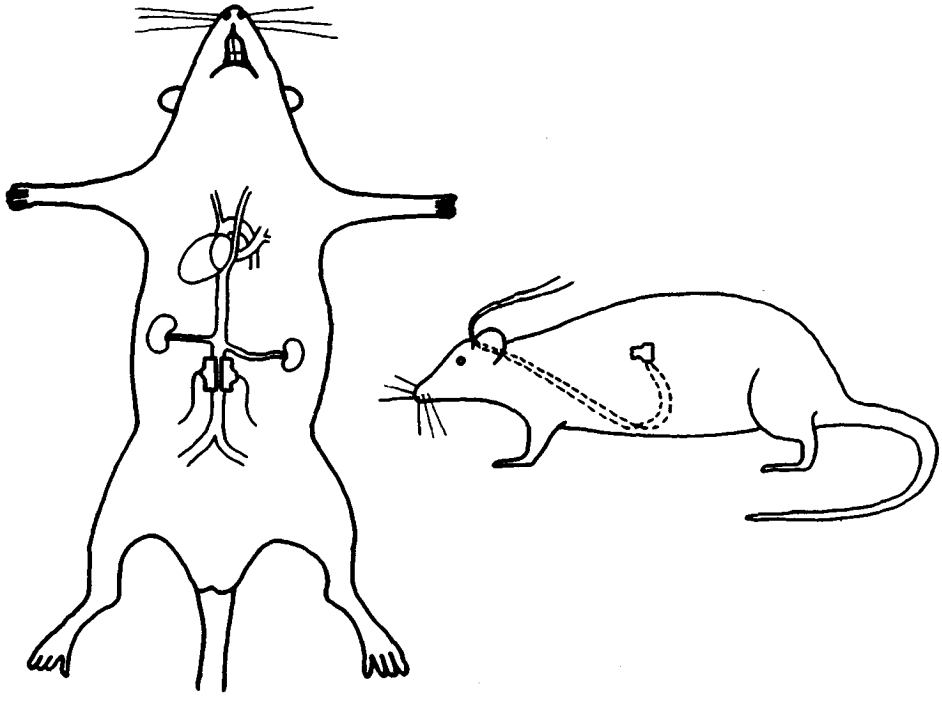


Fig. 1. Surgical preparation of a chronic preparation for detecting venous bubbles during decompression. The Doppler flow probe was implanted on the posterior vena cava caudad to the renal veins. The wire leads from the probe were run subcutaneously to the top of the head between the two ears.

volumes of air through a 25-G needle into the femoral vein. Rats were allowed to recover for at least 24 h before experimentation.

Intravascular bubbles were detected by a Doppler flowmeter model 803 (Parks Electronics, Beaverton, OR) with the output signals fed to an audio amplifier, a cassette tape recorder, and a pen-writing oscillographic recorder. The bubbles were detected by the distinct Doppler-shifted chirping sounds, or from recorded traces (1,2).

### Determination of Decompression Threshold

A step increase in ambient pressure was maintained for 2 h with compressed air at a pressure between 3 and 10 ATA ($P_1$). Chamber pressure was then reduced rapidly (8 ATA/min) to a predetermined lower pressure ($P_2$). If there was no indication of venous bubbles within 1 h, the decompression was considered bubble free. For each saturation pressure ($P_1$), an increasing pressure drop ($\Delta P$) to a lower pressure ($P_2$) was tried on different rats until the greatest $\Delta P$ that produced no bubbles was found. The greatest $\Delta P$ that produces no bubble at a given saturation pressure is called *bubble threshold.*

### Effect of Over-Pressure Spike on Bubble Threshold

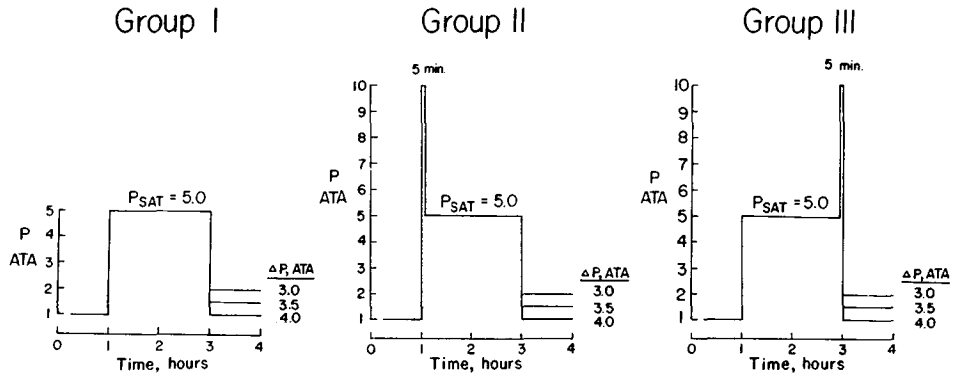
Awake, instrumented rats were exposed to compressed air at 5 ATA for 2 h, followed by rapid pressure reduction ($\Delta P$) of 3.0, 3.5, or 4.0 ATA (*Group I*). In two other groups, an additional over-pressure spike of 5 ATA with a duration of 5 min was superimposed either at the onset of saturation period (*Group II*) or at the end of the saturation period (*Group III*). Decompression was carried out exactly as that in *Group I*. The procedure along with experimental results are illustrated in Fig. 2. Each group consisted of 12 rats.

### Effect of Ambient Temperatures on Bubble Threshold

Experiments were carried out with saturation pressure ranging from 3 to 10 ATA compressed air while rats were exposed to either 15, 24, or 35°C environments. Bubble threshold determination was made as described previously at each temperature with first pressure exposure at 24 h after surgery and the repeat exposure within 48 h after surgery.

### Effect of Ambient Temperature on Nitrogen Elimination and Oxygen Consumption

We determined oxygen consumption and rate of nitrogen elimination to examine a possible relationship between these alterations and temperature-induced threshold changes. Oxygen consumption was determined with a servo-controlled oxygen volume meter (Med-Science Electronics, St. Louis, MO). Carbon dioxide was absorbed by soda lime lining the interior of the animal




| ΔP, ATA | BUBBLE | NO BUBBLE |
|---|---|---|
| 3.00 | 0 | 4 |
| 3.50 | 2 | 2 |
| 4.00 | 3 | 1 |

| ΔP, ATA | BUBBLE | NO BUBBLE |
|---|---|---|
| 3.00 | 1 | 3 |
| 3.50 | 3 | 1 |
| 4.00 | 4 | 0 |

| ΔP, ATA | BUBBLE | NO BUBBLE |
|---|---|---|
| 3.00 | 1 | 3 |
| 3.50 | 3 | 1 |
| 4.00 | 4 | 0 |

Fig. 2. Effect of spike over-pressure on bubble threshold. Experimental protocol is shown on the *upper row* and results on the *bottom row.*

chamber. Nitrogen elimination was measured by a whole-body washout method using pure oxygen (3).

## RESULTS

### Effect of Over-Pressure Spike Bubble Threshold

The number of rats that showed bubbles following decompression is shown in Fig. 3 and summarized in Table I. As the ΔP increased, the number of rats showing intravascular bubbles increased similarly in all three groups. For a given magnitude of pressure reduction, rats in the over-pressure groups not only showed no reduction in frequency of bubbles, but there tended to be a higher number of rats indicating bubbles. For example, at the threshold (ΔP = 3.0 ATA, in this instance), that is, the greatest ΔP that produces no bubbles, there was no indication of bubbles existing intravascularly in *Group I,* but there was one out of four rats in *Groups II* and *III* indicating bubbles (Table I).

### Effect of Ambient Temperature on Bubble Threshold

*First exposure.* At the higher end of saturation pressures ($P_I$), the decompression threshold was reduced at both 15 and 35°C environments as compared to the 24°C condition (Fig. 3). No significant differences were found at lower saturation pressures. These data are summarized in Table II.

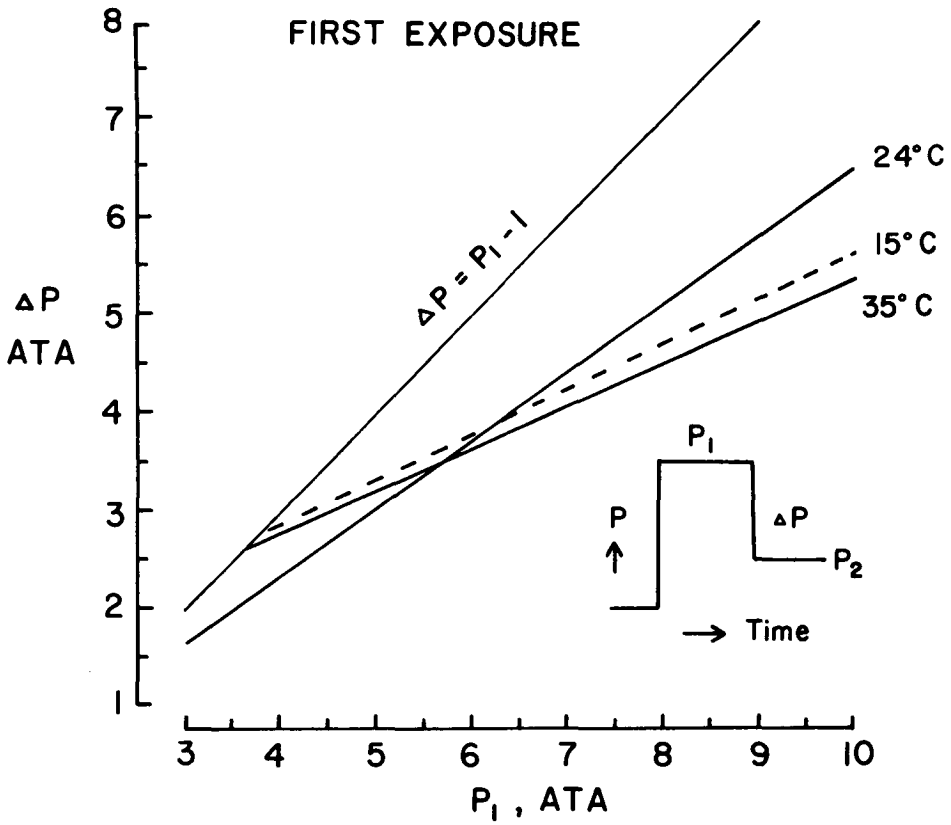



Fig. 3. Effect of ambient temperature on bubble threshold for rats exposed to pressure for the first time. *Regression lines* are the great pressure reduction that produces no detectable intravascular bubbles at 15, 24, and 35°C. The maximum possible pressure reduction is shown by $\Delta P = P_1 - 1$, where $P_1$ is the saturation pressure. *See* Table II for numerical data.

*Second exposure.* With repeat exposure, decompression threshold was lowered in the cold environment. However, the decompression threshold was no different between 24 and 35°C conditions (Fig. 4). These data are summarized in Table II.

*First vs. second exposure.* Comparison of the bubble threshold of the first and the repeat exposures at three ambient temperatures is shown in Fig. 5. Increased decompression thresholds were observed in repeat exposures at 24 and 35°C environments. No difference was found between the first and the repeat exposure at 15°C environments (Fig. 5).

## Oxygen Consumption and Nitrogen Elimination

Oxygen consumption increased from 25.5 mL/min/kg at 24°C ambient temperature to 43.3 mL/min/kg at 15°C. Oxygen consumption decreased to

TABLE I

The Number of Rats in Which Venous Gas Bubbles Were Detected following Decompression

| P, ATA | *Group I* | *Group II* | *Group III* |
|---|---|---|---|
| 3.0 | 0/4 | 1/4 | 1/4 |
| 3.5 | 2/4 | 3/4 | 3/4 |
| 4.0 | 3/4 | 4/4 | 4/4 |

Rats were exposed to compressed air at 5 ATA for 2 h, followed by rapid pressure reduction (ΔP) of 3, 3.5, or 4 ATA (*Group I*). In two other groups, an over-pressure of 5 ATA with a duration of 5 min was superimposed at the onset of the saturation period in one group (*Group II*), and in the other at the end of the saturation period (*Group III*).

20.6 at 35°C (Table III). Lowering ambient temperature from 24 to 15°C reduced nitrogen elimination by 37% (Table III). At the elevated temperature where oxygen consumption was reduced, no change in rate of nitrogen elimination was recorded (Table III).

## DISCUSSION

Intravascular gas emboli are expected to occur during excessive rate of reduction in ambient pressure either from normobaric (aviation, aerospace) or from hyperbaric conditions (diving). Decompression-induced intravascular gas bubbles have indeed been demonstrated in man (8–10), swine (11,12), sheep (13,14), and in the rat (1,2). However, at the threshold of bubble detection the rat shows no symptom indicating decompression sickness. With pressure reduction exceeding the threshold, the animal exhibits behavioral indications of decompression sickness such as respiratory distress, irritability, limping and

TABLE II

Effect of Ambient Temperature on Decompression Threshold

| Exposures | Temp,°C | *n* | Regression equation | *r* | SEE |
|---|---|---|---|---|---|
| First | 15 | 15 | $P = 1.0596 + 0.4564\ P_i$ | 0.9709 | 0.2691 |
| | 24 | 19 | $P = -0.4147 + 0.6930\ P_i$ | 0.9704 | 0.4474 |
| | 35 | 17 | $P = 1.0983 + 0.4281\ P_i$ | 0.8996 | 0.5216 |
| Repeat | 15 | 13 | $P = -0.1729 + 0.6504\ P_i$ | 0.9937 | 0.1573 |
| | 24 | 18 | $P = -0.1870 + 0.7266\ P_i$ | 0.9735 | 0.3263 |
| | 35 | 10 | $P = 0.6216 + 0.5946\ P_i$ | 0.9936 | 0.1501 |

*n:* number of rats in each threshold determination. SEE is the standard error of estimate.

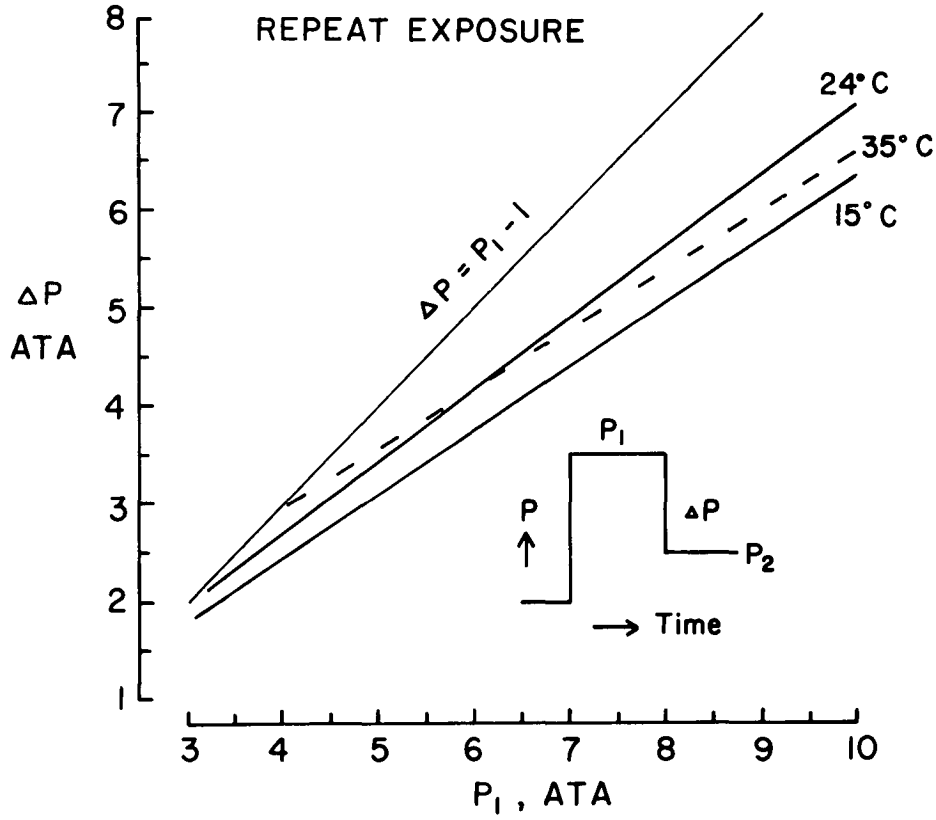



Fig. 4. Effect of ambient temperatures on bubble threshold for rats exposed to pressure for the second time within 24 h following the first exposure. The maximum possible pressure reduction is shown by $\Delta P = P_1 - 1$; $P_1$ is saturation pressure. *See* Table II for details.

inability to maintain posture, and the like. It is not unusual that death of the animal follows shortly after appearance of these symptoms. Thus, detection of low-grade bubbles is an important indicator which precedes symptoms of decompression sickness.

It should be noted that presence of detectable bubbles does not automatically mean that there will be symptomatic expression of decompression sickness. Eatock detected bubbles in 360 out of 585 dives, but only 28 of these resulted in bends (*see* Ref. 15). We have demonstrated the same phenomenon (earlier) by comparing the bubble threshold of rats with the results of Berghage et al. (16). They determined pressure-reduction limits from saturation at 6 to 60 ATA for rats based on behavioral observation. The bubble-defined threshold was below the 5% incidence level of decompression sickness based on behavioral end points. The pressure-reduction limits allowed by the bubble detection were much less than that defined by behavioral criteria (1). Whether decompression tables should be constructed to eliminate bubble detection or to

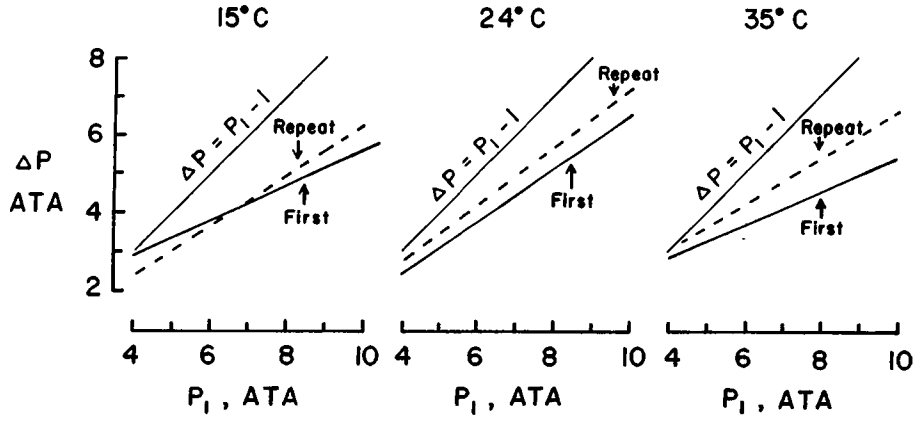



Fig. 5. Effect of ambient temperature and repeat exposures on bubble threshold. The line $\Delta P = P_I - 1$ indicates maximum possible pressure reduction from $P_I$ to sea level; $P_I$ is saturation pressure. *See* Table II for numerical data.

reduce symptoms of decompression sickness has not yet been agreed upon generally.

Results obtained in this study indicate that brief over-pressure is ineffective in altering bubble threshold in the protocol we used. It may be that the over-pressure used in this study was insufficient in magnitude. As compared to the study done by Vann et al. (17), their over-pressure ranged from 12 to 26 ATA. They reported that over-pressure spikes were somewhat effective in reducing incidence of decompression sickness. Their criterion for decompression sickness was death of the rat. In 1969, Evans and Walder (19) demonstrated bubble formation during decompression in transparent shrimp. Bubble formation was drastically reduced by treating the shrimp with brief over-pressure of 389 ATA prior to decompression. Their results imply the dissolu-

TABLE III

Oxygen Consumption and Nitrogen Elimination in Rats

| Temp,°C | $O_2$ Consumption (mL/min/kg) | $N_2$ Elimination (mL/kg) |
|---|---|---|
| 15 | 43.3 ± 1.6* | 8.73 ± 0.67* |
| 24 | 25.5 ± 1.0 | 13.94 ± 0.86 |
| 35 | 20.6 ± 0.9* | 13.22 ± 0.68 |

$X \pm$ SE, 5 rats in each group weighing 350 g on the average. Nitrogen elimination was summed for the first 30 min following the onset of washout. *Indicates statistical significance at $P < 0.05$ compared to 24°C conditions.

tion of micronuclei in vivo under pressure. Yount and Strauss (18) have demonstrated in an in vitro system that increasing crushing pressures reduce the number of bubbles formed in subsequent decompression. These results showed promise but the magnitude of the over-pressure required becomes impractical for treating bend patients in common recompression facilities.

The effectiveness of altering cardiopulmonary functions in changing bubble threshold is also marginal. In any event, in comparison to the bubble threshold that was obtained in 24°C ambient temperature, the altered temperature reduced rather than increased the magnitude of pressure reduction. This is especially clear at the higher end of the saturation pressures (Figs. 3 and 4). Oxygen consumption was elevated by lowering ambient temperature and was depressed by increasing ambient temperature (Table III). But, the end result is the same, i.e., bubble threshold is reduced. Determination of rate of nitrogen elimination did not help explain the bubble threshold obtained in this study. Lowering of ambient temperature depressed the rate of nitrogen elimination. But, this group exhibited bubble thresholds no worse than those in higher ambient temperatures. The mechanism by which ambient temperature affects bubble threshold requires further study.

Repeat exposure to pressure appears effective in elevating bubble threshold. This means that greater pressure reduction is permitted at a given level of pressure saturation after initial exposure (Fig. 5). This result substantiates previous findings in the rat (1) and in man (20–23) that greater pressure reductions can be tolerated on repeat dives better than on first dives. Because the partial pressures of the ambient gases were the same on first and repeat dives and because the solubilities of gases do not change, gas transport and bubble formation must effect the difference. The mechanism of this effect is unclear at present. Hematological changes initiated by circulating bubbles have apparently no effect in this acclimatization process because the effect of an increased threshold seemed to be consistent regardless of the outcome of the initial decompression. Rats undergoing decompression with pressure reduction below bubble threshold (no bubble formed), as well as those demonstrating bubbles, became acclimatized following the first pressure exposure (1). The most likely explanation is an alteration of the number, conformation, or distribution of gas micronuclei shown to be necessary for bubble formation upon decompression. We have no explanation at the present as to why the brief over-pressure of 5 ATA superimposed on saturation pressure at 5 ATA was ineffective, but prolonged initial 5-ATA exposure was effective in elevating bubble threshold 24 h later.

In summary, brief over-pressure superimposed on saturation pressure of 5 ATA is found to be ineffective in altering the bubble threshold. Exposure of the rats in 15 and 35°C consistently depressed bubble threshold as compared to 24°C ambient temperature, especially at the higher range of saturation pressures. Explanation of these changes is not currently available.

## Acknowledgment

This study (HP/R2) was supported in part by University of Hawaii Sea Grant College Program under Institutional Grant NA81AA-D-00070 from NOAA Office of the Sea Grant, Department of Commerce, and a grant from the Hawaii Heart Association.

## References


1. Watt DG, Lin YC. Doppler detection of thresholds for decompression-induced venous gas emboli in the awake rat. Aviat Space Environ Med 1979;50:571–574.
2. Lin YC. Species independent maximum no-bubble pressure reduction from saturation dive. In Bachrach AJ, Matzen MM eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:699–706.
3. Yount DE, Yeung CM, Ingle FW. Determination of the radii of gas cavitation nuclei by filtering gelatin. J Acoust Soc Am 1979;65:1440–1450.
4. Mack GW, Lin YC. Temperature induced changes in inert gas elimination. Physiologist 1982;25:238.
5. Mack GW, Lin YC. Effects of isoproterenol infusion on the kinetics of nitrogen washout in rats. Physiologist 1983;26:A34.
6. Shida KK, Watanabe DK, Lin YC. Effect of ambient temperature on the decompression threshold from a saturation dive in rats. Undersea Biomed Res 1982;9:28.
7. Takehiro TN, Shida, KK, Lin YC. Effects of 2, 4-dinitrophenol on the body temperature and cardiopulmonary functions in unanesthetized rats, *rattus rattus*. Thermal Biol 1979;4:297–301.
8. Spencer MP, Campbell SD. Development of bubbles in venous and arterial blood during hyperbaric decompression. Bull Mason Clinic 1968;22:26–32.
9. Spencer MP, Okino H. Venous gas emboli following repeated breathhold dives. Fed Proc 1972;31:355.
10. Spencer MP. Decompression limits for compressed air determined by ultrasonically detected blood bubbles. J Appl Physiol 1976;40:229–235.
11. Gillis MF, Peterson PL, Karagianes MT. In vivo detection of circulating gas emboli associated with decompression sickness using the Doppler flowmeter. Nature 1968;217:965–967.
12. Powell MR. Doppler ultrasound monitoring of venous gas bubbles in pigs following decompression with air, helium, or neon. Aerosp Med 1970;45:505–508.
13. Spencer MP, Johanson DC, Campbell SD. Safe decompression with the Doppler ultrasonic blood bubble detector. In: Lambertsen CJ, ed. Underwater physiology V. Proceedings of the fifth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1976:311–325.
14. Smith KH, Spencer MP. Doppler indices of decompression sickness: their evaluation and use. Aerosp Med 1970; 41:1396–1400.
15. Hamilton RW. Symposium/workshop on underlying assumptions in calculation of decompression tables. Pressure 1982;11:6–7.
16. Berghage TE, Gomez JA, Roa CE, Everson TR. Pressure reduction limits for rats following steady-state exposures between 6 and 60 ATA. Undersea Biomed Res 1976;3:261–272.
17. Vann RD, Grimstad J, Nielsen CH. Evidence for gas nuclei in decompression rats. Undersea Biomed Res 1980;7:107–112.
18. Evans A, Walder DN. Significance of gas micronuclei in the aetiology of decompression sickness. Nature 1969; 222(519):251–252.
19. Yount DE, Strauss RH. Bubble formation in gelatin: a model for decompression sickness. J Appl Physics 1976;47:5081–5089.
20. Elliott DH. Some factors in the evaluation of oxygen-helium decompression schedules. Aerosp Med 1969;40:129–132.
21. Elliott DH, Hallenbeck JM. The pathophysiology of decompression sickness. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving and compressed air work. Baltimore: Williams & Wilkins, 1975:422.
22. Hempleman HV. Decompression theory: British practice. In Bennett PB, Elliott DH, eds. The physiology and medicine of diving and compressed air work. Baltimore: Williams & Wilkins, 1975:338–343.
23. Walder DN. The prevention of decompression sickness. In Bennett BP, Elliott DH, eds. The physiology and medicine of diving and compressed air work. Baltimore: Williams & Wilkins, 1975:460.

# INVITED REVIEW: DECOMPRESSION SICKNESS, BAROTRAUMA, AND OSTEONECROSIS

*R. R. Pearson*

Although I had occasional contact with diving accidents and decompression illnesses after joining the Royal Navy in 1957, it was not until 1968 that I developed a major interest in underwater medicine. This interest arose from a requirement to treat a case of fulminating decompression sickness. In the event, the diver died under pressure and, in retrospect, I am fairly certain that this would have been the outcome whatever we might have done. It remains the only case of fatal decompression sickness that I have been involved with, but I well remember the feeling of helplessness engendered by this case and the great difficulty in getting any practical advice on what to do. I then discovered how little was known at that time about decompression sickness, particularly its detailed etiology and the treatment of the complicated cases. I determined to find out a little more about the therapeutic aspects of decompression illnesses and was fortunate enough to be able to pursue a career involved with divers and their problems.

On such an occasion as this meeting, it is impossible to resist looking back over the period of my own interest in underwater medicine and pondering how much more we know about decompression illnesses and their therapy. In the last 15 years or so there has been an almost exponential rise in published material relevant to underwater medicine and physiology, and many major advances have occurred in a number of areas. However, it is equally clear that in other areas, many of them of fundamental importance, we remain as ignorant as ever and, on occasion, we are even more confused. Dare I suggest that this ignorance is particularly evident in some of the areas covered by this session even though the various papers all make a contribution to our understanding?

I am well aware that there are many here who are far better qualified than I to indulge in retrospection and many who have had much longer experience of underwater medicine and hyperbaric physiology. However, I hope my thoughts are at least pertinent. If I tend to concentrate on the therapeutic implications of what we have or have not learned about decompression illnesses, it is because my own involvement has largely been in that area. I am well aware that important advances in adjuvant therapy may be just around the corner but this, in my opinion, is a particularly neglected aspect of therapy.

This session is devoted to decompression sickness, barotrauma, and osteonecrosis. In dealing with these topics in that order, I will endeavour to provide a setting against which the various minipapers may be viewed.

## DECOMPRESSION SICKNESS

Interest in decompression sickness as a clinical entity has been with us for well over a hundred years, has been profusely researched and documented, and yet the exact factors involved in its etiology, the interaction of these etiological factors, the early (as opposed to late) associated pathological events, and the optimum therapeutic measures all remain controversial. Indeed, with such a manifestly complex series of events arising from a number of interacting etiological factors and being modified in the individual by a further number of predisposing characteristics, it is no surprise that the only area about which there is common agreement is that decompression sickness is the result of a critical degree of inert gas nucleation. Exactly what degree of inert gas supersaturation and nucleation is necessary to produce the various manifestations of decompression sickness remains unclear as does the precise role of nucleated gas in the intracellular, extracellular, and intravascular compartments. One might then reflect that we might have been further down the road to understanding if the proponents of what I will call *single aetiology theories* had acknowledged that multiple etiological factors and pathologies are involved in any individual case of decompression sickness. That is not to say that any single etiology theory is wholly wrong but neither is it wholly right.

The best starting point in a review of decompression sickness seems to be the mathematics and physics of decompression. Predictive modelling has come a long way since the turn of this century, and it has become what might be called a major growth industry. While I must pay tribute to the many great and richly talented scientists who have made contributions in this area, I wonder if the accuracy they strive for will ever triumph over the infinitely variable complexity of the human diver. Such modelling is, of course, an evolutionary process with a constant need to revise and refine the model in light of human experience. The ability to do this has been enhanced more recently by the various techniques using ultrasonic bubble detection and imaging. It is, therefore, not surprising that two minipapers in this session deal with ultrasonic bubble detection.

The paper by Daniels et al. (1) relates to imaging of bubbles in situ in tissues as opposed to the somewhat more limiting technique of intravascular

detection of transiting bubbles, whether by invasive or noninvasive devices. This paper would suggest that imaging is predictive, whereas one of the great disappointments of recent years has been the failure of intravascular bubble detection to provide a wholly accurate predictive capability. Perhaps the most important issue raised by this paper relates to the ability of imaging bubbles in a limb to predict the outcome of events in the central nervous system (CNS), events which are, after all, the most sinister outcome of decompression sickness. Another thought concerns the early hope that ultrasonic bubble detectors would allow a personal decompression monitor to be carried by divers. Are we now at the stage where we can confidently predict that the usefulness of bubble detection will be confined to monitoring experimental decompressions, or re-assessment of currently used decompression profiles, in strictly controlled conditions?

Turning to the paper by Lin et al. (2), we have another aspect of bubble monitoring using centrally sited, invasive monitoring in an animal model. You may be surprised at the theory which led to the investigation of the effects of an over-pressure spike on decompression after 2 h at a variety of steady pressures. You might also be surprised at the results of this part of the experiment. Conversely, you might not be surprised at the underlying theory and results of that part of the experiment dealing with the effects of ambient temperature on the threshold for bubble detection.

Perhaps a "holy grail" pursued by decompression theoreticians is to incorporate a mathematical model into a foolproof decompression computer with the ultimate aim of providing a diver-mounted personal monitoring system. There is no doubt that the great attraction of such devices lies in the possibility of allowing for multiple depth changes during the course of a dive. A number of these devices are already available and are used with varying degrees of success or failure. The paper by Mano et al. (3) represents an interesting variation on decompression monitoring of individuals. I have always had a simple belief, perhaps over simple, that there can only be a tenuous relationship between events in a homogenous and gelatinous mass and the multicomponent, multicompartmented tissues in man. The relationship is even harder to credit if one adds the dynamic effects of altering degrees of tissue perfusion. It is, therefore, surprising to find in this paper that a practical correlation seems to exist between bubble counts in a small amount of agarose gel and the incidence of bends in the compressed air workers carrying it. Indeed, the workers seem to have eventually used the agarose gel as a form of decompression meter. There are, however, a number of questions I would like to ask about the working pressures used in the period depicted in Fig. 2 of this paper.

Finally, while considering decompression theory, we come to the papers by Bell et al. (4) and Lehner et al. (5). Both groups of investigations use animal models to attempt to solve specific decompression issues. Both concentrate on the gross effects of decompression insults on animals, and both yield quite surprising information. The paper of Bell et al. (4) describes experiments aimed at providing more information on the decompression problems likely to

be encountered in deep submarine escapes or rescue from pressurized compartments. Although the capability for rescue from pressurized compartments exists in the form of the U.S. Navy Deep Submergence Rescue Vehicle and certain other submersibles, the management of survivors in such situations remains problematical. Apart from providing a basis from which human experiments may proceed, this work describes a particularly interesting finding concerning the changing character of induced decompression sickness as predecompression saturation pressures rise. I doubt whether many theories would allow for such an increase in the incidence of mild, as opposed to serious, decompression sickness as the pressure level of saturation rises. Clearly, this finding has important implications and warrants further study.

A similar unpredictable outcome to decompression of sheep is described in the paper of Lehner et al. (5). For me, *chokes* has always been a somewhat mysterious manifestation of decompression, particularly when it is provoked by exposure to altitude and arises after a return to ground level. History tells us to regard the condition as a grave but unpredictable phenomenon, and the Lehner et al. (5) paper demonstrates that the balance between safe and lethal decompressions can rest on a veritable knife-edge. The application to flying after diving is both obvious and timely. This paper also draws attention to the relative independence of the presenting signs of decompression sickness observed in the sheep. This finding parallels a situation in divers which has received growing attention. There is little doubt that the growing amount of air diving in the 30 to 50–msw range has highlighted the number of cases of decompression sickness in which CNS involvement is not accompanied by the milder forms of decompression sickness such as musculoskeletal pain. The slides that we all use for teaching about the incidence of the various presentation of decompression sickness in divers would seem to be in need of revision, and the belief that the vast majority of cases of decompression sickness present as "joint pain only" no longer seems to be valid for diving on air.

Before leaving the Lehner et al. (5) paper, it is interesting to note the suggestion that the conditioning factor in the etiology of chokes arising from altitude exposure is a hypoxic pulmonary vasoconstriction. I am sure that the authors of this paper are aware of the endothelial damage in precapillary arterioles caused by gas emboli, and several authors have, in the past, drawn attention to this phenomenon and the consequent pulmonary interstitial edema. Other authors have looked at the induced bronchoconstriction, but there has been no previous comment on the peculiar effect of altitude exposure.

The paper by Landolt et al. (6) adds yet another dimension to the effects of nucleated gas and raises some interesting issues. It would seem to suggest that audiovestibular manifestations of decompression sickness, if unaccompanied by other signs of CNS dysfunction, may be regarded as evidence of end-organ damage rather than evidence of possible brain-stem involvement. The observation of a poor response to recompression is, therefore, not too surprising in view of the pathological changes described. The therapeutic implications are obvious and these findings go a long way towards explaining the known refractory nature of vestibular problems encountered in decompres-

sion from saturation dives. This paper [Landolt et al.] also gives an interesting pathological differentiation between the auditory and vestibular components of inner ear decompression sickness. I would hope it will lead to further consideration of the therapy of this problem.

This last thought leads naturally enough to the treatment of decompression sickness, and I regard the paper by Gray (7) on the evaluation of recompression procedures used by the U.S. Navy as particularly timely. In a situation in which much of what we do has an empirical basis because of the impossibility of providing suitable controls to allow a true comparison of the various types of recompression therapy, it is most encouraging to see the high success rate of correctly used minimal recompression oxygen tables. Such information is even more encouraging when set against the current vogue to advocate radical changes in the treatment of decompression sickness arising from air or oxygen-nitrogen diving.

I confess to being disappointed that there is only one paper specifically related to therapy. The empirical nature of what we often do is never better illustrated than by the use of adjuvant drug therapy. Recently published papers have drawn attention to the need for research into a number of drugs that are commonly used or might be of benefit. In this respect I would suggest the following list of questions are those to which we should be looking for answers in the future:

1) Do steroids have a place in the treatment of serious decompression sickness and, if so, what type and in what dose?

2) What is the optimum combination of pressure and oxygen partial pressure for the initial treatment of serious decompression sickness?

3) Does so-called *saturation therapy* offer an improved chance of success in treating refractory cases of decompression sickness in comparison with a series of discrete recompressions on oxygen?

4) Is there any advantage to be gained by using oxygen-helium mixtures (or other oxygen-inert gas mixtures) to treat decompression sickness arising from air diving?

5) Is there any true therapeutic benefit to be gained from the use of a number of drugs currently advocated for the treatment of serious decompression sickness (e.g., parenteral aspirin, heparin, nicotinic acid, etc.)?

These questions are all very obvious but concern issues that have been with us far too long without solution.

## BAROTRAUMA

Once again, I have to admit to disappointment that there are no papers on this topic. There can be no doubt that barotrauma and its consequences are the greatest single cause of mortality in recreational divers, yet the subject appears to receive considerably less attention than decompression sickness. In terms of therapy, virtually the same questions may be asked as have already been listed for decompression sickness. Additionally, there are two further questions that seem relative to both forms of decompression illness:

1) Do we know enough about individual predisposing factors in the etiology of barotrauma and decompression sickness?

2) Are we suitably cautious in our advice on whether, and when, victims of serious decompression illnesses should return to diving?

With regard to the latter question, it is my belief that permanent injury to the CNS is a more common sequel to decompression illnesses than we ever suspect. Modern techniques that allow a detailed study of CNS function in ways hitherto impossible are now becoming generally available. I believe their use to determine the degree of damage and subsequent recovery in cases of decompression illness affecting the CNS will lead to a few surprises.

## OSTEONECROSIS

In this section there is the single paper by Fraser et al. (8) This paper attempts to explain some of the odd features related to the etiology and pathology of dysbaric osteonecrosis. Although the theory advanced in this paper would appear to explain the pathological findings of bony damage in the vestibular spaces described by Landolt et al. in a previous paper, I find it hard to see how it explains lesions in the long bones of divers, particularly the "shaft" lesions. Work in the Royal Navy has shown that the lesions of dysbaric osteonecrosis are medullary with reactive endosteal new bone formation. Areas of grossly diminished perfusion can be identified within 48 h of the causative decompression and x-ray changes take at least 3 months to appear in these areas. The obvious lesion appears to be a medullary infarct and, for shaft lesions, the term *bone necrosis* is almost misleading. However, the Fraser et al. (8) paper certainly gives an indication of the forces at work if nucleated inert gas becomes rigidly entrapped during decompression. Once again, I believe there are important unanswered questions to be asked:

1) Should future development of decompression profiles include the freedom from dysbaric osteonecrosis as a criterion for acceptability? This question assumes the ability of scintigraphy to detect lesions at a very early stage.

2) If indeed the initial lesion is a medullary infarct, is it due to extravascular or intravascular events?

3) What is the long-term prognosis if medullary infarcts are the initial lesions?

4) Are there identifiable individual risk factors?

Finally, there is the paper by Bradley and Bornmann (9). It does not fit into any of the main topics covered by this session, but it represents an interesting bit of observation and detective work. It describes a condition that deserves our attention in the future. Certainly, the thought of divers continuing to dive in the prodromal and immediate postrecovery stages of this condition gives food for thought. It is interesting that reference is made to an earlier account of a similar respiratory illness in divers in which decompression sickness and pulmonary barotrauma were listed as possible differential diagnoses.

## CONCLUSION

In conclusion, it is only necessary to cite the following facts as justification for the belief that there is a long way to go before we fully understand decompression and its harmful sequelae:

1) Divers still get bent from air diving with an incidence that does not seem to have changed much for many years.

2) Some divers get bent even when they have clearly obeyed all the rules and carried out relatively innocuous dives.

3) Even with early and correct treatment, there are still cases of serious decompression sickness which prove remarkably refractive to recompression and hyperbaric oxygen.

## References

1. Daniels S, Davies JM, Paton WDM, Smith EB. Recent experiments using ultrasonic imaging to monitor bubble formation in divers. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:249–257.
2. Lin YC, Mack GW, Watanabe DK, Shida KK. Experimental attempts to influence the bubble threshold from saturation dives in animals. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:259–268.
3. Mano Y, Shibayama M, Ishiyama A, Takahashi S, Maeda H. An actual approach to prevent decompression sickness in compressed air workers. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:181–190.
4. Bell PY, Burgess DW, Summerfield M, Towse EJ. The effect of presaturation on the maximum submarine escape depth of goats and the implications for human research. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:241–248.
5. Lehner CE, Will JA, Lightfoot EN, Lanphier EH. Decompression sickness in sheep: Fatal chokes after 24-hour dives with altitude provocation. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:191–200.
6. Landolt JP, Money KE, Radomski MW, Riusech RG, Odell PC, Watson WJ. Inner ear decompression sickness in the squirrel monkey: Observations, interpretations, and mechanisms. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:211–224.
7. Gray CG. A retrospective evaluation of oxygen recompression procedures within the U.S. Navy. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:225–240.
8. Fraser WD, Ward CA, Johnson WR. Bubble-induced local hydrostatic pressure gradients as a possible cause of dysbaric osteonecrosis. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:201–209.
9. Bradley ME, Bornmann RC. Scuba disease revisited (?) In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:173–180.

# Part IV

## CIRCULATORY AND HEMATOLOGIC EFFECTS OF HYPERBARIC EXPOSURE

# STUDIES OF COMPLEMENT AND ACUTE PHASE REACTANT PROTEINS IN THE BLOOD OF DIVER TRAINEES EXPOSED TO PROGRESSIVELY DEEPER AIR DIVES

*M. R. Cross, E. Brown, and L. Booth*

For the past few years, the research laboratory at Fort Bovisand Underwater Centre in Plymouth, Devon, U.K., has carried out a systemic study involving the air diving trainees. We have tried to document as many as possible of the changes that occur in the trainees as they begin their professional career. For most of them, this is the first time that they have been involved in daily diving activities in all conditions and we have been able to observe many changes, some of which are related to the cold and some of which are related to the effects of daily exposure to pressure or to mild degrees of elevated partial pressure of oxygen. In an earlier publication we reported the effects on the levels of hemoglobin and blood volume (1).

In this study, we have systematically looked for indices of tissue damage that may be related to continuous exposure to diving. We have used a battery of tests that have been routinely employed in clinical medicine to reflect tissue damage in different conditions. The acute phase reactant proteins are synthesized by the liver in response to acute tissue damage. We have chosen to study the C-reactive protein, the alpha-1-acid glycoprotein, alpha-1-antitrypsin, and haptoglobin as acute phase reactants. In addition, we measured the levels of plasma fibrinogen and carried out tests for the presence of fibrin degradation products in serum. Since, in experimental dives, we have observed granulocyte responses in relation to provocative exposures, we also decided to study the levels of complement in the plasma. Two fractions of complement were chosen for assay. Fraction C4 is important in the development of immunity and the immune response to infection, whereas C3 fractions are related to the local vascular response to inflammatory conditions and C3 is

involved in local vascular permeability, histamine and bradykinin release, and the function of leucocytes. A decrease in the plasma levels of C3c is observed in autoimmune disease such as Systemic Lupus Erythematosus and Glomerulonephritis, whereas an increase is seen in chronic inflammation and infection.

We had hoped by performing this battery of tests to identify on the basis of a "biochemical fingerprint" the nature of any pathological change induced in the blood of the trainees during the course.

## THE BASIC AIR DIVING COURSE AT FORT BOVISAND

The basic air diving course is particularly well suited for the studies that we perform. The students, all fit young men, are drawn from a normal male population. Most have some history of sport diving and almost all participate in some sport. When they commence their training they are obliged to be resident at the school. They eat the same food and follow a carefully prescribed day, which begins at 0715 h with physical training under an instructor. They then attend lectures and after the lectures the practical activity of the day begins. The course, which is 12 weeks long, is divided into distinct phases. The first 2 weeks involve shallow scuba diving with students wearing wet suits; then follows 2 weeks during which time students learn to dive in dry suits with surface-supplied equipment—however, they do not dive deep. The middle 4 weeks of the course is spent on a "deep phase." During this phase the students dive from a boat and learn to work progressively deeper, starting at 18 m, then progressing to 30 m; for the last 2 weeks they dive daily to depths of 42 m using the U.S. Navy Standard Air Decompression Tables. The last 4 weeks of the course they return to the shallow depths of the harbor and dive daily to 6–8 m while learning to use underwater tools, the "tools phase." The last week does not involve diving: it is spent taking final examinations.

Blood samples were taken from the trainees each week, normally on a Friday morning although occasionally they had to be taken on Thursday or Friday evening according to the daily dive program. In addition to the weekly samples, a sample was also taken on the morning of the first day of the course to provide the control data. Two minor manifestations of decompression sickness occurred during the deep phase, and a retrospective enquiry revealed that some subjects had also experienced minor "niggles," which had not been reported.

## METHODS

Blood was collected into standard lithium-heparin tubes for plasma and samples were also taken in EDTA tubes for hemoglobin and hematocrit assay. Blood for FDP and CRP screening tests was taken into a glass tube with soya-

bean/thrombin to ensure total conversion of biologically active fibrinogen into fibrin; the fibrinogen-free serum was then pipetted off.

Using the technique of radial immunodiffusion, we assayed haptoglobin, alpha-1-antitrypsin, acid glycoprotein, C3c and C4. Nor-Partigen plates were employed for all the assays and the diffusion rings were read on an illuminated reader. Immunogobulin assays were similarly performed on immunodiffusion plates from Immuno Pharmaceuticals. FDP and C-reactive protein were measured with a latex flocculation test. We assayed fibrinogen using the coagulometric method of Clauss (3); all studies were performed in duplicate. Because of the dependence of the acute phase reactant proteins upon liver function, plasma samples were studied by Dr. G. Doran of Charing Cross Hospital. At no time was abnormal liver function demonstrated.

## RESULTS

The results are presented in tabular form. Table I shows the weeks chosen in relation to the course. The changes for the chosen weeks are shown: in Table II, the changes in acute phase reactant proteins; in Table III, the changes in immunoglobulins; and in Table IV, the changes in complement subfractions C4 and C3c.

Figure 1 shows the week-by-week change in one of the parameters, C3c, during the course. It is chosen as representative of many of the changes seen in other measurements, particularly the C4 and the alpha-1-antitrypsin. Most noticeable is the rise in C3c observed in the latter part of the deep phase when the students began regular daily diving at the greater depths—the change is highly significant.

Observations were also made on an individual subject basis to determine if some trainees appeared to show more change than others and, in particular, if a trend that was predictive for the subsequent onset of decompression

TABLE I

Week Number and Course Activity*

| Week Number | Course Activity |
|---|---|
| 0 | Control blood taken on *Day 1* before diving training commenced. |
| 3 | Blood taken on the *last day* of the shallow phase of the course. The students have learned to dive on scuba and surface-supplied equipment to a maximum depth of 18 m using both wet and dry suits. |
| 8 | Last week of *deep phases*. The students have dived daily to depths in excess of 40 m using U.S. Navy air tables. This phase lasted 4 weeks. |
| 11 | End of second *shallow phase* during which time the students have used air tools at shallow depths, 4–7 m. |

*Data reported in Tables II–IV.

TABLE II

Acute Phase Reactant Proteins

| ASSAY | WEEK NUMBER. | | | |
|---|---|---|---|---|
| | 0 | 3 | 8 | 11 |
| $A_1$ ANTITRYPSIN | 2.65/0.22 | 2.78/0.13 | 4.17/0.45** | 2.99/0.17 |
| ACID GLYCOPROTEIN | 0.70/0.03 | 0.74/0.64 | 0.96/0.08 | 0.88/0.07 |
| HAPTOGLOBIN | 0.89/0.23 | 0.73/0.17 | 1.23/0.27 | 1.03/0.18 |
| FIBRINOGEN | 2.60/0.15 | 2.39/0.16 | 2.71/0.16 | 3.21/0.21 |

N= 8 RESULTS GIVEN ARE MEAN + SEM

ALL RESULTS IN GM/LITRE PLASMA

sickness could be demonstrated. Inevitably, in a relatively small sample of men performing air dives without surface decompression, the number of instances of decompression sickness is small. In fact, two members of the course under study showed manifestations of Type 1 decompression sickness during the deep phase, one in *Week 7* and one in *Week 8*. The results in Table V were obtained from one of the two subjects who suffered from a relatively minor manifestation of decompression sickness during the deep phase. The elevation of certain parameters the week before the bend suggests biochemical "decompression stress." The alteration of other parameters only after the bend suggests that they may reflect its consequences.

TABLE III

Immunoglobulin Assays

| IMMUNOGLOBULIN | WEEK NUMBER | | | |
|---|---|---|---|---|
| | 0 | 3 | 8 | 11 |
| IgG | 1.17/0.10 | 1.10/0.08 | 1.10/0.9 | 1.21/0.85 |
| IgA | 286/41 | 247/35 | 266/35 | 271/42 |
| IgM | 185/34 | 172/28 | 188/32 | 190/27 |

N = 8. ALL RESULTS ARE MEAN + SEM

ALL RESULTS ARE IN MG/DL

TABLE IV

Complement Fractions

| FRACTION | WEEK NUMBER. 0 | 3 | 8 | 11 |
|---|---|---|---|---|
| C3c | 0.55/0.03 | 0.63/0.04 | 0.96/0.04* | 0.95/0.04* |
| C4 | 0.30/0.03 | 0.39/0.05 | 0.49/0.05* | 0.44/0.04* |

N = 8. ALL VALUES ARE MEAN + SEM * P < 0.01 VS CONTROL.
ALL VALUES ARE GM/LITRE.

It can be seen that the subject who presented with shoulder pain in *Week 7* shows elevations in the alpha-1-antitrypsin and the haptoglobin levels in the *Prebend Week*, and in the *Week of Bend* he shows considerably larger elevations, particularly in the alpha-1-antitrypsin and the fibrinogen. The blood sample taken in the *Week of Bend* shows significant elevations in the acute reactive proteins, fibrinogen, and, also for the first time, C-reactive protein.

## DISCUSSION

The results presented in this paper are a small part of a continuous program of study into adaptation to diving carried out at the Fort Bovisand Underwater Centre. In this particular series of observations, we were endeavoring to determine if any evidence could be found of tissue damage at a subclinical level occasioned by one of the stresses imposed by air diving. Experience has taught us that the diversity of environmental perturbation experienced in entering and descending in cold water can cause many changes. We have documented those changes that we believe to be attributable to hypothermia and physical fitness (1).

The series of observations presented here reflect some parameters whose principal change appears to be depth related. Certainly, during the deep phase of the course the level of physical activity is no more than, or perhaps less than, in the initial phases, so simple hard work cannot explain the changes—neither can hypothermic exposure, because during the deep-phase training dry suits are worn and, of necessity, exposures are considerably reduced in time as compared with the shallow-tools phase.

Acute phase reactants are synthesized by the liver in response to tissue injury or damage. Serial estimations of these enzymes have been used to

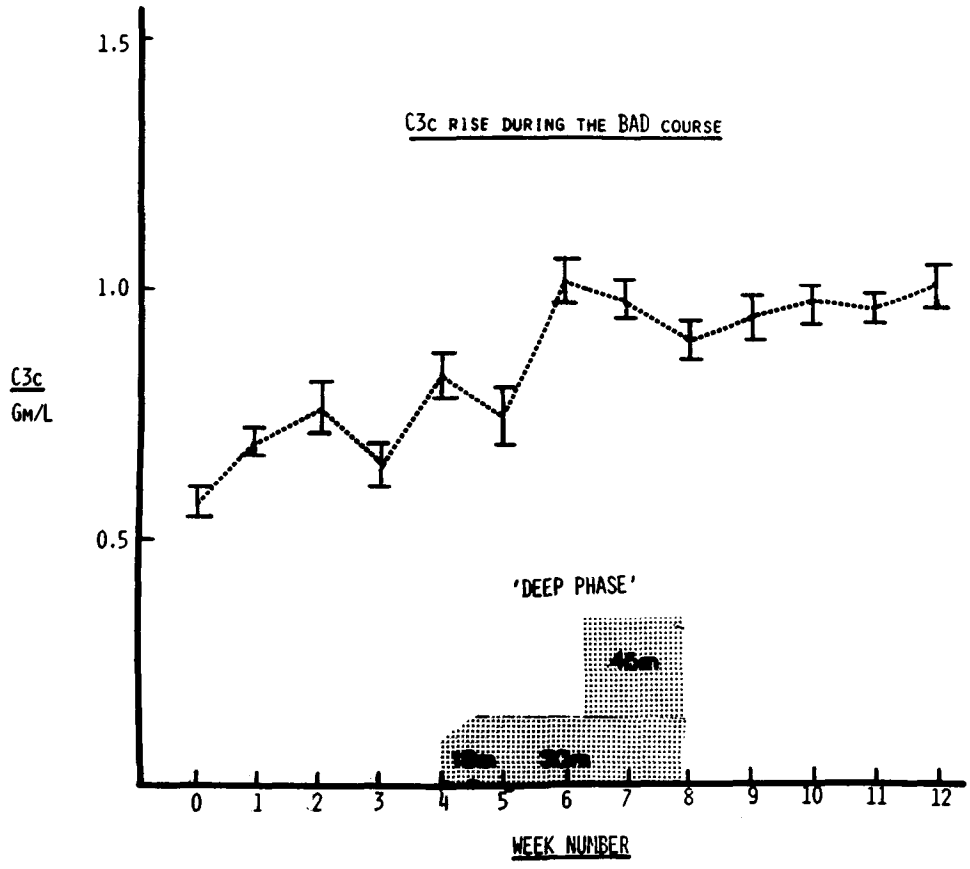



Fig. 1. Week-by-week changes in complement subfraction C3c.

monitor the progress of a number of clinical disorders, particularly myocardial infarction. In an important study, Vougliari et al. (2) studied the changes in a number of acute phase reactants after diverse disease processes, including myocardial infarction, hip replacement, hernia repair, and infections with protozoal, bacterial, and viral agents. In a study similar to the present one, they hoped to find a pattern of change characteristic of a particular type of insult, a sort of "biochemical fingerprint." In fact, in the published study, it was shown that the response is general and such a biochemical discrimination could not be devised; however, when we reviewed the results of Vougliari et al. (2), it was noticeable that alpha-1-antitrypsin showed more of a response to hip replacement than to other disease processes studied. This finding raises the question of the significance of the elevated levels of this protein found in our study, both in relation to the overall course and in relation specifically to the results from the subject who suffered minor decompression sickness. We

TABLE V

Biochemical Changes in Week of Decompression Sickness*

| | Prebend Week (gL) | Week of Bend (gL) | Control (Week 0) (gL) |
|---|---|---|---|
| Alpha-1-antitrypsin | 2.75 | 3.52 | 1.45 |
| Acid glycoprotein | 1.03 | 1.22 | 0.94 |
| Haptoglobin | 1.93 | 2.47 | 1.80 |
| Fibrinogen | 2.86 | 3.83 | 2.61 |
| C3c | 0.94 | 1.06 | 0.49 |
| C4 | 0.40 | 0.45 | 0.31 |
| CRP | neg | neg | + + (= 5.31) |
| FDP | neg | neg | neg |

**Subject McPh.* Type 1 decompression sickness in *Week 7.*

believe that this particular acute phase reactant may be the most valuable chemical marker of bone damage identified to date; studies are in progress that involve experimental dives, and it is proposed that studies be extended to crises in sickle cell anemia, where bone infarction is known to occur.

The depth-related rises in the complement fractions, particularly the C3c are difficult to explain simply. Increases in plasma complement levels are seen in many diseases involving both inflammatory and autoimmune pathologies. The most acute response is naturally a consumptive phenomenon and complement levels fall; however, prolongation of the pathological stimulus results in a stimulation of production and the high levels shown in our study may reflect some continued pathological process involving the vascular wall, which occurs with diving and which is depth-dependent. A process linked to decompression or to oxygen partial pressure would account for this finding and studies are in progress to determine if oxygen alone can produce this response. Studies from this laboratory have shown that both oxygen administration and decompression may be associated with a transient granulocytic response, and it is known that platelet depletion may occur after decompression. Both observations would be consistent with some form of vascular wall damage. The absence of a significant immunoglobulin response suggests that the pathological changes observed in the complement fractions and in the acute phase reactants support the idea that antigen-antibody responses are not involved in the induced biochemical pathological response. This suggests that if the complement change reflects a vasculopathy associated with the deeper air diving, then it is probably not an autoimmune process but a simple response to vessel wall damage.

Further studies are in progress to determine the value of these observations both in selecting divers and in predicting which men are at risk of developing decompression sickness during a diving program.

The plasma fibrinogen results are the most difficult to interpret: this may be because of the slow speed with which fibrinogen is produced by the liver.

It also may be because fibrinogen production is stimulated not only by tissue damage but also by nonspecific stress situations in which a liberation of catecholamines provokes mobilization of fat in the body and, thence, of free fatty acids. An elevation of plasma free fatty acids is associated with increased fibrinogen production; this may account for the fibrinogen elevation without an acute phase reactant response seen in the last week of the course, the "examination phase."

## References

1. Cross MR, Booth LA. Biochemical and hematological responses to shallow water diver training. Proceedings of the annual scientific meeting, European Undersea Biomedical Society, Cambridge, 1981.
2. Vougliari F et al. Serum levels of acute phase and cardiac proteins after myocardial infarction, surgery, and infection. Brit H J 1982;48:352–356.
3. Clauss A. Gerinnungsphysiologische Schnellnekemethod zur Bestimmung bes Fibrinogens. Acta Hematol 1957;17:237.

# ELECTROCARDIOGRAPHIC CHANGES IN NORMAL AND CARDIOMYOPATHIC HAMSTERS EXPOSED TO HYPERBARIC OXYGEN

*T. J. Doubt and D. C. Legrys*

Heart failure is a pathological state in which the heart muscle is unable to generate enough force to provide adequate perfusion of all body tissues (1). This failure is generally due to an intrinsic defect in the heart muscle, but may also be caused by an overload imposed on the heart. The former is the more prevalent cause of heart failure and has been associated with defects in calcium translocation, abnormalities of oxidative pathways, depressed enzyme kinetics, and alterations in autonomic nervous function (1–4).

Approaches to the management of congestive heart failure utilize various combinations of cardiac glycosides, calcium entry blockers, and agents that reduce preload or afterload on the heart (5,6). Recently there have been reports that hyperbaric oxygen (HBO) exposure improves the hemodynamic and contractile status of patients with left ventricular failure (7,8).

The cardiomyopathic Syrian hamster provides a suitable animal model for the study of congestive heart failure (9,10). This animal model exhibits a well-defined, temporal sequence of events that culminates in the development of severe congestive cardiomyopathy. At 30 to 60 days of age, these animals develop focal myolytic lesions and ventricular dilatation. This dilatation is replaced by hypertrophy from 60 to 120 days of age, followed by development of frank congestive failure. Death occurs at approximately 200 days due to heart failure.

Studies using this animal model have reported depressed control of cellular respiration and calcium transport processes (11), depression of fatty acid oxidation (12), and reduced catecholamine turnover (13). It has been postulated that the focal myocardial necrosis noted in the early stages of this disease may

be the result of focal vascular spasms within the coronary microcirculation (14).

No specific electrocardiographic (ECG) changes were reported in the early stages of the cardiomyopathy (15). Notching of the QRS complex was noted, however, during the development of myolytic lesions in the hamsters.

The purpose of our study was to examine ECG changes in 50-day-old normal and myopathic hamsters exposed to HBO. This age was chosen because it represents an early and potentially reversible stage in the development of congestive failure in the myopathic hamsters.

## METHODS

The 50-day-old, cardiomyopathic hamsters (strain BIO 14.6) and the normal hamsters (strain Fl.B) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). Respirations were spontaneous and unassisted. Each animal was placed in a prone position and needle electrodes were inserted subcutaneously in each limb. The standard frontal leads of the ECG were recorded at a paper speed of 100 mm/s. Calibrated records were obtained at the surface while animals were breathing air, after 1 min of HBO exposure, and after 30 min of HBO exposure.

One group of both normal and myopathic hamsters was exposed to 1 ATA $O_2$. Two normal and two myopathic animals were used for each hyperbaric chamber exposure. After obtaining recordings at the surface while animals breathed air, we vented the chamber with a helium-oxygen (heliox) mixture containing 87% $O_2$. After 5 min of ventilation, the chamber was compressed to 1.2 ATA with heliox. Timing of the HBO exposure began upon arrival at 1.2 ATA ($P_{O_2}$ = 1 ATA). The chamber was vented continuously during HBO exposure. Surface equivalent levels of $CO_2$ never exceeded 0.5%. Ambient chamber temperature was maintained at 30 to 32°C.

Another group of animals was exposed to 3 ATA $O_2$. Paired normal and myopathic hamsters were placed in the chamber, where ECG records were obtained, and the chamber was vented with 100% $O_2$ at the surface. The chamber was then compressed with 100% $O_2$ to a depth of 3 ATA ($P_{O_2}$ = 3 ATA). The chamber was vented continuously and ambient temperature remained at 30 to 32°C during the HBO exposure.

After the HBO exposure, the chamber was decompressed to the surface and the animals were sacrificed with an overdose of pentobarbital. The hearts were removed, rinsed in cold saline, blotted dry, and weighed.

Each frontal lead of the ECG was hand-digitized and processed with the aid of a computer. Standard conduction intervals, heart rate, amplitudes, and ST segment changes were recorded for each lead. The mean frontal axis of the QRS complex was determined by trigonometric methods. The ECG traces of each animal were reviewed for changes in rate and rhythm. The morphology of the QRS complex of each animal was evaluated both for changes due to HBO exposure and for relative differences between normal and myopathic animals.

## RESULTS

Table I presents the body and heart weights of both groups of animals. The body weight of the myopathic hamsters was significantly less than the age-matched normal animals. There was, however, no difference in heart weight. Thus, the ratio of heart weight to body weight was significantly greater in the myopathic animals. This finding suggests a relative cardiac enlargement in the myopathic group.

Table II shows the heart rate and conduction interval data for both normal and myopathic animals. There was no statistically significant difference in heart rate between normal and myopathic animals at the surface breathing air. No significant changes were noted in either group after a 30-min exposure to 1 ATA $O_2$. A 30-min exposure to 3 ATA $O_2$ produced a 20 ± 4% increase in heart rate in the myopathic animals, but essentially no change in the normal hamsters.

We found no significant differences in the P-R interval between the normal and myopathic animals. Exposure of both groups of animals to 1 ATA $O_2$ and 3 ATA $O_2$ produced significant decreases in the P-R interval. The degree of the reduction in the P-R interval was similar in both normal and myopathic animals.

At the surface, the duration of the QRS complex was significantly longer in the myopathic animals than in the normal animals. The wider QRS complex in the myopathic animals suggested ventricular strain. Exposure of the normal animals to 1 ATA $O_2$ produced essentially no change in the duration of the QRS complex, whereas the myopathic QRS was shortened significantly. Exposure to 3 ATA $O_2$ produced a slight but statistically significant reduction in the QRS duration of the normal animals, and a greater reduction in the myopathic hamsters. As shown in Table II, the myopathic QRS values after HBO exposure were equivalent to those obtained in the normal animals.

TABLE I

Body and Heart Weights of Normal and Myopathic Hamsters

| | Body Weight (g) | Heart Weight (mg) | Ratio Heart/Body |
|---|---|---|---|
| Normal ($n = 15$) | 84 ± 1* | 260 ± 5 | 0.00314 ± 0.0001 |
| | $P < 0.001$ | NS+ | $P < 0.001$ |
| Myopathic ($n = 13$) | 74 ± 2 | 254 ± 8 | 0.00344 ± 0.0001 |

*Values are mean ± SE. +NS = not significant by unpaired *t*-test.

TABLE II

Heart Rate and ECG Intervals of Normal and Myopathic Hamsters

| | | 1 ATA Air | 1 ATA $O_2$ | 3 ATA $O_2$ |
|---|---|---|---|---|
| Heart Rate (beats/min) | Normal | 426 ± 9 ($n$=14) | 472 ± 18 ($n$=6) | 435 ± 34 ($n$=8) |
| | Myopathic | 412 ± 22 ($n$=13) | 472 ± 35 ($n$=6) | 440 ± 21* ($n$=7) |
| P-R Interval (ms) | Normal | 49 ± 1 | 42 ± 1* | 43 ± 1* |
| | Myopathic | 47 ± 1 | 43 ± 1* | 45 ± 2* |
| QRS Duration (ms) | Normal | 17 ± 1 | 16 ± 1 | 15 ± 1* |
| | Myopathic | 21 ± 1† | 15 ± 1* | 17 ± 1* |
| QT Interval (ms) | Normal | 94 ± 3 | 80 ± 3* | 88 ± 3* |
| | Myopathic | 103 ± 2 | 91 ± 2† | 92 ± 4* |

Values are mean ± SE. *Significantly different from 1 ATA air. †Significantly different from normal animals.

While at the surface, the myopathic hamsters had a significantly longer QT interval than the normal animals. Exposure to 1 ATA $O_2$ significantly reduced the QT interval in both groups of animals. Least-squares regression analysis of the data indicated that a portion of the change in the QT interval was independent of changes in heart rate. At this level of HBO exposure, the QT interval in the myopathic hamsters remained longer than the QT interval in the normal animal. Exposure to 3 ATA $O_2$ significantly reduced the QT interval in both groups of animals. There was, however, no difference in the QT interval between the normal and myopathic animals at this higher level of HBO.

The mean electrical axis of the myopathic QRS complex in the frontal plane tended to be displaced to the right of the mean electrical axis of the normal animals. At the surface, the myopathic QRS axis ranged from +40 to +90 degrees, whereas the QRS axis for the normal hamster ranged from about −20 to +20 degrees. Ventricular depolarization in the myopathic animals was typically characterized by the presence of Q waves in lead aVL, while the normal animals typically displayed an R complex. Exposure to HBO did not consistently alter the mean electrical axis or ventricular depolarization complexes in either myopathic or normal animals. There was, however, a tendency for the myopathic QRS axis to be rotated slightly to the left with

HBO exposure. Four of the myopathic hamsters exposed to 1 ATA $O_2$ had slight alterations of the QRS complex, which suggested leftward rotation. Five of the myopathic animals exposed to 3 ATA $O_2$ had similar axis rotations. Two of the animals in this latter group exhibited a transition from an R complex to an Rs complex in lead aVL during the HBO exposure.

No consistent changes occurred in P wave or T wave amplitudes in either group of animals exposed to HBO. Furthermore, no changes occurred in the ST segments of either group.

Two of the normal hamsters exposed to 1 ATA $O_2$ developed sinus arrhythmias during the first 15–30 min of exposure. The arrhythmias were characterized by variations in the R-R interval, without substantial changes in P wave amplitude or in P-R intervals. Five of the eight normal animals exposed to 3 ATA $O_2$ also developed marked sinus arrhythmias. Of these five animals, three developed ECG evidence of wandering pacemaker activity marked by beat-by-beat alterations in P wave amplitude and variability in the P-R interval. No sinus arrhythmias were found in any of the myopathic animals exposed to either HBO level. No other types of arrhythmias were noted in either the normal or myopathic hamsters.

## DISCUSSION

The results of this study indicate that exposure to hyperbaric oxygen can significantly alter the ECG patterns in both normal and myopathic hamsters. Previous reports (9,14) indicate that myopathic hamsters near 50 days of age develop focal lesions and ventricular dilatation. The present findings of an increased heart weight to body weight ratio, plus the wider QRS duration, are consistent with these indications. The marked decreases in the duration of the myopathic QRS with HBO exposure would suggest that high oxygen levels can at least transiently reverse the ventricular strain seen in the myopathic group.

A recent report suggests that the focal myocardial lesions in the myopathic hamsters may possibly be due to the development of local spasms within the coronary microcirculation (14). One may speculate that exposure to high oxygen levels enhances the tendency for focal spasms to occur through the direct effects of oxygen on the vascular smooth muscle. We cannot rule out the possibility that HBO contributed to focal vascular spasms. The absence of increases in conduction intervals, alterations in T-wave amplitude, and isoelectric shifts in the ST segment, however, suggest that interactions of HBO with coronary vasculature did not result in gross vasoconstriction.

One may also speculate that the shortening of the P-R and QT intervals in both normal and myopathic hamsters is a result of the direct effects of hyperbaric oxygen on the enhancement of cardiac conduction. It is known that increases in the activity of the sympathetic nervous system can also decrease both P-R and QT intervals. The sympathetic nervous system would have to be activated asymmetrically, however, to decrease the conduction intervals with-

out increasing the heart rate. Additional research would be required to determine whether hyperbaric oxygen selectively enhances sympathetic activity in either the right or left stellate ganglion, or in areas of the hypothalamus.

The present findings indicate that substantial differences exist between the sensitivity of normal and myopathic hamsters to HBO-related arrhythmias. Normal hamsters developed sinus arrhythmias after exposure to either level of high oxygen. In addition, the incidence of the arrhythmias was higher at the higher oxygen pressure. The characteristic ECG changes noted with these arrhythmias (changes in R-R and P-R intervals, and P wave amplitude) are consonant with the idea of altered tone in the autonomic nervous system, particularly in the parasympathetic branch.

The 50-day-old myopathic hamsters were uniquely resistant to the arrhythmogenic effects of hyperbaric oxygen. This finding may be related to previous reports of depressed catecholamine turnover in the myopathic heart (4,13). On the other hand, one might speculate that the myopathic pacemaker cells are in some way more resistant to the direct effects of hyperbaric oxygen.

Additional experiments are required before definitive statements can be made concerning the possible therapeutic effects of HBO. Certain reports in the literature (7,8) indicate that patients with longstanding depressed cardiac function can benefit from hyperbaric oxygen exposure. The exact contractile mechanisms from which the benefits of the HBO derive are not yet known. Nor is it known if hyperbaric oxygenation would be of any inotropic benefit in the early stages of cardiomyopathy. Based solely on ECG evidence, the tentative hypothesis offered here is that HBO may be of some benefit in the early stages of congestive heart failure. Additional oxygen doses and exposure times will be required to determine if this favorable effect in the early stages of failure is transient or represents a potential reversal of myopathic defects.

## Acknowledgments

Research was supported by NMRDC Work Unit MR00001.01.1284. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official views of the Department of the Navy or the Naval Service at large.

## References

1. Katz AM. Congestive heart failure. N Engl J Med 1975;293:1184–1190.
2. Katz AM, Messined FC. Myocardial membrane function and drug action in heart failure. Am Heart J 1981;102:491–500.
3. Francis GS, Goldsmith SF, Ziesche SM, Cohn HN. Response of plasma norepinephrine and epinephrine to dynamic exercise in patients with congestive heart failure. Am J Cardiol 1982;49:1152–1156.
4. Bristow MR, Ginsbur R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K, Billingham ME, Harrison DC, Stinson EB. Decreased catecholamine sensitivity and β-adrenergic receptor density in failing human hearts. N Engl J Med 1982;307:205–211.
5. Vanhoutie PM. Calcium entry blockers and the cardiovascular system. Fed Proc 1981;40:2851–2888.

6. Weber KT, Andrews V, Janicki JS. Cardiotonic agents in the management of chronic cardiac failure. Am Heart J 1982;103:639–649.
7. Efuni SN, Kun IS, Rodionov VV, Topchiashvili GS, Migachev VN, Poctchabiia OA. Hyperbaric oxygenation in treatment of chronic left ventricular failure in rheumatic heart defects. Sov Med 1982;2:47–49.
8. Deepika K, Myers RAM, Cooley RA. Cardiovascular effects of hyperbaric oxygen in septic patients. Undersea Biomed Res 1982;9(Suppl):44.
9. Homburger F. Myopathy of hamster dystrophy: history and morphologic aspects. Ann NY Acad Sci 1979;317:2–17.
10. Forman R, Parmley WW, Sonnenblick EH. Myocardial contractility in relation to hypertrophy and failure in myopathic Syrian hamsters. J Mol Cell Cardiol 1972;4:203–211.
11. Schwarts A, Lindenmayer GE, Harigaya S. Respiratory control and calcium transport in heart mitochondria from the cardiomyopathic Syrian hamster. Trans NY Acad Sci 1968;30:951–954.
12. Kako JR, Thornton MJ, Heggtveit HA. Depressed fatty acid and acetate oxidation and other metabolic defects in homogenates from hearts of hamsters with hereditary cardiomyopath. Circ Res 1974;34:570–580.
13. Sole MJ, Kamble AB, Hussain MN. Possible change in rate-limiting step for cardiac norepinephrine synthesis in cardiomyopathic Syrian hamster. Circ Res 1977;41:814–817.
14. Factor SM, Minase T, Cho S, Dominitz R, Sonnenblick EH. Microvascular spasm in cardiomyopathic Syrian hamster: preventable cause of focal myocardial necrosis. Circulation 1982;66:342–354.
15. Angelakos ET, Daniels J, Robinson S, Bajusz E. Electrocardiographic changes during progressive focal myocardiopathy in hamsters. Recent Adv Stud Card Struc Metab 1973;2:507.

# CARDIAC OUTPUT ADJUSTMENT TO SUDDEN IMMERSION DETERMINED BY IMPEDANCE CARDIOGRAPHY

*Z. Hajduczok, D. D. Hickey, and C. E. G. Lundgren**

This paper deals with the ability of the heart to adjust to the sudden changes in blood distribution induced by immersion. Several publications exist that address the steady-state circulatory performance, notably cardiac output, during water immersion. By contrast, the dynamics of the cardiac output adjustment to immersion have not been studied in man. This adjustment is of considerable interest in that it reflects the ability of the circulatory system to cope with sudden, marked changes in venous return.

Although the classical methods based on Fick's principle and dye- or thermodilution techniques for measuring cardiac output do not allow analysis of beat-to-beat changes in cardiac performance, stroke volume measurements by impedance cardiography allow such analysis given that certain conditions have been met. Impedance cardiography is a widely recognized means to provide good information about relative changes in cardiac output in nonimmersed resting man; however, we believed it necessary to evaluate the method with regard to the effects of wetting the electrodes and of electrolytes in the water, as well as with regard to thermal balance if a dry suit must be used.

A systematic study of these factors was made in 5 resting, healthy, male volunteer subjects 20–25 years of age. They were monitored sitting nonimmersed under thermoneutral conditions (28°C) and after a minimum of 5 min of immersion under thermoneutral conditions, either naked (35°C water) or wearing a diver's dry suit. Thermoneutrality with regard to the suited subjects was determined according to Ramanathan (1). The results obtained are shown in Table I.

*Authors' names listed in alphabetical order.

Table I

Cardiac Output Measurements

| | |
|---|---|
| Nonimmersed, naked | 5.74 L/min ± 1.69 (SD) |
| Nonimmersed, dry suit | 5.55 L/min ± 1.73 (SD) |
| Immersed, naked | 8.29 L/min ± 1.3 (SD) |
| Immersed, dry suit | 7.89 L/min ± 1.56 (SD) |

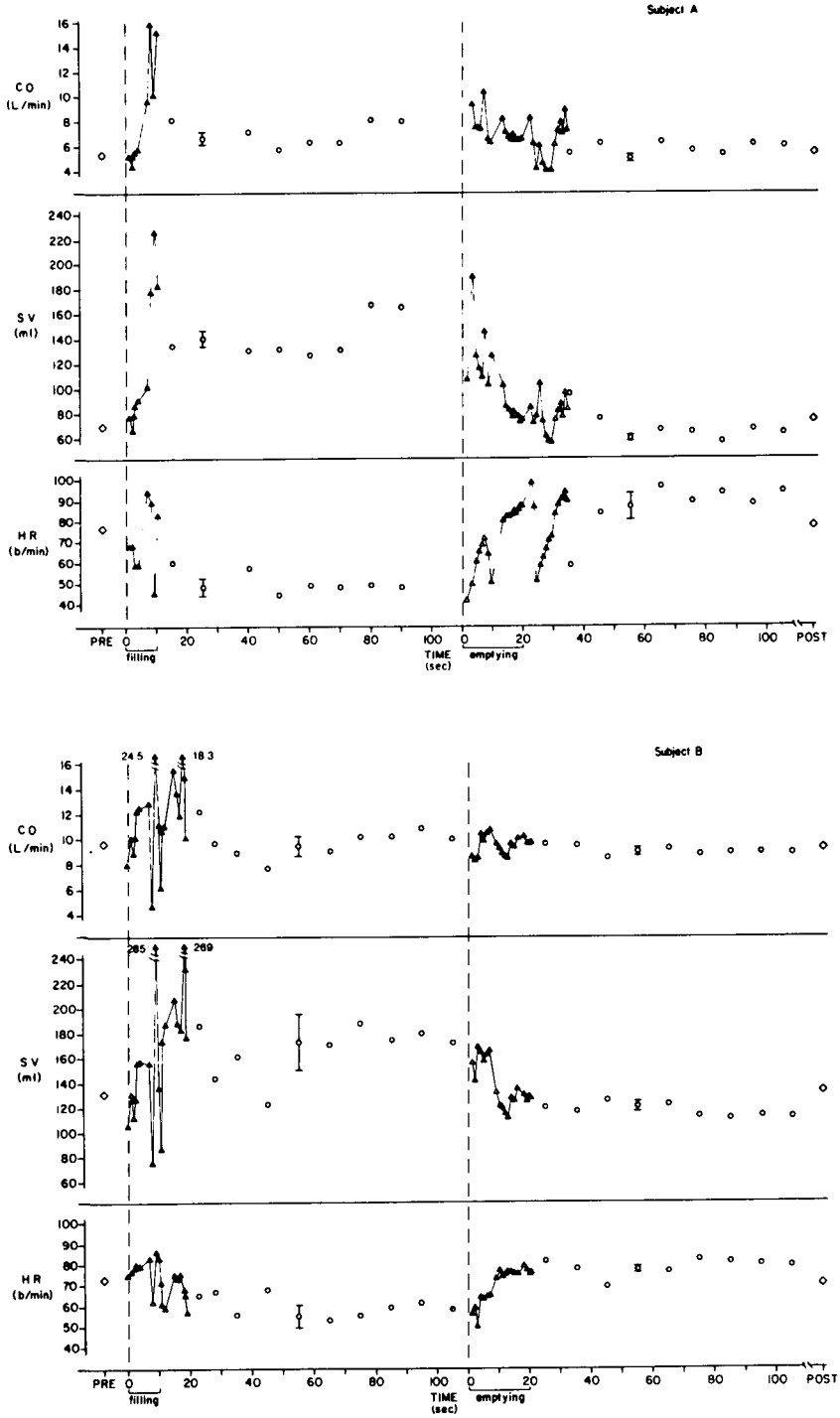
The mean increase ($P < 0.001$, paired $t$-test) in cardiac output due to immersion in the naked subjects was 50.0% ±22.9 (SD) and in the subjects wearing a dry suit it was 45.7% ±20.8 (SD) and there were no significant differences between naked and suited controls or between immersion experiments in the naked and the suited subjects. As little as 0.5% sodium chloride in the water made measurements in the naked subject impossible. Cardiac output measurements in two subjects wearing a dry suit in fresh water respectively salt water, containing between 5% and 3.5% sodium chloride, were compared in five experimental runs. No significant differences (Student's $t$-test) between the effects of fresh water and salt water were observed during these conditions.

The increase in steady-state cardiac output during head-out immersion recorded in our subjects is in good agreement with the reports from other laboratories employing different techniques (2,3). Consequently, we conclude that the impedance cardiography technique is well suited for analysis of relative changes in beat-by-beat cardiac output in naked subjects who are immersed in fresh water or dry-suited subjects immersed in either fresh water or salt water.

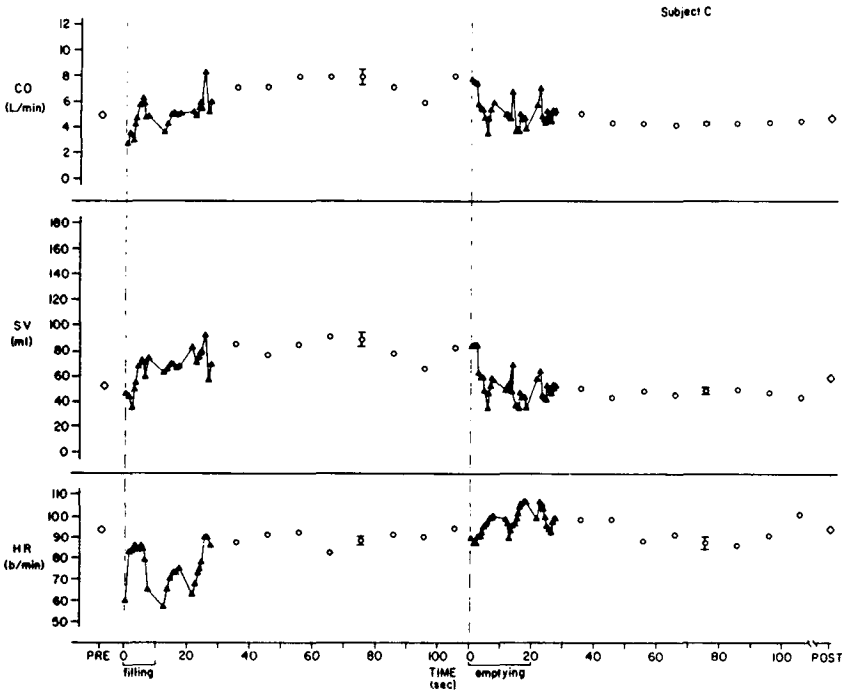
The experimental protocol for the studies of sudden immersion consisted of fitting the subject with the impedance electrodes and a waterproofed precordial microphone and placing him in a watertight body container. The subject wore a snorkel tube connected to a pneumotachograph, the signal of which allowed identification of possible disturbances in the cardiograph tracing caused by respiratory movements. The subject took one breath every 10 s and relaxed at functional residual capacity between breaths. A raised water container allowed filling of the body container to the subject's chin in 10 s. The water could be drained in 20 s. The water temperature (35°C) as well as the air temperature (29°C) in the nonimmersed control runs were in the thermoneutral range.

A striking finding in the three subjects studied at the time of writing is the marked circulatory response immediately linked with the immersion. This response consisted of large fluctuations in heart rate, stroke volume, and cardiac output (*see* Fig. 1*A*, *B*, *C*).

The maximal stroke volumes (only represented by a few beats) reached during the filling phase were: 225 mL vs. 70 mL in the preimmersion control




[Explanation of figure on page 298.]




Figs. 1*A*, *B*, *C*. Cardiac output, stroke volume, and heart rate in three sitting subjects (*A*, *B*, *C*) exposed to sudden immersion to the chin in thermoneutral water. *Circles* are 10-s mean values—*bars* are representative SEs; *triangles* are data from individual heart beats (*connecting lines* between *triangles* were drawn for clarity—data are missing where subject breathed); *diamonds* are pre- and postimmersion control values, which are means of several consecutive beats. Immersion was introduced between 0 and 10 s, and drainage of water between 105 s and the consecutive 20-s mark.

(a 221% increase in *Subject A*); 285 mL vs. 130 mL (a 119% increase in *Subject B*); and 93 mL vs. 52 mL (a 79% increase in *Subject C*). The cardiac output values, while also subject to fluctuation, showed maximal readings of 16 L/min vs. 5.5 L/min, (a 191% increase in *Subject A*); 24.5 L/min vs. 9.5 L/min (a 158% increase in *Subject B*); and 8.2 L/min vs. 4.8 L/min (a 71% increase in *Subject C*); these levels are up to four times higher than those commonly recorded during steady-state immersion (*cf.* Table I).

The general trend during the ensuring 100 s of steady-state immersion was for heart rate to fall below control level while stroke volume remained elevated. The resulting average cardiac output during this time period was either not different from the preimmersion control level (*Subject B*); or moderately elevated by about 25% (*Subject A*); or markedly elevated by about 65% (*Subject C*).

The reaction to the drainage of the water was in all three subjects an increase in heart rate with a tendency for exceeding the pre- and postimmersion control levels somewhat. The stroke volumes fell simultaneously with the

heart rate increases so that the resulting cardiac output postimmersion was not different from the controls. The 3-min postimmersion cardiac output values were generated by final adjustments in heart rate and stroke volume to the preimmersion levels.

The fluctuations in heart rate, stroke volume, and cardiac output in connection with drainage, and for a short period afterwards, were marked. However, after roughly the first minute of the drainage period these parameters exhibited much less fluctuation than during the immersion period, as is evident from a comparison of the standard error bars (SE) in the two conditions (the largest SE's encountered for any 10 s set of heart beats are shown).

The most interesting findings in these subjects are the very large stroke volumes encountered in the latter half of the 10-s phase of water filling. It is at the beginning of this period that the water reached the level of the abdomen that Farhi and Linnarsson (3), studying the effects of graded steady-state immersion, have shown must be reached before a marked increase in cardiac output is manifest during steady-state immersion. The stroke volumes (maximally 285 mL in *Subject B* who is of large body build: 85.7 kg, 192 cm) are surprising but feasible in view of increases in diastolic heart volume by up to 360 mL recorded in subjects of smaller build by Risch et al. (4) using x-ray cinematography during immersion.

It is conceivable that the distension and preload caused by such large volumes could damage a heart weakened by prior pathological processes, and it is possible that such a mechanism could play a role in otherwise hard-to-explain fatalities in connection with swimming and diving (*cf.* Ref. 5). Indeed, extrasystoles (2,6) as well as other types of arrhythmia (6) have been observed in response to acute immersion of both resting and exercising healthy subjects and right atrial pressure recordings can exhibit a pattern similar to that seen in constrictive pericarditis with right heart congestion (2). It is also noteworthy that animal studies have demonstrated that straining the right heart can elicit extra systoles (7).

## Acknowledgment

This work was supported by ONR contract N00014–78–C–0205 with funds provided by the Naval Medical Research and Development Command.

## References

1. Ramanathan, NL. A new weighting system for mean surface temperature of the human body. J Appl Physiol 1964;19:531–533.
2. Aborelius M Jr, Balldin UI, Lilja B, Lundgren CEG. Hemodynamic changes in man during immersion with the head above water. Aerosp Med 1972;43:592–598.
3. Farhi LE, Linnarsson D. Cardiopulmonary readjustments during graded immersion in water at 35°C. Respir Physiol 1977;30:35–50.

4. Risch WD, Koubenec HJ, Beckmann U, Lange S, Gauer OH. The effect of graded immersion on heart volume, central venous pressure, pulmonary blood distribution and heart rate in man. Pfluegers Arch Eur J Physiol 1978;374:115–118.
5. Eldridge L. Sudden unexplained death syndrome in cold water SCUBA diving. Undersea Biomed Res 1979;6(Suppl):41.
6. Jung K, Stolle W. Behavior of heart rate and incidence of arrhythmia in swimming and diving. Biotelem Patient Monit 1981;8:228–239.
7. Heinrich F. Hertzrythmusstorungen infolge akuter Dilatation des Herzens. Klin Wochenschr 1970;48:573–579.

# RED CELL MORPHOLOGICAL AND HEMATOLOGICAL STUDIES IN MEN DURING AND AFTER A 60-M NITROX CHAMBER DIVE

*J. A. Paciorek, H. Ornhagen, O. Eiken, and M. Liner*

Routine hematological studies during both shallow and deeper chamber dives have yielded a plethora of data (1–4), which often has shown little or no significant changes during the pressure exposure. During this work we have tried to compare decompression methods on serum electrolyte levels, red cell indices, cell counts, and red cell morphology. Erythrocyte morphology has been followed for up to 1 year postdive.

## METHODS

In this dive 2 men underwent a 24-h cold exposure in heliox at 150 msw. On decompression to 60 msw, they joined 4 other subjects in a nitrox saturation. The dive profile for both the Nisahex and Nitrox components of the dive are shown in Fig. 1. Details about the dive are given in Muren et al. (5) in this *Proceedings*.

All six subjects were experienced divers, of which four were naval divers. The two subjects that performed the intrachamber hematological monitoring were final-year medical students. Execution of the chamber blood testing was done before breakfast on days as shown in the tables [to be discussed later in this paper]. No set diet or fluid balance regime was used. While under nitrox exposure the divers carried out blood sampling, measuring the erythrocyte sedimentation rate in citrate (ESR), hemoglobin concentration, and hematocrit (Hct) in the chamber. Blood was also collected into heparin tubes and divided into separate tubes. These tubes were decompressed at two different rates, either at 1 m/min in a small pressure vessel that was locked out

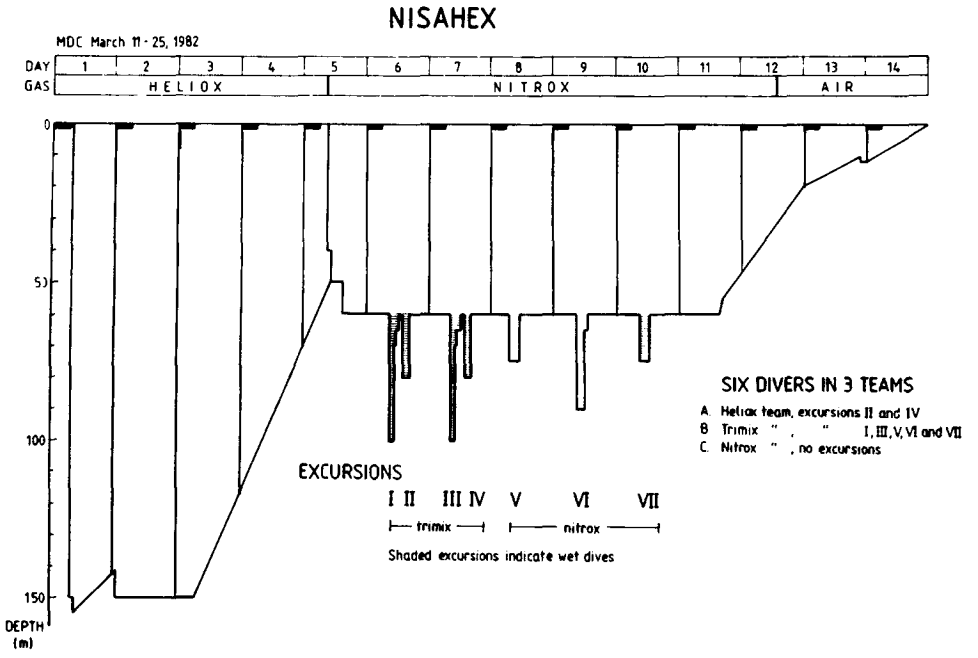



Fig. 1. Dive profile for Nisahex and Nitrox components of dive.

from the chamber, or at 60 m/min in the chamber medical lock (6). Heparinized blood was used in the measurement of packed red cell volume (PRCV); mean cell hemoglobin (MCH); mean corpuscular volume (MCV); and mean cell hemoglobin concentration (MCHC). Blood for hemoglobin (Hb) mass, erythrocyte, leucocyte, and thromobocyte counts was collected into EDTA tubes and for serum assay of electrolytes, erythropoietin, lipids, lactate dehydrogenase (LDH), and transaminases (ALAT and ASAT) into gel tubes. Red cells in heparin were fixed in a 8% glutaraldehyde phosphate buffer system (pH 7.2) after the decompression procedures and viewed in a Jeol scanning electron microscope (SEM) according of previously published techniques (7). Erythrocyte ghosts (8) were prepared for the investigation of any changes in the cytoskeletal and membrane proteins by electrophoreses on sodium dodecyl sulphate polyacrylamide gels in 6% cylindrical gels and 4–15% gradient Laemmli slab system (9).

## RESULTS

### Routine Hematology

*Comparative Techniques of Blood Tested at Pressure and after Decompression to Surface*

Measurement of Hb in the chamber and of Hb in blood decompressed fast and slowly and measured at a Clinical Chemistry Department did not show

any differences. By the Student's *t*-test there was no significant difference between predive, dive, or postdive Hb values. Samples were tested for ESR by the Westergren method for 1 h, in which the normal fall range is 0–20 mm. Results are presented in Table I.

A micro method using capillary tubes was employed in the chamber Hct determination. Comparison of these values to hospital PRCV results gave no great difference. Elevation of PRCV on the 1st and 4th day at pressure may be indicative of a general hemoconcentration in each subject. Reduction of plasma volume may also be indicated by the high $Na^{+}$and $Ca^{++}$ levels but the low MCHC.

*Red Cell Parameters and Plasma Lipid, Electrolyte, and Enzyme Investigation*

A summary of the averaged red cell indices and cell counts as well as some plasma ions and lipids is given in Table II. Results are based on samples collected from all six subjects before breakfast and decompressed at 1 m/min. Predive values could not be obtained because of technical problems.

Serum lipids showed high normal values of cholesterol with normal low triglyceride levels. Cholesterol levels 1 month and 1 year postdive showed a markedly lower level in each subject, when compared to the normal but high values during the dive.

## Enzyme Levels

Levels of ASAT and ALAT remained within the normal limits for all six divers. Values of LDH were increased on the 4th day following compression. Electrophoresis of the five isoenzymes using sigma agrose kits and densitometry showed significant increases in LDH 3 in all six men, which is indicative

TABLE I

Measurements for ESR*

| Subject | Phase of Dive and Date of Sample | | | | |
|---|---|---|---|---|---|
| | Predive (mm) | At Pressure (mm) | | | Decompression (mm) |
| | 3/15 | 3/16 | 3/18 | 3/20 | 3/23 |
| A | 1 | 14 | 13 | 15 | 10 |
| B | 4 | 10 | 18 | 13 | 6 |
| C | 4 | 7 | 13 | 11 | 6 |
| D | 4 | 17 | 7 | 8 | 8 |
| E | | 6 | 11 | 8 | 7 |
| F | | 20 | 12 | 13 | 11 |

ESR: erythrocyte sedimentation rate in citrate. Normal fall range: 0–20 mm.

TABLE II

Summary of Averaged Hematological Investigation

| Parameter | Phase of Dive and Date of Sample | | | | | Normal Range Units |
|---|---|---|---|---|---|---|
| | Dive | | | | Postdive | |
| | 3/16 | 3/18 | 3/20 | 3/23 | 3/25 | |
| ESR | 12.3 | 12.3 | 11.3 | 8.0 | — | 0–20 mm |
| PRCV | 48.0 | 47.7 | 48.7 | 46.2 | 46.8 | 37–50 % |
| MCH | 29.3 | 28.7 | 29.3 | — | 29.2 | 26–50 pg |
| MCV | 90.1 | 92.8 | 93.4 | 92.5 | 92.0 | 76–96 fl |
| MCHC | 312 | 312 | 315 | 317 | 318 | 320–360 g/L |
| Hb mass | 148 | 150 | 154 | 148 | 150 | 115–165 g/L |
| Eryth ct | 5.1 | 5.1 | 5.3 | 5.0 | 5.2 | 3.4–5.5 $10^{12}$/L |
| Leuco ct | 4.5 | 5.9 | 5.5 | 5.5 | 7.0 | 3.0–9.0 $10^{9}$/L |
| Thromb ct | 185 | 227 | 223 | 221 | 250 | 150–400 $10^{9}$/L |
| $Na^{+}$ | 152 | 149 | 147 | 147 | 146 | 133–146 mmol/L |
| $K^{+}$ | 5.1 | 4.6 | 4.4 | 4.5 | 4.4 | 3.6–5.1 mmol/L |
| $Ca^{++}$ | 2.5 | 2.49 | 2.46 | 2.56 | — | 2.07–2.55 mmol/L |
| Triglycerides | 0.9 | 0.43 | 0.65 | 1.13 | | 0–2.2 mmol/L |
| | | | | | 14/4 | 2/2/83 2.6–7.8 mmol/L |
| Cholesterol | 5.95 | 6.00 | | 6.30 | 4.5 | 5.25 |

Code: Eryth ct: erythrocyte count; Leuco ct: leucocyte count. Predive values not obtainable because of technical problems.

of necrosis in cells from blood, lung, intestinal lymphnode, and/or spleen tissue.

Figure 2 represents the average proportion of LDH 3 isoenzyme to total average LDH estimated by electrophoresis separation. Values of LDH 3 measured 1 year postdive show a return to normal limits. Values of the frozen plasma total LDH compared well with the serum LDH values, which were determined at Huddinge Hospital during the dive (shown as *A** and *B**).

## Erythrocyte Structure

### *Red Cell Morphology*

Fixed cells were stored at 4°C and at a pH of 7.2. The pH of this buffering system was checked before SEM preparation; it was always found to be 7.2 ± 0.02. Cell counts were based on counting 1000 cells per preparation, which was done in duplicate.

Various cell morphologies are shown in Fig 3. This represents the sequence of changes in the red cell population of one man compressed directly to 60 msw on nitrox, both during and up to 9 months postdive.

In Table III the percentage of abnormal erythrocytes are given. The percentage of red cells that could not be classified is shown in parentheses. The remaining percentage is discocytes. Throughout the *stay at pressure,* cell

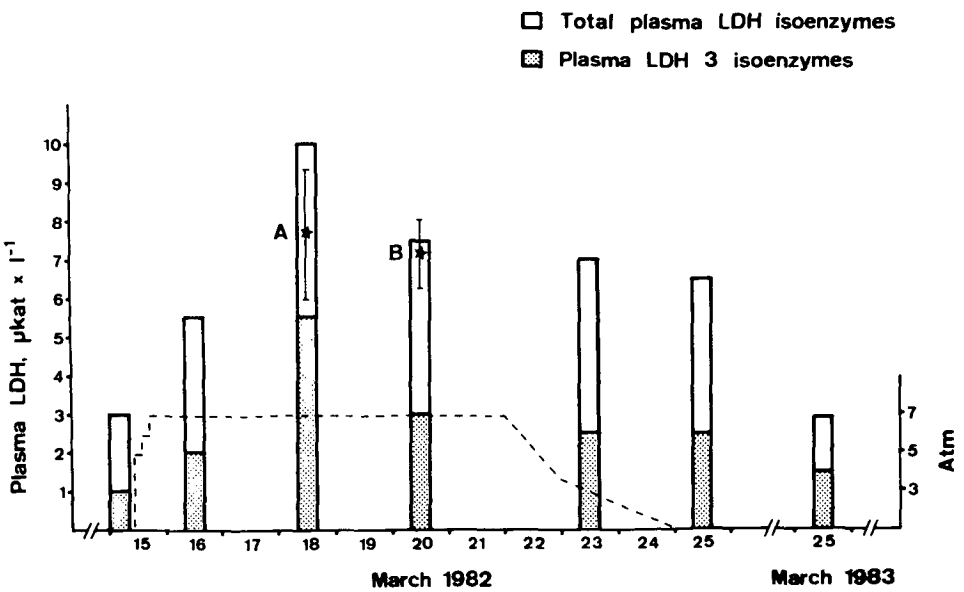



Fig. 2. Average proportion of LDH 3 isoenzyme to total average LDH estimates by electrophoresis separation.

morphology and membrane proteins remained essentially normal. During decompression, the appearance of an increasing number of acanthocytic cells were noted. These cell types are larger in volume due to the incorporation of cholesterol into the lipid bilayer and are associated with a low relative MCHC.

Elliptical cells (10–15% of total RBC numbers) were observed 4 weeks postdive. These stayed in the circulation up until the May sampling. The number of aberrant cell types was 2–5% higher, at the August sampling, than is usually found in the general circulation.

Nine months and/or 1 year postdive, the SEM of red cell morphology in all our subjects was completely normal, i.e., the cells were all discocytes.

## DISCUSSION

All subjects suffered a degree of hemoconcentration on compression, which continued during the dive (raised PRCV, $Na^+/Ca^{++}$ levels but decreased MCHC). Changes in some red cell shapes were seen after 2 days at pressure; the cells became flattened, fluted, and larger (red cell volume increased). Cell types like these are seen in liver disease or hypercholesterolaemia; these cells are called acanthocytes (10). Increased cell cholesterol reduces membrane fluidity (11) and so may affect the ability of the cells to pass through the microcirculation. However, the acanthocyte cells disappeared or returned to normal cell types during decompression.

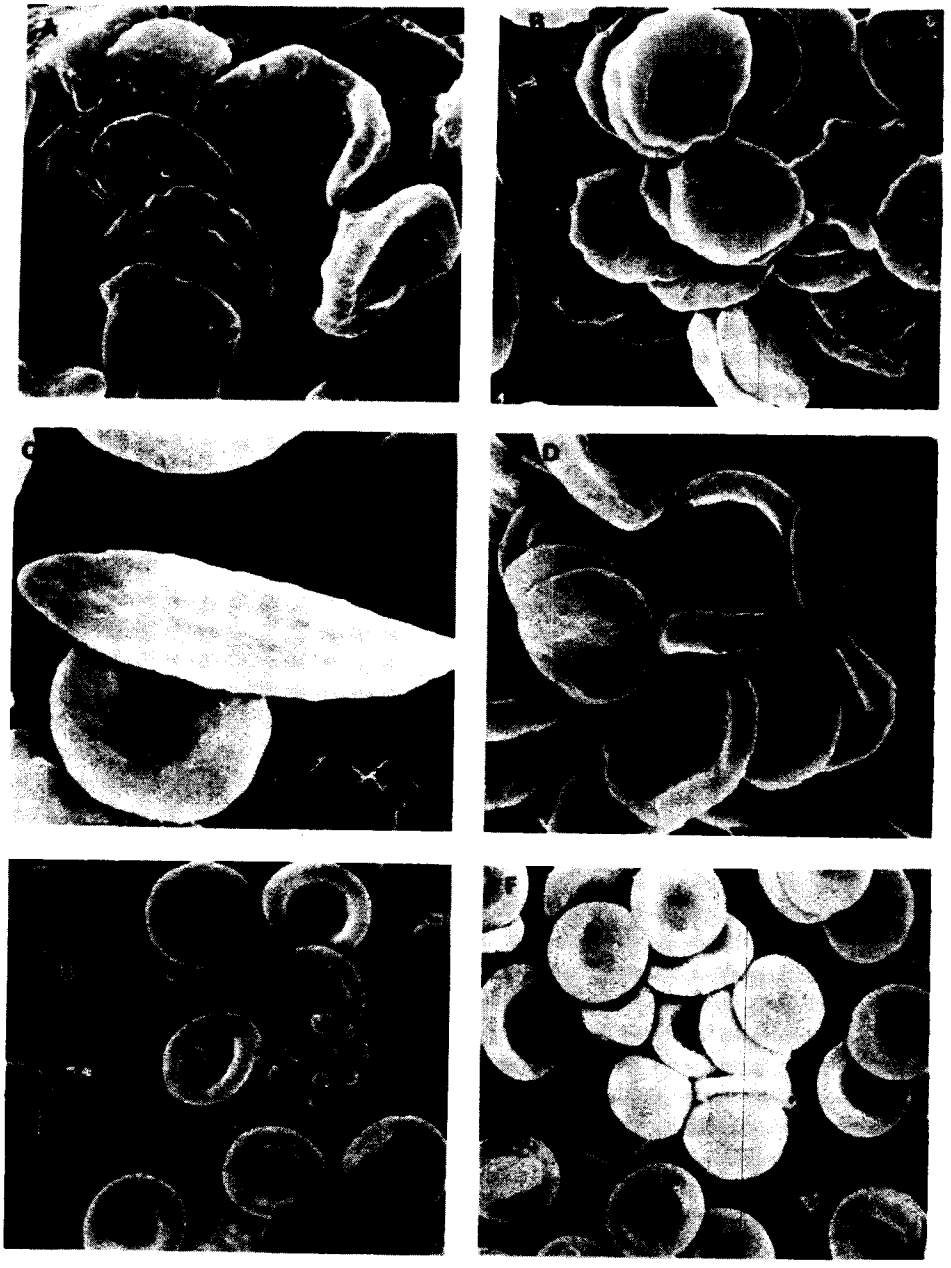



Fig. 3A. On arrival at 60 msw cells were discoid but with a fluted edge (volume increase). 3B. 15 days postcompression the presence of acanthocytes may have indicated increased intracellular cholesterol levels. 3C. 28 days postcompression; up to 15% of elliptocytes were seen. 3 D. 56 days postcompression the presence of elliptocytes was greatly reduced but red cell shape was not completely discoid. 3E. 5 months postdive the sample contained a higher than normal proportion of echinocytes. 3F. 9 months postdive all the red cells are discocytes.

Table III

Percentage of Abnormal Erythrocytes*

| Subject | Phase of Dive and Date of Sample | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Dive | | | | Postdive | | | |
| | 3/16 | 3/20 | 3/25 | 3/31 | 4/14 | 5/25 | 8/8 | 3/1983 |
| A | (7) 1 | (2) 4 | (5) 5 | (5) 5 | (3) 12 | (1) 9 | (1) 4 | — — |
| B | (2) 2 | (4) 3 | (3) 7 | (2) 8 | (0) 11 | (0) 8 | (0) 4 | (0) 0 |
| C | (3) 3 | (2) 6 | (4) 6 | (8) 5 | (8) 10 | (1) 9 | (1) 4 | (0) 0 |
| D | (1) 2 | (0) 8 | (3) 7 | — — | (4) 11 | (1) 15 | — — | (1) 1 |
| Cold Exposure | | | | | | | | |
| E | (1) 1 | (5) 5 | (4) 7 | — — | (5) 12 | (2) 11 | — — | (1) 1 |
| F | (1) 4 | (4) 5 | (2) 10 | — — | (0) 16 | (2) 12 | — — | (2) 1 |
| Average Classifiable Abnormal Cells | | | | | | | | |
| | 2.1 ± 0.2 | 5.1 ± 0.7 | 7.0 ± 1.7 | | 12.0 ± 2.1 | 10.6 ± 2.6 | | |

*Percentage of red cells that could not be classified are in ( ). All dates are 1982 unless otherwise stated.

A normal low reticulocyte count was observed in all subjects at the end of the dive. The occurrence of elliptocytes 4 weeks postdive is a novel observation. As yet no adequate explanation of this phenomenon can be made, except that maybe the erythropoiesis was interrupted as a result of an increased $Po_2$, and this was found.

Hereditary elliptocytosis is due to a deficiency of the cytoskeleton protein 4.1, which results in decreased cell deformability, increased hemolysis, anemia, reduced fragmentation, and an abnormal tetramer-dimer spectrin configuration (12). During the dive no differences in spectrin forms or degradation of the protein 4.1 were noted. There were no elliptocytes at this time. It was unfortunate that we were unable to study the protein 4.1 postdive, as an anomaly in protein 4.1 may have occurred in those cells being differentiated during the pressure exposure; these cells may not have been fully matured and released into the general circulation until well into the postdive phase. Elevated abnormal red cell morphologies up to 5 months postdive were also recorded. The cause of persistent abnormal red cell morphologies can only be speculative on the present observation; however, unsatisfactory erythropoiesis does have its origins in a) elevated or depressed hormone levels on stem cell development (13); b) impaired cytoskeletal development (14) such as in hereditary elliptocytosis, which is at a messenger level of protein synthesis; c) control of calcium entry/ejection from cells, which then controls the cytoskeletal gelation reaction (15).

All of our subjects returned to diving within a week of the nitrox dive. Obviously, a more detailed, longer series of hematology and morphology studies are necessary pre-, during, and postnitrox exposure, so that we can differentiate between purely adaptive and detrimental pressure effects.

## Acknowledgments

In conducting and participating in this dive we thank the divers and the staff of the Swedish Navy Diving Centre. We also thank the Huddinge Hospital for extending their facilities to us.

## References

1. Philp RB. A review of blood changes associated with compression-decompression: relationship to decompression sickness. Undersea Biomed Res 1974;1:117–150.
2. Nichols G. In Hempleman et al., Human physiological studies at 43 bar. 1980;5:180. (AMTE report no. R8040,).
3. Philip RB, Gowdy CW, Prasad M. Changes in blood lipid concentration and cell counts following decompression sickness in rats and the influence of dietary lipid. Can J Physiol Pharmacol 1967;45:1047.
4. Barnard EEP, Weathersby PK. Blood cell changes in asymptomatic divers. Undersea Biomed Res 1981;8:187–198.
5. Muren A, Adolfson J, Ornhagen H, Gennser M, Hamilton RW. *Nisahex*: Excursions with nitrox and trimix from deep nitrox saturation. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Biomedical Society, Inc., 1984;713:729.
6. Morild E, Paciorek JA, Onarheim J. Changes in hematology, plasma chemistry, and erythrocyte morphology in Deep Ex 81. Hematology, plasma chemistry, membrane studies, and red cell carbonic anhydrase activity. Ytre Laksevag, Norway: Norwegian Underwater Technology Centre, 1982. (NUTEC report no. 15a–82.)
7. Paciorek JA, Morild E, Onarheim J. Changes in hematology, plasma chemistry, and erythrocyte morphology in Deep Ex 81. Pressure induced echinocytogenesis. Ytre Laksevag, Norway: Norwegian Underwater Technology Centre, 1982. (NUTEC report no. 15b–82.)
8. Paciorek JA. Red cell structure and the effect of hyperbaric exposure in helium 0.4 bar oxygen. In: Elliott DH, ed. Proceedings of European Undersea Biomedical Society 7th annual congress. 1981;1:83–100.
9. Laemmli UK. Cleavage of structural proteins during assembly of the head of bacteriophages T4. Nature 1970;227:680.
10. Biemer JJ. Acanthocytosis—biochemical and physiological consideration. Ann Clin Lab Sci 1980;10:238.
11. Cooper RA. Influence of increased membrane cholesterol on membrane fluidity and cell function in human red blood cells. J Supramol Struct 1978;8:413.
12. Tchernia G, Mohandas N, Shonet SB. Deficiency of skeletal proteinband 4.1 in homozygous hereditary elliptocytosis. J Clin Invest 1981;68:454.
13. Robinson SH, Tsong M. Hemolysis of stress reticulocytes, a source of erythropoietic bilirubin formation. J Clin Invest 1970;49:1025.
14. Lux SE. Spectrin-actin membrane skeleton of normal and abnormal red cells. Semin Hematol 1979;16:75.
15. Fowler V, Taylor DL. Spectrin plus band 4.1 cross linked actin. J Cell Biol 1980;85:361.

# THE EFFECT OF $\beta_1$ BLOCKADE ON THE DISTRIBUTION OF CARDIAC OUTPUT AT NORMAL AND INCREASED AMBIENT PRESSURE IN CONSCIOUS RATS

*J. Risberg, C. Hordnes, and I. Tyssebotn*

Studies of the effect of increased ambient pressure and changing atmospheric gas composition on cardiac output and its distribution are scattered. Onarheim and Tyssebotn (1) described this effect on anesthetized rats, and Hordnes et al. (2) reported a preliminary study on awake, trained animals. An increased myocardial blood flow (MBF) and reduced renal blood flow (RBF) combined with unchanged cardiac output (CO), mean arterial pressure (MAP), and heart rate (HR) were found when ambient pressure was increased to 5 ATA and the partial pressure of oxygen was maintained at the normal 0.2 ATA.

We undertook this study to determine whether the increased MBF at increased ambient pressure was due to altered $\beta_1$-receptor activation or not. Atenolol (Tenormin$^R$, ICI) was used for $\beta_1$-blockade to selectively block the cardiac receptors.

## METHODS

The experiments were made on male Albino-Wistar rats weighing 250–300 g. Each rat was individually trained for 21 consecutive days before the chamber experiments to ensure that they accepted the experimental situation without visible stress reactions. During the experiment, the animals were restricted in a Plexiglas cylinder, slightly larger than the rat (Fig. 1).

The surgery was undertaken in shortlasting ether anesthesia the day before the chamber experiment. One PE50 catheter was put into the tail artery for

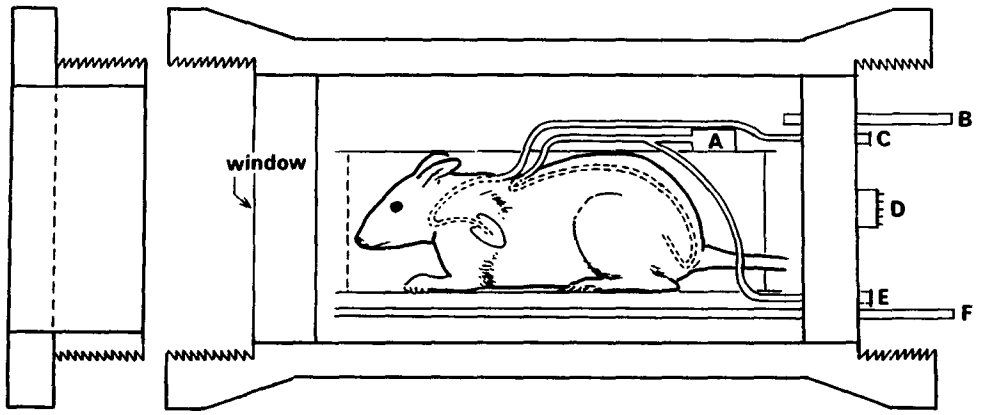



Fig. 1. Schematic drawing of the pressure chamber. *A:* Arterial pressure transducer; *B:* Gas inflow; *C:* Cannula for MS injection; *D:* Plug for electrical connections; *E:* Cannula for arterial blood sampling; *F:* Gas outflow.

blood sampling and one PE50 catheter was introduced in the left ventricle retrograd via the right carotid artery for injections. Both catheters were lead subcutaneously to the back of the animal to give an easy approach to the catheters when the chamber experiment started.

Organ blood flow and CO were measured by the microsphere method (3). About 200 000 microspheres (MS) in 0.2 mL 10% Ficoll were injected in the left ventricle in 10 s. Just before the MS injection, blood sampling from the tail artery was started. Blood was sampled for 2 min at a rate of 0.5 mL /min by a syringe pump. The MS's were labelled with $^{85}Sr$ and $^{141}Ce$. We changed the order of isotope injection for each experiment to avoid systematic errors. Mean arterial pressure and HR were monitored continuously by a pressure transducer (AE840, AME) and recorded on a Hewlett-Packard recorder (HP7754A) during the chamber experiment. Each rat served as its own control. Control measurements were performed at normal ambient pressure and gas composition and in the same rat were compared to one of the following experimental situations:

**Group A: Control—Atenolol ($P_{O_2}$ = 0.2 ATA, $P_{N_2}$ = 0.8 ATA; Ambient Pressure 1.0 ATA)**

Five minutes after the control measurements, atenolol was given i.a. 1 mg/kg body weight. Fifteen minutes after the blockade, the second MS injection was made. *Series A* was made to evaluate the effect of $\beta_1$-receptor blockade on awake rats at 1 ATA ($n=8$).

**Group B: Control—Compression to 5 ATA ($P_{O_2}$ = 0.2 ATA, $P_{N_2}$ = 4.8 ATA; Ambient Pressure 5.0 ATA)**

Immediately after the control measurements, the animal was compressed to 5 ATA within 5 min. After 15 min at 5 ATA, the second MS injection was

given. *Series B* was made to evaluate the effect of high ambient pressure with normoxic atmosphere on organ blood flow and CO. This is a preliminary series as described by Hordnes et al. (2); ($n=7$).

### Group C: Atenolol—Control—Compression to 5 ATA ($P_{O_2}$ = 0.2 ATA, $P_{N_2}$ = 4.8 ATA; Ambient Pressure 5.0 ATA)

Fifteen minutes after the atenolol injection, control blood flow was measured. The animals were compressed to 5 ATA within 5 min. The second MS injection was given 15 min after compression. *Series C* was made to elucidate if the $\beta_1$-receptor blockade had blocked the increased MBF compared to MAP, HR, AND CO ($n=6$).

After the experiments, the rats were decompressed in 5 min and immediately killed by Mebumal given intracardial. The heart and kidneys were dissected free, weighed, and counted in a $\gamma$-counter (Searle 1185R). Organ blood flow and cardiac output were calculated according to these equations:

$$F_o = \frac{C_o \times V_r}{C_r} \times \frac{1}{W_o} \qquad CO = \frac{C_i \times V_r}{C_r} \times \frac{1}{W_a}$$

Where: $F_o$: Organ blood flow (mL/min × g); CO: Cardiac output (mL/min × 100 g); $C_i$: Total activity of the injected isotope (cpm); $C_r$: Counted activity in the reference blood sample (cpm); $C_o$: Counted activity in the actual organ (cpm); $V_r$; Sampling velocity of the reference blood sample (mL/min); $W_a$: Animal weight.

All statistic evaluations were made with the Student's *t*-test for paired data.

## RESULTS

The results are summarized in Table I and Fig. 2.

### Group A

Heart rate decreased 17% from 408 to 339 bpm ($P < 0.05$), indicating a $\beta_1$-receptor blockade. Mean arterial pressure fell by 9% from 111 mmHg to 101 ($P < 0.05$); CO fell 24% from 42.24 to 32.03 mL/min × 100 g ($P < 0.05$); while total peripheral resistance (TPR) increased by 23% from 2.6 to 3.2 mmHg/(mL/min × 100 g) (NS). Myocardial blood flow in the left ventricle (L.VENT) fell 19% from 7.60 to 6.13 mL/min × g ($P < 0.05$). Renal blood flow fell 25% from 4.58 mL/min × g to 3.41 ($P < 0.05$). The reduction of MBF is well correlated to the fall in HR, MAP, and CO.

TABLE I

Central Hemodynamic Parameters and Blood Flow to the Myocardium and the Kidney

| | *Group A* | | *Group B* | | *Group C* | |
|---|---|---|---|---|---|---|
| Ambient Pressure: | 1 ATA | 1 ATA | 1 ATA | 5 ATA | 1 ATA | 5 ATA |
| HR | 408 ± 16.6 | 339 ± 15.5* | 399 ± 22 | 401 ± 17 | 319 ± 15.1 | 349 ± 18.6* |
| MAP | 111 ± 6 | 101 ± 6.9* | 106 ± 5 | 109 ± 6 | 105 ± 7.4 | 99 ± 5.2 |
| CO | 42.2 ± 4.1 | 32.0 ± 5.6* | 31.6 ± 2.6 | 31.1 ± 2.6 | 28.2 ± 1.6 | 24.8 ± 2.3 |
| TPR | 2.6 ± 0.3 | 3.2 ± 0.3 | 3.4 ± 0.4 | 3.5 ± 0.4 | 3.7 ± 0.2 | 3.9 ± 0.2 |
| MBF | 7.6 ± 0.9 | 6.1 ± 0.8* | 4.0 ± 0.2 | 5.1 ± 0.5* | 4.5 ± 0.2 | 5.2 ± 0.3 |
| RBF | 4.6 ± 0.3 | 3.4 ± 0.4* | 4.2 ± 0.2 | 3.2 ± 0.3* | 5.1 ± 0.3 | 4.0 ± 0.7 |

Mean values ± SEM are given. HR: Heart rate; MAP: Mean arterial pressure; CO: Cardiac output; TPR: Total peripheral resistance; MBF: Myocardic blood flow; RBF: Renal blood flow. *Group A, B, and C (see text).* *$P$-value 0.05.

## Group B

Heart rate remained unchanged, 399 versus 401; MAP and CO insignificantly changed, respectively, from 106 to 109 and from 31.57 to 31.11. Total peripheral resistance changed only 4% from 3.36 to 3.50 mmHg/(mL/min × 100 g). At the same time MBF increased 29% from 3.95 to 5.08 mL/min × g ($P < 0.05$) and RBF fell from 4.19 to 3.22 mL/min × g ($P < 0.05$).

## Group C

Heart rate increased 9% from 319 to 349 ($P < 0.05$); MAP and CO fell, respectively, 6% from 105 to 99 mmHg (NS) and 12% from 28.16 to 24.77

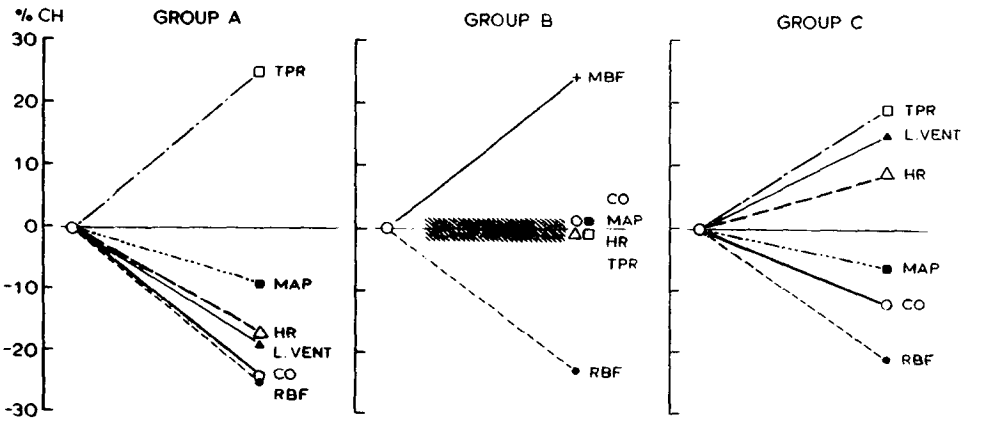



Fig. 2. The percentage change of central hemodynamic parameters and blood flow to the myocardium and the kidneys in three groups of animals. CO: Cardiac output; MAP: Mean arterial pressure; HR: Heart rate; TPR: Total peripheral resistance; MBF: Myocardic blood flow; RBF: Renal blood flow; L.VENT: Left ventricle of the heart.

mL/min × 100 g (NS). Total peripheral resistance increased 19% from 3.68 to 4.38 mmHg/(mL/min × 100 g) (NS). Myocardial blood flow in the left ventricle increased 15% from 4.55 to 5.24 mL/min × g (NS), and RBF fell 21% from 5.11 to 4.01 mL/min × g (NS) so that the ratio MBF/CO increased by 31%.

## DISCUSSION

The microsphere method (3) was modified for use in hyperbaric chambers by Onarheim & Tyssebotn, who thoroughly discussed the validity of this method in their publication (1). In our study a cardioselective $\beta_1$-receptor blockade reduced HR, MAP, CO, and MBF significantly during conditions at 1 ATA pressure. These findings agree well with the results reported by Singh et al. (4) and Izumi et al. (5) when they used anesthetized dogs. A HR reduction of 17% indicates an adequate $\beta_1$-receptor blockade at the chosen atenolol dose of 1 mg/kg body weight.

Atenolol administration has reduced the workload of the heart significantly since CO, MAP, and HR all fell significantly. This finding indicates a reduced oxygen consumption of the heart of the same degree. If we assume that the oxygen extraction is unchanged, the MBF also should decrease to the same extent. This decrease is in good correlation with the reduction of MBF of 19% (Fig. 2, *left panel*).

*Series B* results demonstrate the common changes observed when ambient pressure increases to 5 ATA and $P_{O_2}$ is kept constant at 0.2 ATA. These changes agree well with the results from anesthetized rats during identical experimental procedures (1). The workload of the heart remains unchanged as CO, HR, and MAP did not change. However, the MBF increased at the same time by 29% if one assumes an increased oxygen consumption if oxygen extraction from the blood is unchanged. This discrepancy was not abolished after selective $\beta_1$-receptor blockade, as demonstrated in *Group C*. An increased HR of 9% and decreased MAP of 6% would closely balance each other. A reduced CO of 12% might therefore be compared to the rise in MBF of 15%, which means a relative overperfusion of the heart by about 30% compared to its workload. This value is identical to the overperfusion of 29% in *Group B* without $\beta_1$-receptor blockade, which strongly indicates that $\beta_1$-receptor stimulation did not count for the increased MBF at high ambient pressure.

## CONCLUSIONS

When pressure rises from 1 to 5 ATA, MBF increases, whether the animal is $\beta_1$-blocked or not. On the contrary, RBF changes differently in the $\beta_1$-blocked animal than in the reference animal. The change in MBF is

mediated outside the $\beta_1$ system, while the RBF change at least partially must be due to altered activation of the $\beta_1$ system.

## References

1. Onarheim J, Tyssebotn I. Effect of high ambient pressure and oxygen tension on organ blood flow in the anesthetized rat. Undersea Biomed Res 1980;7:47–60.
2. Hordnes C, Tyssebotn I, Onarheim J. Effect of high ambient pressure and oxygen tension on organ blood flow in conscious rats. Acta Physiol Scand 1982;114:23A.
3. Rudolph AM, Heymann MA. The circulation of the fetus in utero. Circ Res 1967;21:163–184.
4. Singh BN, Nisbet HD, Harris EA, Whitlock RML. A comparison of the actions of ICI 66082 and propranolol on cardiac and peripheral β-adrenoceptors. Eur J Pharmacol 1975;34:75–86.
5. Izumi T, Nishimura T, Sakai K, et al. The effect of atenolol on the cardiovascular system. Folia Pharmacol Jpn 1980;76:505–513.

# P-50 IN DIVERS DECOMPRESSING FROM 650 MSW

*B. W. Stolp, R. E. Moon, J. V. Salzano, and E. M. Camporesi*

Availability of oxygen to tissues during exposures to increased ambient pressures has not been assessed directly in the hyperbaric environment. We assume, however, in line with the principles appearing operative at sea level pressure, that for the prevailing $P_{O_2}$ of any tissue, the amount of oxygen that can be extracted from capillary blood will be dependent on four factors: regional blood flow, concentration of hemoglobin (Hb), saturation of arterial blood, and the shape of the Hb dissociation curve. This last factor may greatly affect oxygen availability during work in the hyperbaric environment.

A few data are available on the effects of hyperbaric gases on the function of Hb in solution: Johnson and Schlegel (1) showed that hydrostatic pressures up to 680 ATA do not produce a significant effect on the Hb-$O_2$ equilibrium of diluted solutions. Wells (2), on the other hand, in his thesis publication showed that at 100 ATA the Hb-$O_2$ affinity of diluted Hb solutions was increased for low oxygen tensions (left shift in the Hb-$O_2$ dissociation curve).

In a comprehensive study on intact red blood cell suspensions Kiesow (3) showed that the exposure to increased pressures of nitrogen resulted in significant increases in the affinity of Hb for oxygen. The magnitude of this left shift depended on nitrogen partial pressure and further differed at various saturation levels: the sigmoidal shape of the dissociation curve normally observed at 1 ATA approached a hyperbolic-type curve at 100 ATA $N_2$, a finding that indicates loss of cooperativity among the Hb subunits at low oxygen tensions. In this study, the use of helium as background gas did not alter the dissociation curves at 1 ATA, but did cause a much reduced left shift at 100 ATA compared to nitrogen. The loss of cooperativity at pressure, however, was still pronounced. Kiesow's experiments indicated that both absolute pressure and inert gas species may interact with the dissociation curve and reduce the ability

of oxygen to dissociate from the carrier Hb at increased ambient pressure, especially at the low levels of oxygen tensions that might be encountered in working muscles and in venous blood.

During the last several years we were able to quantitate exercise responses in trained subjects exposed to hyperbaric environments ranging from 45 to 69 ATA (4–6). During steady-state hyperbaric exercise levels, the subjects maintained their $Pa_{O_2}$ above 250 Torr by breathing gas mixtures with an oxygen partial pressure equivalent to 0.5 ATA. At heavy work rates, a significant metabolic acidosis developed at pressure, with arterial lactic acid levels much higher than those recorded for the same exercise levels while subjects were breathing air at sea level. One possible cause of this observation is that oxygen availability to the working muscles might be impaired at depth. This possibility prompted the present investigation, in which Hb dissociation curves of blood samples drawn from divers at pressure were measured directly in the hyperbaric chamber.

## METHODS

A TCS Hemox Analyzer was used to determine the oxygen dissociation curves of whole blood from three subjects (Table I) during the *Atlantis IV* Trimix-5 Dive (7-day compression of 650 msw, 65.6 ATA). This analyzer is a dual wavelength spectrophotometer, which compares the differential absorption of Hb at 560 and 570 nm and records the changes in optical properties of the molecule during oxygenation or deoxygenation. 20 μl of fresh whole blood (obtained from either finger stick or venipuncture) were added to an optical cuvette containing 4 mL of buffer (Hemox solution, pH = 7.4). A special stopper contained the humdified gas exchange tubing through which oxygen pressures of the solution in the cuvette were increased (saturation) or decreased (desaturation). The oxygen pressure of this magnetically stirred solution was measured continuously from a Clark oxygen electrode and was simultaneously plotted against the optical signal (proportional to percentage Hb saturation). The temperature of the entire cuvette and gas exchange system was continu-

TABLE I

Characteristics of the Three Subjects

| Diver | Age (yr) | Height (cm) | Weight (kg) | HB* (g/dl) |
|---|---|---|---|---|
| SP | 27 | 185 | 88.2 | 14.1 |
| PB | 36 | 177 | 77.2 | 13.1 |
| GL | 25 | 173 | 63.6 | 14.4 |

*Control values obtained before compression. Slightly higher values were measured during compression.

ously monitored and maintained close to 37° C by an Exacal EX-100 constant-temperature water bath.

The data reported are obtained from the downward or dissociation curves of Hb. These curves were obtained by bubbling inert gas (nitrogen or helium at 1 ATA; helium at pressure) through the cuvette after equilibration had been attained with gases that were premixed to produce about 300 Torr partial pressure of oxygen at each study depth.

We modified the Hemox analyzer to obtain dissociation curves at the pressure at which samples were drawn. This modification consisted of placing the cuvette, stirrer, heater, and gas exchange apparatus in the hyperbaric chamber while maintaining the pressure-sensitive photomultiplier tubes and electronic circuitry at normal atmospheric pressure. These changes were achieved by transmitting light to and from the cuvette with bundles of non-coherent fiber optics of 4 mm diameter. Signals were transmitted through the plexiglass ports of the chamber by careful alignment of the respective bundles on either side of the port. This correct positioning was achieved by mounting the polished ends of each fiber bundle in a hole drilled into solid porthole covers juxtaposed on either side of the window (see Fig. 1).

A major drawback to this technique is that significant amounts of light are lost at each fiberoptic junction; this loss results in a diminished intensity of the light incident to each photomultiplier tube (PMT). Use of a high-intensity incident light and higher gain settings on the analyzer were more than sufficient to compensate for this decreased high intensity. Control data obtained at 1 ATA with the cuvette fiberoptically isolated were indistinguishable from those we obtained using the unmodified analyzer.

Partial pressures of oxygen were measured at characteristic saturation values (25, 50, and 75%) of the oxyhemoglobin curves to yield $P_{25}$, $P_{50}$, and $P_{75}$.

We used the modified Hemox analyzer to obtain Hb dissociation curves under the following four conditions:

1) *Predive control blood samples* (*column A*, Table II) were obtained at 1 ATA and analyzed at 1 ATA with the cuvette isolated in the chamber.

2) *Decompressed blood samples* (*column B*, Table II) were drawn from venipunctures during compression depths between 530 and 650 msw (54 and 66 ATA). Because of space and scheduling considerations, the isolated cuvette and gas exchange apparatus could not be placed in the chamber during this stage of the dive. Therefore, we decompressed the blood samples to the surface (20 min) using fluorocarbon FC–80 as a gas sink. For these determinations, the cuvette was placed outside the chamber at normal barometric pressure. However, we juxtaposed the fiberoptic bundles on either side of an isolated plexiglass port to simulate conditions prevailing when the signal originated from the cuvette inside the chamber. Further, FC–80 itself did not appear to affect the shape or position of the Hb dissociation curve.

3) *Depth measurements* (*column C*, Table II) were obtained during a 22-day period of decompression from 530 to 120 msw. Fresh blood samples were drawn from finger sticks and analyzed with the isolated cuvette at pressure.

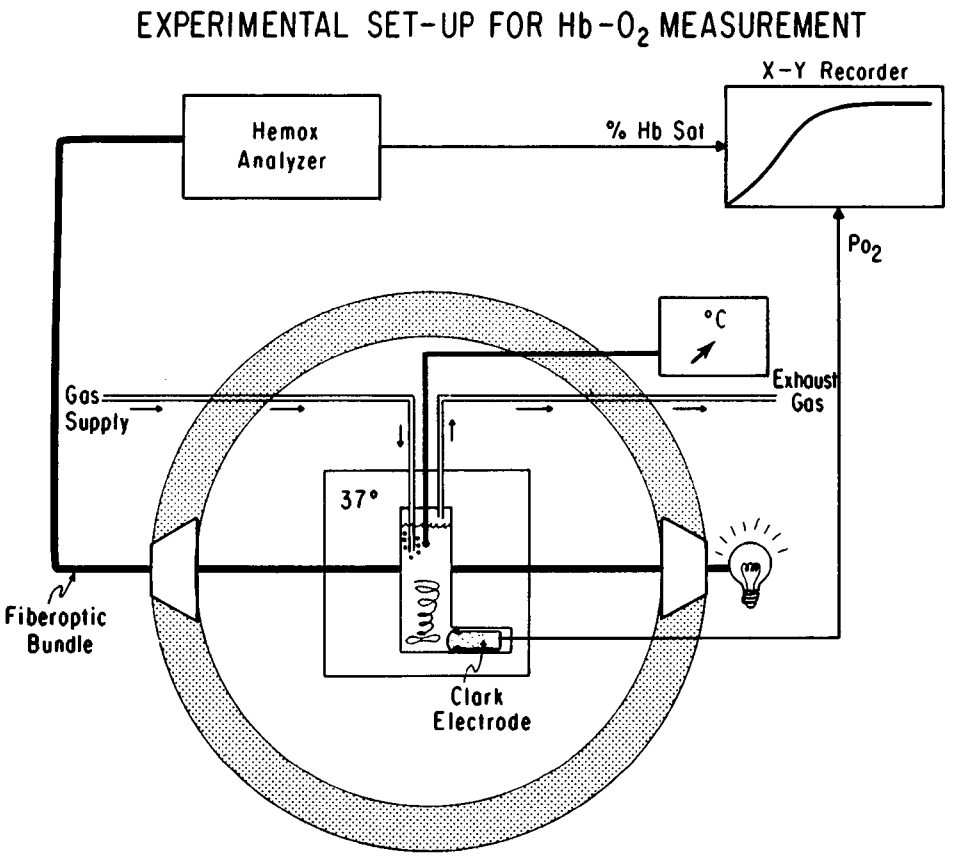



Fig. 1. Schematic diagram of the modified Hemox Analyzer. A cuvette containing buffer solution, gas supply, and exhaust system surrounded by thermoregulatory devices is located inside the pressure vessel. Incident light, outside the chamber, is transmitted by fiberoptic bundles through a chamber viewport and then to the cuvette by another fiberoptic bundle. The viewport was covered, externally and internally, by a sheet of nontransparent material. The fiberoptic bundles were inserted into a hole drilled into this material such that optimum alignment of the bundles was obtained. The light transmitted through the cuvette was carried to the photomultiplier tubes (PTM) of the instrument by fiberoptic bundles in a manner similar to the transmission of incident light. The $Po_2$ of the solution, measured by a Clark electrode inside the cuvette, was applied to the abscissa of an x-y recorder by electrical coupling though the chamber wall, Hb-$O_2$ saturation was displayed on the ordinate.

4) *Postdive samples (column D*, Table II) were drawn for 5 days after the dive and analyzed as described for predive controls.

## Atmospheric CO Determination

Throughout the dive 100-cc samples of the chamber atmosphere were withdrawn into lactic acid sealed glass syringes at various depths and analyzed for carbon monoxide (CO) concentration on a VARIAN 3700 gas chro-

matograph. For this determination, we passed the sample through a nickel catalyst methanizer to convert CO into methane, which we then separated on a 5A molecular sieve column and analyzed using a flame ionization detector (FID). Calibration gases (up to 1 ppm CO) were supplied by the Environmental Protection Agency (Research Triangle Park, N.C.).

### Carboxyhemoglobin Determination

We decompressed (20 min) 5–cc samples of venous blood in fluorocarbon FC–80 and then measured them on an IL 282 coximeter. FC–80 was shown not to affect carboxyhemoglobin concentration at 1 ATA when mixed with blood in the same manner as was used for decompression.

## RESULTS

We obtained 63 complete dissociation curves that were acceptable for analysis. Control values at 1 ATA for $P_{50}$ ranged between 24.5 and 29.2 Torr, well within the range of the norm. All $P_{50}$ values measured in the chamber, plotted as a function of the depth of measurement, are presented in Fig. 2. No significant trend between $P_{50}$ and depth of measurement can be discerned in the two subjects with multiple replications. All depth data are summarized in *column C* of Table II, which also presents statistical comparisons and the control measurements for the individual subjects. Figure 3 displays mean values of $P_{25}$, $P_{50}$, and $P_{75}$ for all three subjects. $P_{50}$ values measured at depth were always less than during predive control. Only for *Subject PB*, however, did this difference attain statistical significance (Sheffé method for multiple comparisons: F test; $P < 0.05$). Pooled values for all three divers also reached significance (Fig. 3). Similar trends can be observed for $P_{25}$ and $P_{75}$ values. No difference was observed between control and samples that were collected at depth, but decompressed and measured at the surface (*Decompressed* sample in Fig. 3). Postdive values were equal or slightly higher than predive control.

Figure 4 presents percentage saturation as a function of $P_{O_2}$, for all subjects, during predive controls, and during the prevailing pressure exposures in the hyperbaric chamber (condition 3).

## DISCUSSION

### Validation of Measurements

Use of the Clark electrode at pressure has been validated previously, and this technique has been used repeatedly in the hyperbaric chamber (7). Measurement of Hb saturation makes use of absorbance of Hb at two closely spaced wavelengths: one at which absorbance is related to oxygen saturation; the other at which absorbance is constant (isosbestic or reference point). Use

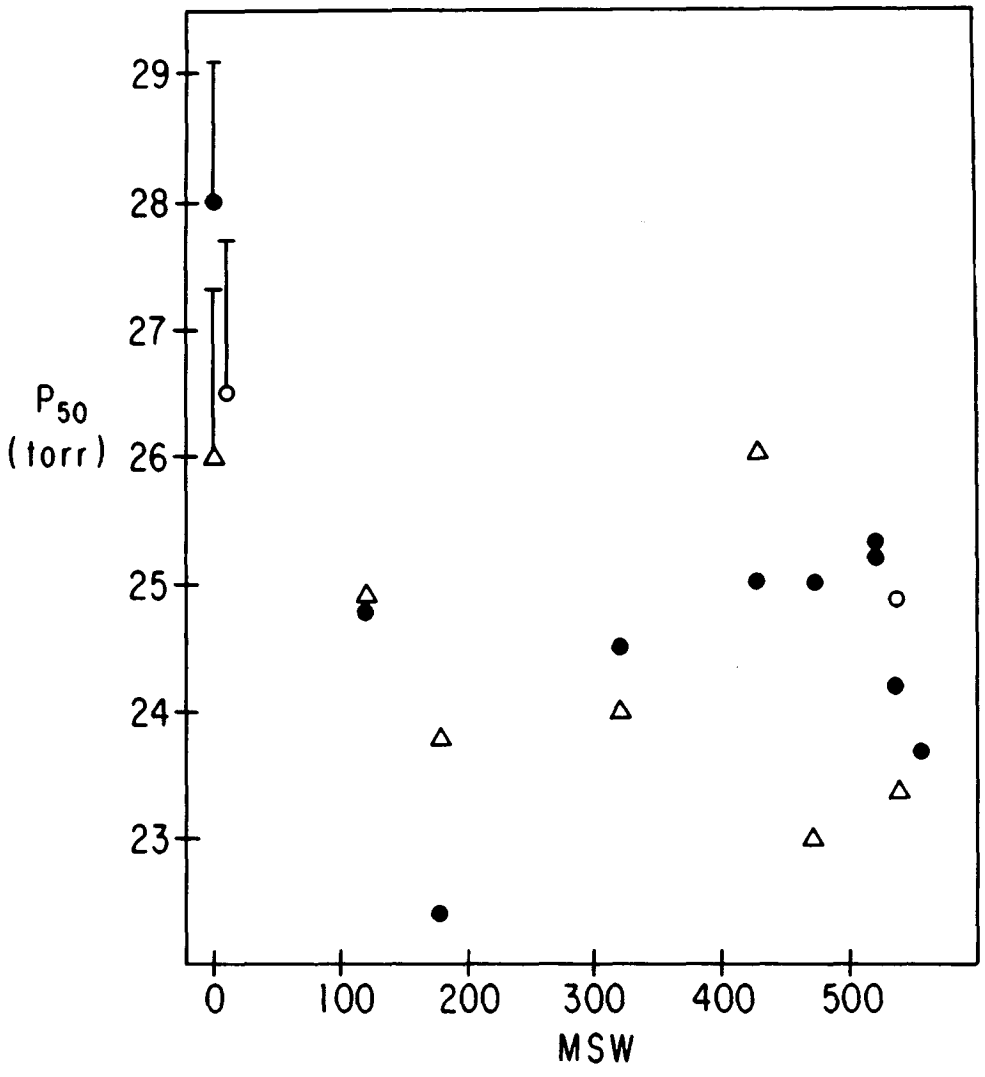



Fig. 2. $P_{50}$ values for the three subjects plotted as a function of the depth at which they were collected and analyzed (meters of sea water = msw). Mean values for predive control $P_{50}$ ± SD are included for comparison (△ = SP; ● = PB; ○ = GL). No consistent correlation between $P_{50}$ and depth can be observed.

of the Hemox Analyzer at pressure assumes that the Hb molecule has the same absorption characteristics at these two wave-lengths at pressure as it does at 1 ATA. Very small changes in the absorption spectra after compression to 1000 ATA have been reported (8), but no significant effects are apparent in the range of 0–100 ATA (3). The curves obtained using our modified apparatus at 1 ATA produced $P_{50}$ values similar to those reported in the literature. The method, therefore, appears valid both at the surface and at depth.

TABLE II

$P_{O_2}$ Values for 25, 50, and 75% Saturation ($P_{25}$, $P_{50}$, and $P_{75}$) for the Three Divers

| | Diver | Predive *A* (Torr) | Dive *B* (Torr) | Dive *C* (Torr) | Postdive *D* (Torr) |
|---|---|---|---|---|---|
| $P_{25}$ | SP | 15.8 ± 0.6(3) | 16.2 ± 1.4(8) | 14.8 ± 1.1(6) | 17.6 ± 0.6(5) |
| | PB | 17.4 ± 0.8(3) | 16.8 ± 0.8(11) | 15.3 ± 0.6(9)* | 16.9 ± 1.0(4) |
| | GL | 16.7 ± 0.9(3) | 17.4 ± 1.4(6) | 15.6 (1) | 17.9 ± 1.8(4) |
| $P_{50}$ | SP | 26.0 ± 1.3(3) | 25.9 ± 2.3(8) | 24.2 ± 1.1(6) | 28.2 ± 1.3(5) |
| | PB | 28.0 ± 1.1(3) | 27.0 ± 1.3(11) | 24.5 ± 0.9(9)* | 27.4 ± 0.8(4) |
| | GL | 26.5 ± 1.2(3) | 28.1 ± 2.0(6) | 24.9 (1) | 27.1 ± 1.5(4) |
| $P_{75}$ | SP | 38.9 ± 2.5(3) | 39.4 ± 3.5(8) | 37.6 ± 2.2(6) | 42.2 ± 2.1(5) |
| | PB | 41.9 ± 0.6(3) | 41.9 ± 2.7(11) | 37.3 ± 1.6(9)* | 41.3 ± 1.2(4) |
| | GL | 39.3 ± 1.8(3) | 42.2 ± 2.8(6) | 37.8 (1) | 40.6 ± 1.1(4) |

*A:* Samples collected and analyzed at 1 ATA. *B:* Samples collected at depth and analyzed at 1 ATA. *C:* Samples collected and analyzed at depth. *D:* Samples collected and analyzed at 1 ATA. Means ± 1 SD are presented; Numbers in parentheses indicate replications. *$P < 0.05$: significantly different from predive control.

Although temperature was monitored and recorded it was difficult to maintain the cuvette at 37°C during each experimental run. Additionally, different batches of buffer varied slightly in pH. Therefore, we used a standard nomogram to correct various points on the oxyhemoglobin curve to a pH of 7.40, and a temperature of 37°C. This procedure can be applied at depth only if temperature and pH affect Hb-$O_2$ binding in the same way at pressure as they do at 1 ATA. We did not systematically investigate these effects. Because correction factors were small (less than 4%) and random in direction, they would not likely explain the systematic leftward shift seen at pressure.

Possible differing hydrogen activity at pressure might explain the shift. This was investigated by Kiesow and found not to be a factor at pressures less than 100 ATA.

Another possible cause of a leftward shift of the Hb-$O_2$ curve is the presence of trace amounts of CO in the chamber atmosphere produced endogenously by the divers. The highest values measured in the chamber atmosphere were equivalent to 20 ppm at 1 ATA. Using the traditional affinity values for CO and Hb binding, valid at 1 ATA, and assuming that the subjects' blood were in equilibrium with the atmospheric CO, one would predict Hb-CO to be about 2% of their total Hb. Four measurements on decompressed blood obtained during this dive showed that this was indeed the case. The calculated leftward shift (9) at this point during the dive, assuming 1 ATA binding properties of CO, was approximately 1.4 Torr. However, these considerations might not apply entirely, because during the measurements we equilibrated a small blood sample with excess CO-free gas for 20–30 min.

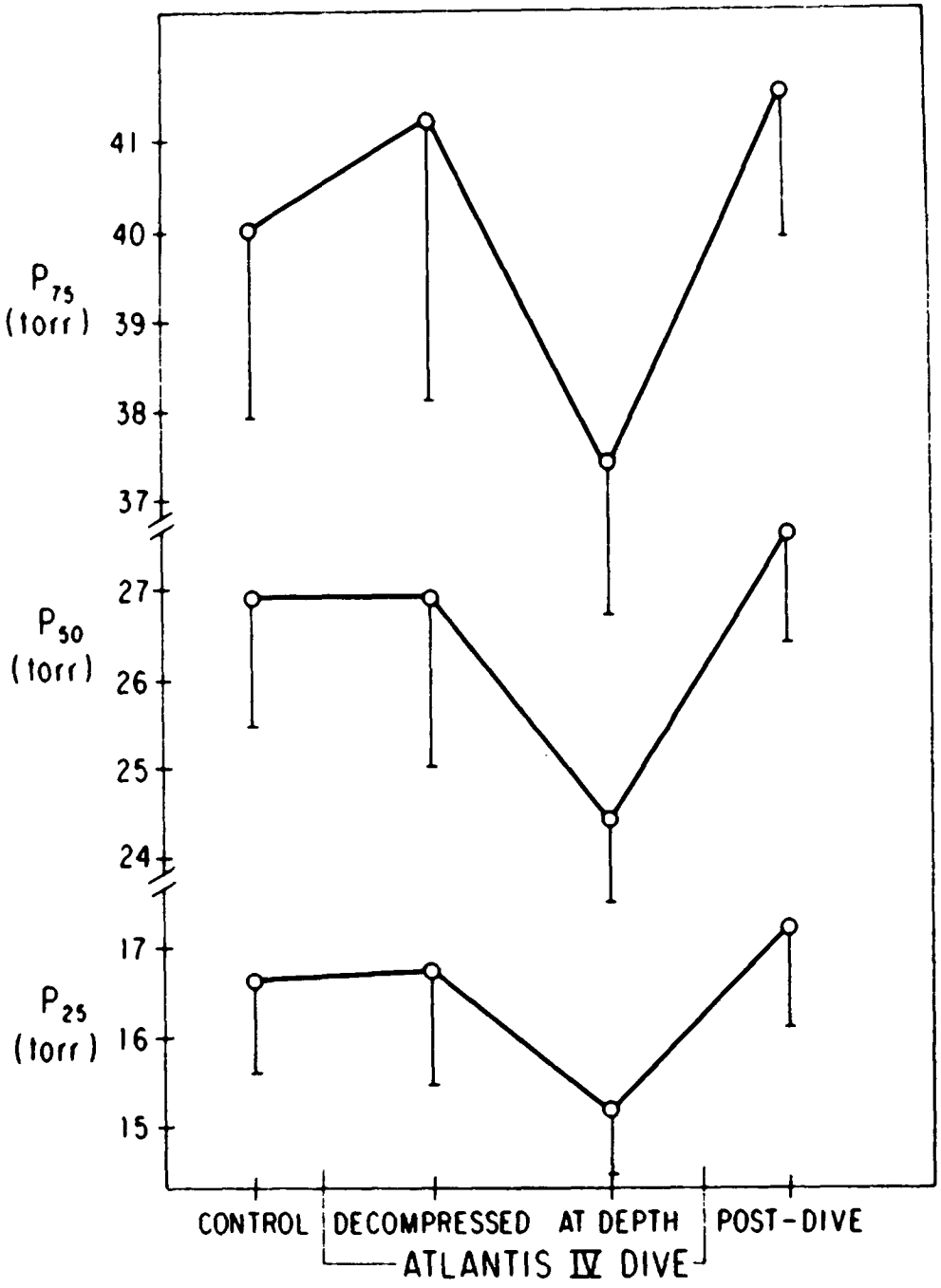



Fig. 3. Mean values of $P_{25}$, $P_{50}$, and $P_{75} \pm$ SD, for the three subjects under the various conditions studied. Values measured *At Depth* are significantly different from predive *Control*. (Sheffé F test for multiple comparisons, $P < 0.05$)

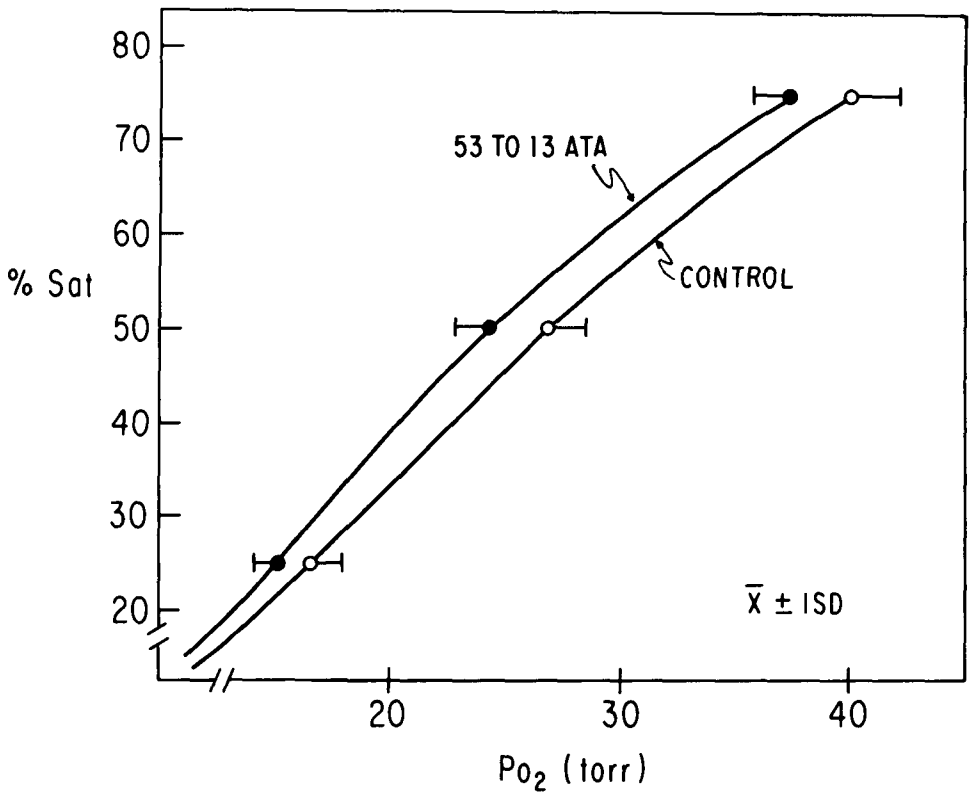



Fig. 4. Mean ± SD for $P_{25}$, $P_{50}$, and $P_{75}$ for all subjects at surface (*column A*, Table II) and depth (*column C*, Table II). The slight leftward shift of the Hb dissociation curve is evident.

The remaining possible cause of the leftward shift is the direct effect of pressure on Hb-$O_2$ binding, as suggested by Kiesow (3) and Wells (2).

The physiological impact of the leftward shift in the Hb-$O_2$ curve at pressure is debatable. Given that arterial $P_{O_2}$ values in our subjects were high, then a leftward shift in the curve would result in lower oxygen availability for any given tissue blood flow and venous $P_{O_2}$. Bile flow rate (10,11) and redox state of cytochrome $aa_3$ (12) have been shown to be affected by $P_{50}$ of hemoglobin at constant tissue blood flow. The importance of the effect on oxygen delivery to muscle is unclear (13,14). Patients with congenitally left-shifted Hb-$O_2$ curves may be only mildly symptomatic (15,16). The explanation must be that several compensatory mechanisms are available: a) increased tissue blood flow, b) increased tissue oxygen extraction, and c) erythrocytosis. However, even in a setting in which the compensatory mechanisms are fully activated a left shift of the Hb-$O_2$ curve may still impair tissue oxygenation. Oxygen transport parameters during maximal exercise in these patients with congenitally left-shifted curves have not been reported in the literature. Thus

the question of whether, in humans, a left-shifted curve may lead to impairment of tissue oxygenation under stressed conditions remains open.

An outline of gas exchange at the tissue is shown in Fig. 5. Both at 1 ATA and 65.6 ATA arterial blood is essentially 100% saturated. From the

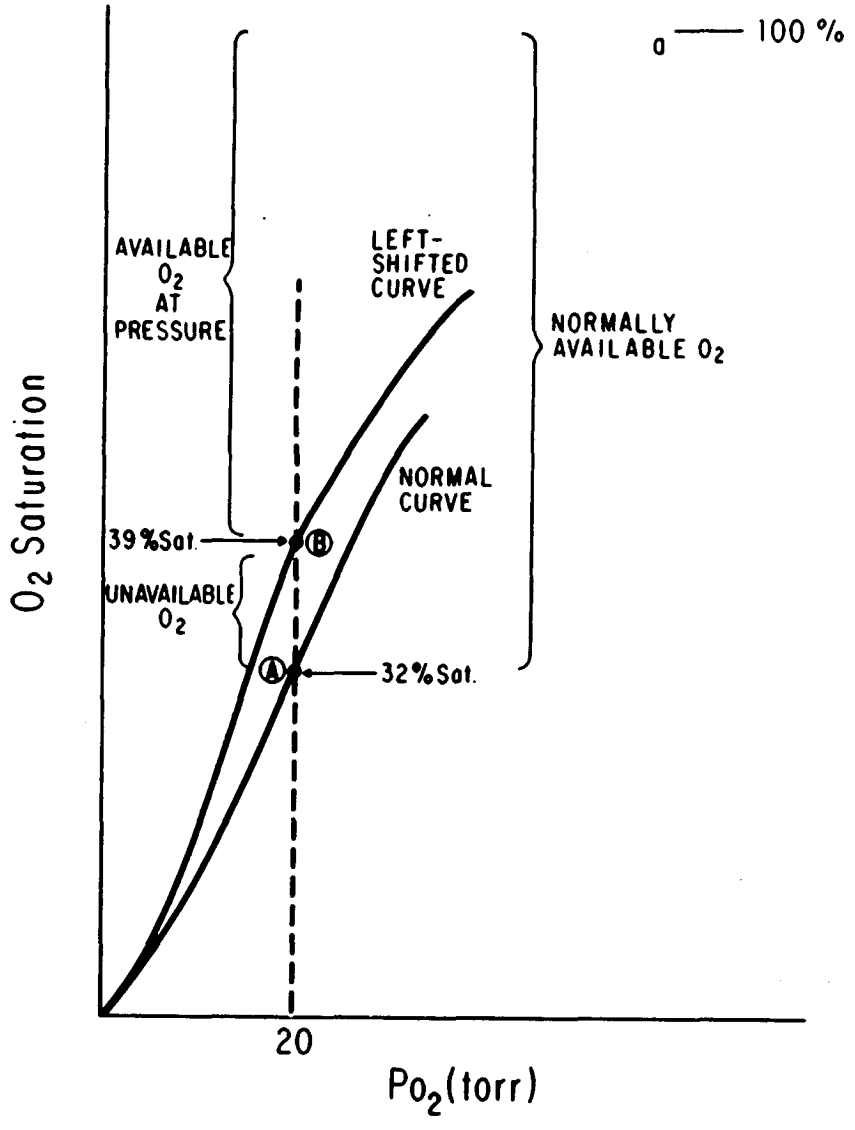



Fig. 5. Schematic diagram of tissue oxygen extraction with normal and left-shifted hemoglobin-oxygen dissociation curves.

normal curve, assuming a venous $P_{O_2}$ of 20 Torr (*point A*), it can be seen that oxygen available to the tissue is about 100 − 32 = 68% of the total oxygen delivered. With the degree of left shift at pressure observed in this experiment the venous saturation at the same venous $P_{O_2}$ is approximately 39% (*point B*). Available oxygen is therefore 100 − 39 = 61% of total delivered oxygen. Under these conditions, if one assumes a Hb concentration of 15 g/L, tissue oxygen extraction may amount to 16 mL $O_2$/L of blood less with the left-shifted curve demonstrated in our subjects than with a normal curve. If, at maximal exertion, blood flow to the exercising tissue is 25 L/min, then oxygen consumption may be lower than predicted by about 400 mL/min. These calculations are consistent with our *Atlantis III* data, which showed that oxygen consumption at 67.6 ATA at 1440 kp · m/min was lower, on average, by about 300 mL/min than at 1 ATA. In addition, there was a significant lactic acidosis, perhaps accounting for the balance of oxygen deficit.

This type of conjecture assumes that the Hb-$O_2$ curve during exercise is shifted to the left by the same amount as at rest. Of course, during actual exercise, pH and temperature changes in the working muscle are likely to produce a relatively large rightward shift in the curve, probably compensating for the slight baseline leftward shift. That is, the curve from capillary blood during exercise at pressure may not be significantly different from the curve at 1 ATA, given the increased acidity and $CO_2$ content for any given work rate at pressure (5,6). The significance of these observations is therefore unresolved. The reversibility of the shift after decompression to 1 ATA (*column B*, Table II and Fig. 3) confirms the data of Kiesow (3), suggesting that the changes seen are related to the acute effects of pressure on Hb conformation or binding.

These findings suggest that our previously reported increases in arterial lactate at pressure are not solely, if at all, caused by an effect of hydrostatic pressure on the shape and position of the oxyhemoglobin dissociation curve.

## References

1. Johnson FH, Schlegel FM. Hemoglobin oxygenation in relation to hydrostatic pressure. J Cell Comp Physiol 1948;31:421–425.
2. Wells JM. Pressure and hemoglobin oxygenation. Dissertation. San Diego: University of California, 1969.
3. Kiesow LA. Hyperbaric inert gases and the hemoglobin-oxygen equilibrium in red blood cells. Undersea Biomed Res 1974;1:29–43.
4. Moon RE, Camporesi EM, Stolp B, Salzano JV. Arterial lactate and pyruvate levels after exercise at 65.6 ATA. Physiologist 1981;24:67.
5. Camporesi EM, Salzano JV, Moon RE, Stolp B. Arterial $P_{O_2}$ and gas exchange at 46.7 and 65.6 ATA. Undersea Biomed Res 1982;9(Suppl):35.
6. Salzano JV, Camporesi EM, Moon RE, Stolp B. Arterial acid-base parameters at depths to 650 meters. Undersea Biomed Res 1982;9(Suppl):36.
7. Overfield EM, Saltzman HA, Kylstra JA, Salzano JV. Respiratory gas exchange in normal men breathing 0.9% oxygen in helium at 31.3 ATA. J Appl Physiol 1969;27:471–475.

8. Gibson QH, Carey FG. Effect of hydrostatic pressure on spectra of heme compounds. J Biol Chem 1977;252:4098–4101.
9. Roughton FJW. Transport of oxygen and carbon dioxide. In: Fenn WO, Rahn H,eds. Handbook of physiology: Respiration. Washington, DC: American Physiological Society, 1964: sect. 3, vol. 1, 767–825.
10. Bakker JC, Gortmaker GC, Vrolijk ACM, Offerijns FGJ. The influence of the position of the oxygen dissociation curve on oxygen-dependent functions of the isolated perfused rat liver. I. Studies at different levels of hypoxic hypoxia. Pfluegers Archiv 1976;362:21–31.
11. Bakker JC, Gortmaker GC, Offerijns, FGJ. The influence of the position of the oxygen dissociation curve on oxygen-dependent functions of the isolated perfused rat liver. II. Studies at different levels of hypoxia induced by decrease of blood flow rate. Pfluegers Archiv 1976;366:45–52.
12. Versmold H, Brauser B, Riegel KP. Influence of hemoglobin oxygen affinity on oxidation reduction state of cytochrome $aa_3$ in perfused liver. Adv Exp Med Biol 1975;75:145–150.
13. Thomson JM, Dempsey JA, Chosy LW, Shahidi NT, Reddan WG. Oxygen transport and oxy-hemoglobin dissociation during prolonged muscular work. J Appl Physiol 1974;37:658–664.
14. Ross BK, Hlastala MP. Increased hemoglobin-oxygen affinity does not decrease skeletal muscle oxygen consumption. J Appl Physiol: Respir Environ Exercise Physiol 1981;51:864–870.
15. Woodson RD, Heywood JD, Lenfant C. Oxygen transport in hemoglobin Koln. Arch Int Med 1974;134:711–715.
16. Poyart C, Bursaux E, Teisseire B, Freminet A, Duvelleroy M, Rosa J. Hemoglobin creteil: Oxygen transport by erythrocytes. Ann Int Med 1978;88:758–763.

# RECURRENT PULMONARY EDEMA IN SCUBA DIVERS; PRODROME OF HYPERTENSION: A NEW SYNDROME

*P. T. Wilmshurst, M. Nuri, A. Crowther, J. C. Betts, and M. M. Webb-Peploe*

The sport of underwater swimming using self-contained underwater breathing apparatus (scuba) is a growing pastime. Many of its participants, like the 40 000 in the United Kingdom, dive in relatively cold conditions. We report a previously undescribed syndrome, of divers who developed episodes of pulmonary edema while scuba diving. We investigated the arterial and venomotor responses in four such divers and compared their responses with a group of eight normal divers.

## METHODS

### Subjects: Case Reports

Four amateur divers whose clinical details are shown in Table I developed up to seven episodes of pulmonary edema while scuba diving. During these episodes they developed dyspnea, rapid regular palpitations, and expectoration of frothy, bloodstained sputum, symptoms leading in one case to syncope on two separate occasions. Symptoms always started on the bottom, often within 5 to 10 min of the start of the dive, and before their ascent. Each diver was certain that he or she had not aspirated water. Each had been accompanied and in no case did the ascent rate exceed 15 m/min. The episodes occurred only in cold water (below 12°C) and at depths of 10 to 35 m. Dives in warmer water and to shallower depths did not produce symptoms. During the episodes each diver was using standard scuba equipment including a full neoprene wet suit. Two divers used twin-hose demand valves and two used two-stage, single-hose

TABLE I

Clinical Details of the Divers Who Developed Pulmonary Edema

| | Sex | Age When Studied (yr) | Diving Experience (yr) | No. of Episodes of Pulmonary Edema | Resting Blood Pressure (mmHg) | | |
|---|---|---|---|---|---|---|---|
| | | | | | At Presentation | When Investigated | Currently |
| 1 | F | 52 | 10 | 7 | 120/80 | 135/85 | 150/100 |
| 2 | M | 52 | 3 | 2 | 170/110 | 145/90 | 140/90* |
| 3 | M | 48 | 15 | 2 | 130/80 | 140/85 | 145/90 |
| 4 | M | 38 | 5 | 1 | 125/70 | 130/80 | 125/80 |

*Current BP recorded while taking antihypertensive treatment.

demand valves. One diver developed episodes while using a number of different demand valves. In all cases the equipment was checked and found to be in working order and the compressed air used during the dives was from different sources. Most divers noticed an improvement in their dyspnea immediately on leaving the water, although symptoms did not entirely disappear until 6 to 8 h later. Physical examination by a doctor during this period revealed in each case a third heart sound and bilateral basal crepitations in the chest, but no cardiac murmur. At this time the electrocardiogram was normal but the chest x-ray showed pulmonary edema although heart size was normal (Fig. 1). In each case the chest x-ray became normal without treatment in 2 to 4 weeks (Fig. 2).

Between diving-related episodes of pulmonary edema each diver was, and currently remains, asymptomatic without added heart sounds. One month before his first episode of pulmonary edema one diver (*No. 2*) was noted to be hypertensive but was untreated. He is currently receiving metoprolol. The remaining divers were normotensive although two have since become mildly hypertensive during up to 7 years follow-up. The diver (*No. 4*) most recently discovered to have this condition remains normotensive.

Normal investigations in each case included blood count, thyroid function, chest x-ray, resting electrocardiogram, M-mode echocardiogram, and phonocardiogram. During treadmill exercise testing, each achieved stage V of the Bruce Protocol with a normal heart rate response and electrocardiogram. Exercise and rest thallium–201 scintigraphy showed normal myocardial perfusion.

## Procedures

The four subjects who had experienced episodes of acute pulmonary edema when scuba diving (*Group 1*) were compared with eight males who had a similar length of diving experience without any suggestion of cardiac or respiratory problems (*Group 2*). The ages of the subjects in the two groups

Fig. 1. The chest x-ray of a diver (*No. 1*) who had pulmonary edema while diving in the English Channel. The x-ray was taken 6 h after leaving the water.

were not significantly different: *Group 1*, 38–52 years (mean 47 years); *Group 2*, 23–63 years (mean 37 years). The heights and weights of the two groups were not significantly different and each subject was within 20% of his or her predicted weight for height. Each subject gave informed written consent for the study, which had the approval of the Hospital Ethical Committee. Medication was withdrawn 3 days before the study in the diver who was hypertensive (*No 2*). No other subject received any medication.

The studies were conducted in a sound-proofed, air-conditioned room, with the room temperature maintained at 26–28°C. The subjects were supine and in the postabsorptive state, and were allowed to rest quietly for 30 min

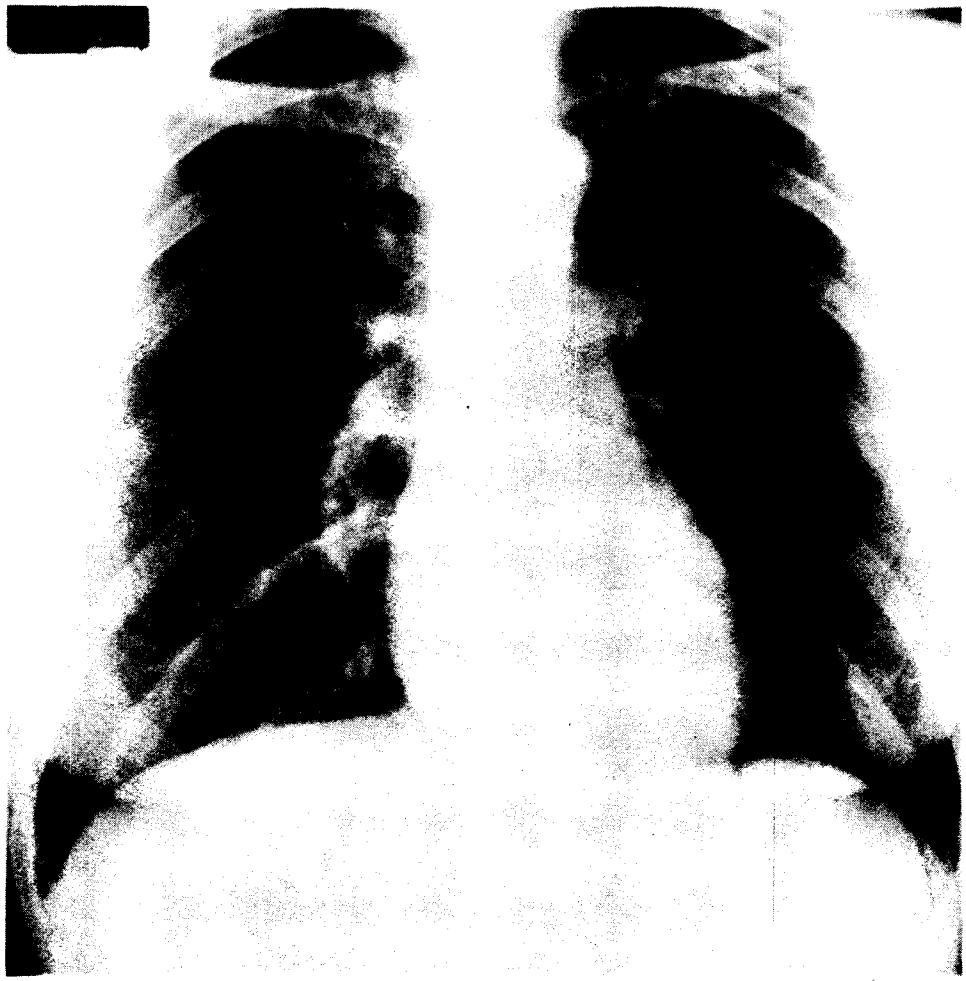


Fig. 2. The chest-ray of diver *No. 1* taken 4 weeks after the x-ray shown in Fig. 1. In the interim the subject had received no medication.

before the study. The electrocardiogram was continuously displayed on an oscilloscope, recorded by an Oxford Medilog 24-h tape-recorder, and subsequently analyzed off-line for rate and rhythm with a Reynolds Pathfinder ECG Analyzer. Equality of blood pressure in the arms was confirmed before the study. Blood pressure during the study was measured in the right arm by an automatic sphygmomanometer (sphygmotron BP 200-SM). Mean blood pressure was calculated as diastolic pressure plus one-third the pulse pressure. The left arm was supported comfortably just above the level of the heart. A venous occlusion pneumatic cuff was applied to the left upper arm and a mercury-in-rubber strain gauge was applied to the left forearm at the point of maximum

girth. Forearm venous pressure was measured by a leader-cath (ORX gauge 18 Vygon SA) inserted into an arm vein near the strain gauge and connected to a pressure transducer (AME Horton Norway AE 840). The occlusion cuff, the strain gauge, and the pressure transducer were connected to a venous occlusion plethysmograph (Medimatic SP2, Medimatic A/S, Copenhagen). The outputs from the pressure transducer and the strain gauge, representing venous pressure and alterations in forearm volume, respectively, were displayed on the chart recorder of the plethysmograph. Pressures were measured with reference to the level of the catheter tip. We measured forearm blood flow by instantaneously inflating the venous occlusion cuff to 60 mmHg and calculating the consequent rate of increase in forearm volume. Forearm vascular resistance (units) was calculated from mean arterial pressure divided by forearm blood flow.

Measurements of forearm venous responses were performed by inflation of the venous occlusion cuff to pressures of 20, then 40, and, finally, 60 mmHg, and then deflation in steps of 10 mmHg. Sufficient time was allowed for equilibration of limb volumes at each cuff pressure. The cuff and venous pressures were identical at each cuff pressure when equilibration of limb volume had occurred. Such cycles of inflation and deflation required 10–15 min with at least 15 min between successive cycles. Forearm venous responses were calculated in two ways. Venous compliance (VV60) was defined as the increase in venous volume (mL/100 mL tissue) per 1 mmHg rise in pressure at a venous pressure of 60 mmHg. The increase in volume (venous capacity, VC) was measured from the volume-time recording where both forearm volume and pressure had reached equilibrium. Thus:

$$\text{VV60} = \frac{\text{VC} \times 1000}{60} \qquad \text{mL/100 mL/mmHg/10}^{-3}$$

where 60 is the venous pressure (mmHg) and a scaling factor of 1000 is employed. Loop area (a measure of venous hysteresis) was measured by plotting for each inflation-deflation cycle equilibrated venous pressure on the abscissa (5 cm to 20 mmHg) against corresponding increase in venous volume on the ordinate (5 cm to 1 mL/100 mL tissue) and determination of the area enclosed within the pressure-volume loop using an X-Y plotter attached to a microprocessor (Numonics Data Analyser Model 1239). In our Laboratory these techniques give highly reproducible results and sensitively detect alterations in arterial and venous tone induced by a wide variety of interventions in a number of cardiological conditions (1).

Measurements were made during a number of *interventions:*

*1) Exercise.* Subject maintained a 75% maximum voluntary isometric right handgrip contraction for 2 min using an Ergometer (Model EM50 Psytech). Venous responses were not measured during this intervention because subjects were unable to maintain the required level of exercise during the period of a venous occlusion inflation-deflation cycle.

2) *Breathing air from standard scuba equipment* (compressed air cylinder and Scubapro Mark IV demand valve).

3) *Ice.* While subject breathed air as in *Intervention 2*, the head and neck were covered in towels soaked in ice-cold water.

4) *Oxygen.* Subject breathed 67% oxygen + 33% nitrogen. Measurements were made 7 min after the start of oxygen breathing.

5) *Oxygen + ice.*

Between interventions control measurements were made with the subjects breathing room air. *Interventions 4* and *5* were not performed in two of the normal divers. Reproducibility studies were performed on five normal and three abnormal divers on a subsequent day.

We analyzed results using a paired $t$ for changes within groups and the unpaired $t$ test for small samples for differences between *Groups 1* and *2*. Differences were considered significant when $P < 0.05$. Results are expressed as mean ± SEM.

## RESULTS

In both groups of divers, breathing air from scuba equipment did not alter any hemodynamic parameter when compared with control (i.e., breathing room air).

The changes in heart rate induced by interventions are shown in Table II. The control heart rates in the two groups were not significantly different. An increase in heart rate was seen during isometric exercise in both groups but was only statistically significant in *Group 2*. Heart rate rose significantly during cold exposure in both groups of divers. Heart rate was not significantly altered during oxygen breathing in either group, and administration of oxygen prevented the increase in heart rate seen during cold exposure. The heart rates during all physical interventions were similar in both groups of divers. No cardiac dysrhythmias were detected at any stage, with all subjects remaining in sinus rhythm.

Mean arterial pressures were not significantly different in the two groups of divers in the control state (Fig. 3). In the normal divers (*Group 2*) mean arterial pressure rose during isometric exercise, cold exposure, and the combination of oxygen + ice. Oxygen breathing did not alter mean arterial pressure in *Group 2*. In the abnormal divers *(Group 1)* all interventions, including oxygen breathing, caused significant increases in mean arterial pressure. During each of the interventions mean arterial pressure was significantly higher in *Group 1* than in *Group 2*.

Forearm blood flow was lower in the control state in *Group 1* compared with *Group 2*, although the difference just failed to achieve statistical significance ($P < 0.1$) (Fig. 4). Forearm blood flow was increased during isometric exercise and was reduced during cold exposure and oxygen + ice in both groups. A reduction in forearm blood flow also occurred in all divers during oxygen breathing, although, because of large scatter, the reductions for the

TABLE II

Effects of Physiological Interventions on Heart Rate (beats per minute) in the Two Groups of Divers

| | Control 1 | Exercise | Control 2 | Ice | Control 3 | Oxygen | Control 4 | Oxygen + Ice |
|---|---|---|---|---|---|---|---|---|
| Group 1 | 61.8±1.3 | 67.8±3.6 | 61.8±1.3 | 73.8±1.1* | 60.0±1.2 | 58.0±2.5 | 60.0±1.2 | 53.5±5.0 |
| Group 2 | 67.8±2.6 | 77.1±4.4** | 68.1±2.4 | 75.0±4.0** | 61.8±3.9 | 59.3±4.1 | 61.5±3.9 | 56.5±3.6 |
| Difference Between Groups | NS | NS | NS | NS | NS | NS | NS | NS |

Within Groups: significant difference from control state $*P < 0.05$, $**P < 0.01$.

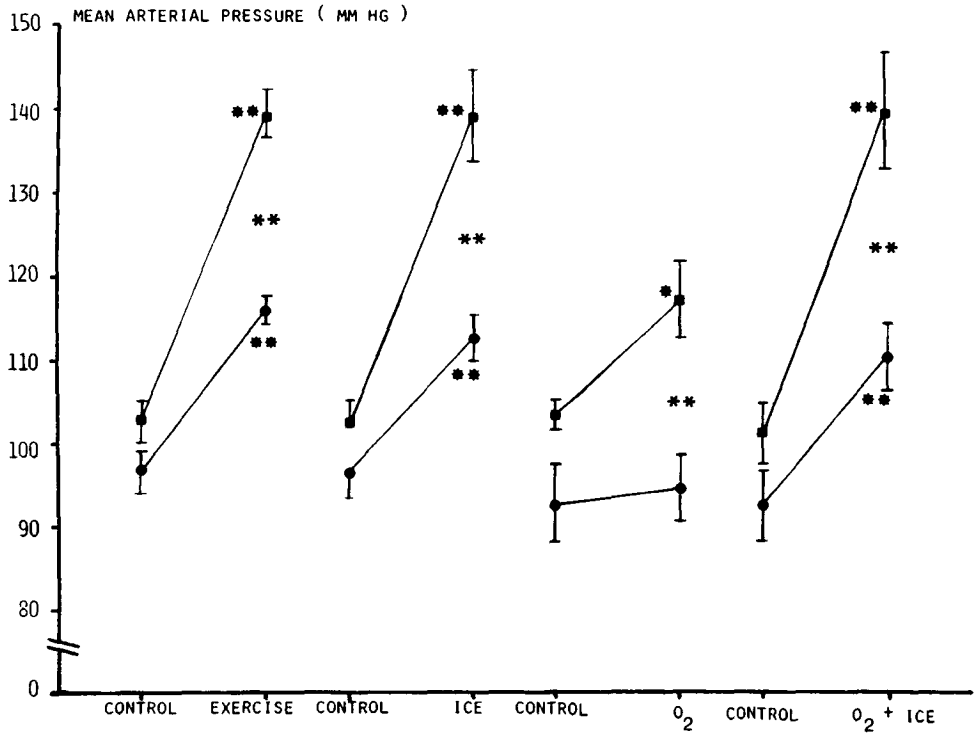



Fig. 3. Responses of mean arterial pressure in the divers. *Group 1* ■, *Group 2* ●; difference between groups ** $P < 0.01$; within-group change from control * $P < 0.05$, ** $P$ 0.01.

groups as a whole were not significantly different from control. During all interventions forearm blood flow was significantly lower in *Group 1* than *Group 2* divers.

Changes in forearm vascular resistance in the divers are shown in Fig. 5. In the control state forearm vascular resistance was significantly higher in *Group 1* than *Group 2*. Forearm vascular resistance was significantly reduced by exercise and increased by the other interventions in both groups. During all states the difference between the groups was maintained, with exceedingly high levels of forearm vascular resistance being found in *Group 1* during cold exposure and the combination of oxygen + ice.

The venomotor responses in the divers are shown in Fig. 6. Venous compliance (Fig. 6*a*) was reduced significantly from control values in both groups during cold and oxygen + ice. The reduction in VV60 during oxygen breathing just failed to achieve statistical significance ($P < 0.1$). Loop area (Fig. 6*b*) was significantly reduced by all pressor interventions in both groups. Throughout the study, as assessed by reduced VV60 and loop area, *Group 1* had greater venomotor tone than *Group 2*.

In both groups of divers, cold and oxygen had constrictor effects on both the arterial and venous systems. The constrictor effects of cold were greater

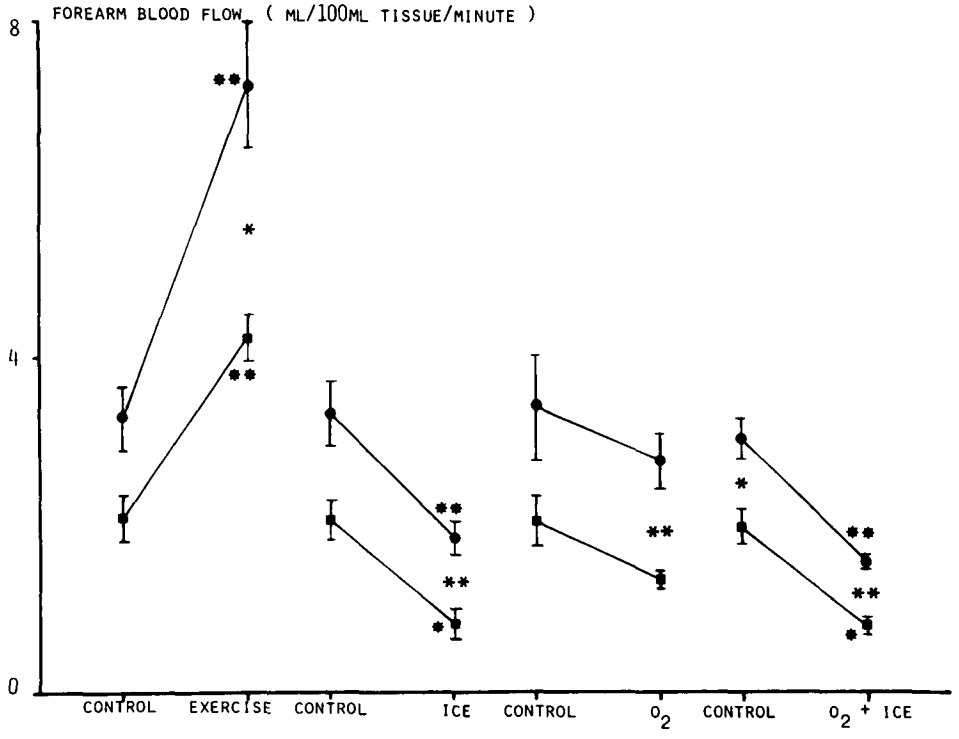



Fig. 4. Responses of forearm blood flow in the divers. *Group 1* ■, *Group 2* ●; difference between groups * $P < 0.05$, ** $P < 0.01$; within-group change from control * $P < 0.05$, ** $P < 0.01$.

than oxygen alone and the addition of oxygen breathing to cold exposure did not produce any detectable additional pressor effect. However, during the combination of oxygen + ice, all four *Group 1* divers developed third heart sounds, two (*Nos. 1* and *2*) developed soft mitral systolic murmurs, and one (*No. 3*) developed acute pulmonary edema, which was treated with an infusion of nitroprusside.

In three *Group 1* and five *Group 2* divers repeat studies showed the results to be highly reproducible.

## DISCUSSION

Pulmonary edema may develop in surface-supplied divers during equipment failure or unanticipated rapid descents and in breath-holders who dive deep (2). In these cases the intrapulmonary gas volumes are excessively reduced as a result of increases in ambient water pressure relative to the initial intrapulmonary pressure; this reduction results in a shift of blood to the thorax,

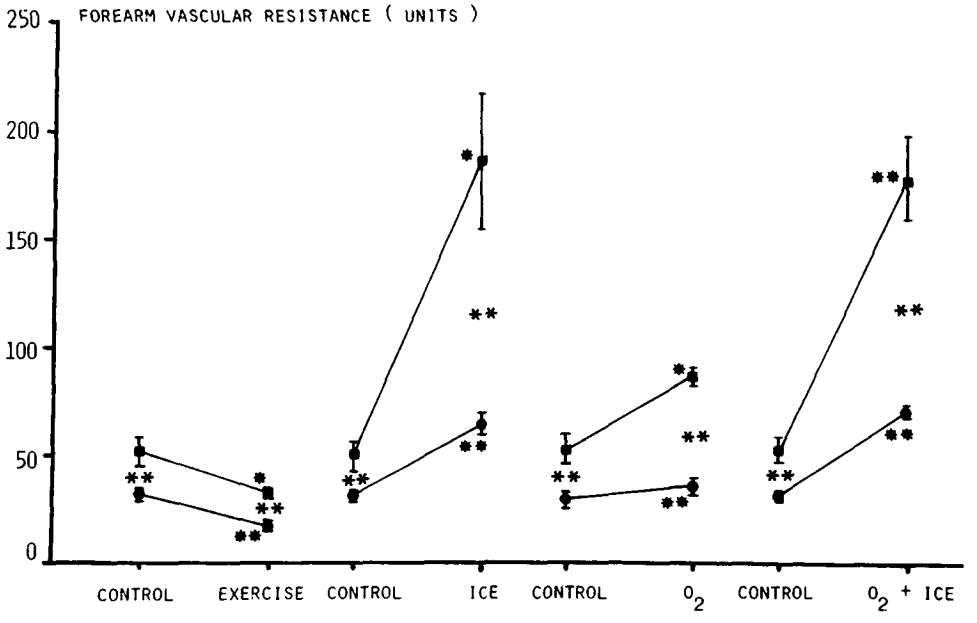



Fig. 5. Responses of forearm vascular resistance in the divers. *Group 1* ■, *Group 2* ●; difference between groups ** $P < 0.01$; within-group change from control * $P < 0.05$, ** $P < 0.01$.

engorgement of pulmonary capillaries, and fluid transudation into the alveoli. The scuba demand valve ensures that the air delivered to the diver's mouth and lungs is at approximately the pressure of the surrounding water, so such a mechanism cannot be invoked as the cause of pulmonary edema in the *Group 1* divers.

It was clear from the histories obtained from the divers who had experienced pulmonary edema that dyspnea occurred soon after the start of a dive and before the ascent. The dyspnea only occurred in cold water and at depths greater than 10 m; it was associated with palpitations and was relieved considerably by the diver's removal from the water. It thus seemed clear that in this syndrome a number of factors might be implicated, including immersion, cold, altered partial pressures of gases, and cardiac dysrhythmias. It did not seem likely, in view of the lack of cardiorespiratory symptoms at other times, good exercise performance, and normal investigations, including myocardial perfusion scan, that these subjects had intrinsic cardiac disease, although coronary arteriography was not performed.

Immersion, like weightlessness for other reasons, causes a rapid redistribution of blood from the lower limbs to the thorax (3). Immersion with the head above water increases intrathoracic blood volume by between 200 and 700 mL; even in warm water (35°C) immersion increases mean right atrial pressure by 18 mmHg and mean pulmonary artery pressure by 17 mmHg with

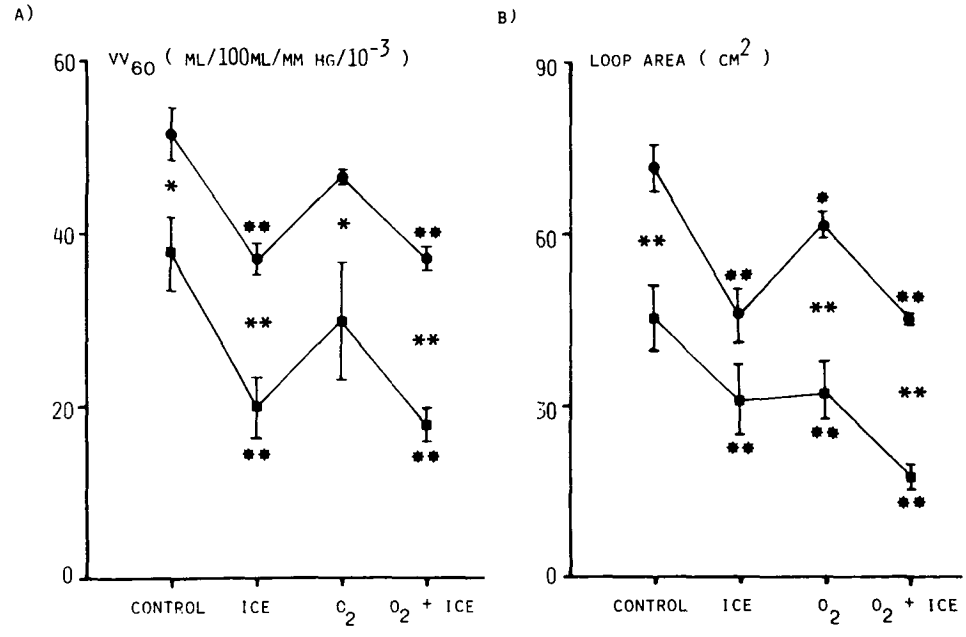



Fig. 6. The venomotor response in the divers. *Group 1* ■, *Group 2* ●; difference between groups * $P < 0.05$, ** $P < 0.01$; within-group change from control * $P < 0.05$, ** $P < 0.01$.

an increase in stroke volume and cardiac output of more than 30% (4,5). In our study the subjects lay supine, so that gravitational effects were minimized and right heart filling pressures increased.

In amateur divers wearing wet suits, the area of the body exposed to flowing water and to which a large amount of cooling occurs is the face and head (6). We thus elected to cool only the head and neck with ice-cold water. In this way the limbs in which hemodynamic measurements were made were affected only reflexly by cold exposure. During such exposure other workers (7) have shown reduced peripheral blood flow and raised systemic blood pressure as a result of sympathetically mediated peripheral arterial and venous constriction. However, cold exposure alone has not been described as causing pulmonary edema, in cold water swimming, for example, and so additional factors may be implicated in divers who develop pulmonary edema.

In divers at a raised ambient pressure the partial pressure of all inhaled gases is increased proportionately. Amateur scuba divers breathe only air. Nitrogen is relatively inert and carbon dioxide is present only in small amounts. We considered that if altered partial pressures were implicated in development of pulmonary edema in our subjects, the gas most probably involved would be oxygen, which can, per se, exert vasoconstrictor effects (8). Our study used 67% oxygen, giving a partial pressure similar to that found at depths of 20–30 m, where most amateur scuba diving is conducted.

Breath-hold diving is known to be associated with alterations in cardiac rhythm. Vagally mediated bradycardia and sympathetic peripheral vasoconstriction form part of the diving reflex (9,10). The bradycardia is most marked at low water temperatures (9,11). In cold water other dysrhythmias are also common, occurring in up to 74% of cases (12). Because we were unsure of what part such dysrhythmias might play in the development of pulmonary edema in our subjects, the electrocardiogram was recorded continuously.

No cardiac dysrhythmias were seen in either group of divers during the study. The heart rate responses of the two groups were identical for all stimuli. It was interesting that in both groups of nonbreath-holding divers cooling of the face and head with cold, wet towels increased the heart rate. This heart rate increase was prevented by simultaneous oxygen administration.

Mean arterial pressure was similar in both groups of divers at rest, but forearm vascular resistance was increased and forearm blood flow was reduced in *Group 1*. Similarly, in the control state both indices of venomotor activity suggested greater venomotor tone in the divers who had experienced pulmonary edema. Mean arterial pressure reached higher levels during all interventions in *Group 1* that it did in *Group 2*, and only in *Group 1* did oxygen administration raise blood pressure. Forearm vascular resistance also increased considerably more in *Group 1* than in *Group 2* during cold exposure and simultaneous administration of oxygen + ice. Other differences between the groups were maintained during all interventions.

It is of interest that during oxygen + ice the level of forearm vascular resistance achieved by *Group 1* was considerable, in our experience, but no greater than when ice alone was administered. However, during simultaneous administration of both stimuli, all four *Group 1* divers developed evidence of cardiac decompensation. All had a third heart sound without a tachycardia. The techniques we employed, of course, only measure alterations in hemodynamics in the limbs and do not measure alterations in other vascular beds, e.g., splanchnic vessels. It is possible that the combined stimuli also caused an additional increase in resistance to flows through other vascular beds. Another possibility is that the combined stimuli could have produced coronary spasm, but was not associated with chest pain.

The data suggest that the divers who developed pulmonary edema had higher resting arterial and venous tone, and showed exaggerated constrictor responses to the stimuli we employed. The increased preload and afterload, coupled with the increased central blood volume during weightlessness were, we believe, sufficient to produce pulmonary edema without need to postulate any intrinsic cardiac abnormality. There are other situations where large increases in vasomotor tone are known to produce pulmonary edema, e.g., following cerebrovascular accidents. Since each of the divers had enjoyed many years of trouble-free diving before these episodes of pulmonary edema, it seems likely that these abnormal vascular responses had developed over a period of time. These responses are similar to those which we have seen in "labile" hypertensives. It is therefore of some interest that on follow-up of up

to 7 years, two of the three normotensive individuals have become hypertensive. This syndrome in divers may be prodromal of hypertension.

Pulmonary edema amongst amateur scuba divers, although previously undescribed, may be quite common. We have seen an additional four divers who each give a history of having had at least two episodes similar to those in the *Group 1* divers, although none had sought medical advice until sometime later. This was because each felt much improved on leaving the water. Only two divers have been studied in the manner described above. Their resting blood pressures were 110/65 and 165/105 and their hemodynamic responses were identical to those seen in *Group 1*. (Because we had no objective medical proof that these divers also had pulmonary edema, although the history was highly suggestive, we have not included them in *Group 1*).

Failure of divers to report this problem may be due to isolation from medical facilities when diving and rapid resolution of symptoms on leaving the water. The immersion diuresis occuring under weightless conditions (13) and rapid redistribution of body fluids on returning to normal gravity undoubtedly play a part in this recovery. Fear of a ban on future dives may also account for under-reporting of this condition. It is possible that some deaths in scuba divers previously attributed to drowning may, in fact, be the result of pulmonary edema.

## References

1. Nuri M, Crowther A, Webb-Peploe MM. Forearm vascular responses in congestive cardiac failure. Br Heart J 1981;45:349.
2. U.S. Navy U.S. Navy diving manual. NAVSHIP 250-538, Part 1. Washington, DC: Navy Department, 1963.
3. Johnston RS, Dietlein LF, eds. Biomedical results from Skylab. Washington, DC: Scientific and Technical Information Office of the National Aeronautic and Space Administration, 1977.
4. Hong SK, Cerretelli P, Cruz JC, Rahn H. Mechanics of respiration during submersion in water. J Appl Physiol 1969;27:535–538.
5. Arborelius M, Balldin UI, Lilja B, Lundgren CEG. Hemodynamic changes in man during immersion with the head above water. Aerosp Med 1972;43:592–598.
6. British Sub-Aqua Club. Hypothermia, Chap 2. British sub-aqua club diving manual, 10th ed. Bristol: J.W. Arrowsmith, 1977:113–117.
7. Keatinge WR, McIlroy MB, Goldfien A. Cardiovascular responses to ice-cold showers. J Appl Physiol 1964,19:1145–1150.
8. Bird AD, Telfer ABM. Effect of hyperbaric oxygen on limb circulation. Lancet 1965;1:355–356.
9. Song SH, Lee WK, Chung YA, Hong SK. Mechanism of apneic bradycardia in man. J Appl Physiol 1969;27:323–327.
10. Brick I. Circulatory responses to immersing the face in water. J Appl Physiol 1966;21:33–36.
11. Kawakami Y, Natelson BH, DuBois AB. Cardiovascular effects of face immersion and factors affecting diving reflex in man. J Appl Physiol 1967;23:964–970.
12. Hong SK, Song SH, Kim PK, Suh CS. Seasonal observations on the cardiac rhythm during diving in the Korean Ama. J Appl Physiol 1967;23:18–22.
13. Strauss RH. Diving medicine. New York: Grune & Stratton, 1976.

# Part V

## THERMAL EFFECTS OF THE HYPERBARIC ENVIRONMENT

# SHIVERING THRESHOLD IN MAN

*G. Bolstad, A. O. Brubakk, A. Påsche, and B. Holand*

In man, several physiological defense mechanisms against cold are active: vasoconstriction of cutaneous vessels, shivering and nonshivering thermogenesis. Shivering is primarily controlled by hypothalamic centers, while higher brain centers may be involved in modulating this response (1,2). Both central and peripheral receptors are active in thermal regulation, although the peripherals are considered to be absolutely dominating (3). It is still not known, however, precisely what kind of stimulus activates these receptors. Several possibilities have been suggested including absolute temperature, temperature change, or rate of temperature change (3–5).

To investigate further the effect of these possible stimuli on shivering threshold, we have exposed subjects to gradual cooling in various atmospheres and at different pressures.

## MATERIALS AND METHODS

Two male divers were studied. Their physical characteristics can be seen from Table I.

The study was performed at the surface on air, at 26 and 51 ATA during two simulated heliox saturation dives.

The divers were dressed in shorts only, and the following parameters were recorded: chamber temperature, rectal temperature, four skin temperatures, and muscle temperature of vastus lateralis of the right leg. The subjects rested sitting on the bunks interrupted by a series of three maximal isometric contractions of the knee extensors and the hand grip muscles every 20 min. Control values of all parameters were recorded immediately before the

TABLE I

Physical Characteristics of the Subjects

| Diver No. | Age | Height (cm) | Weight (kg) | Average Skinfold (mm) | Body Surface Area ($m^2$) |
|---|---|---|---|---|---|
| 1 | 28 | 176 | 74.5 | 14.5 | 1.91 |
| 2 | 35 | 185 | 85.0 | 20.1 | 2.09 |

cooling started. Start of shivering was reported by the subjects and observed visually by the investigators.

The cooling rate of the chamber can be seen from Fig. 1. The cooling rate was essentially the same in all three situations, but due to some technical problems only approximately linear. Rectal temperature was recorded with a thermistor type YSI–701 (Yellow Springs Instruments Co., Yellow Springs, OH). Skin temperatures were recorded with thermistors (type YSI–709) on the following locations: chest (T1), lower arm (T2), thigh (T3), and front calf (T4). Both rectal ($T_{re}$) and skin temperature ($T_s$) were recorded by a digital voltmeter. Mean skin temperature was calculated according to the Ramanathan Technique (6) using the following equation: Mean skin temperature ($T_s$) = 0.3 T1 + 0.3 T2 + 0.2 T3 + 0.2 T4). Muscle temperature ($T_m$) was recorded in the vastus lateralis of the right leg, about 20 cm from the patellae, by a barehead Fenwall (fast response time) thermistor, type BC 32 L, with a response time in the muscle of 0.4 s. The thermistor was inserted 3–5 cm into the muscle by a hypodermic needle. This was done under a local anesthetic, and the needle was withdrawn afterward. The leads of the thermistor were then taped to the skin and connected through an amplifier to a two-channel Hewlett-Packard recorder, model 17402A. We measured skinfold thicknesses using Harpenden Skinfold Calipers on the upper thorax, just below the scapula; over the left triceps muscle; and on the abdomen, just left of the navel. Mean skinfold was calculated as the arithmetic mean of these three measurements.

## RESULTS

The results are presented in Tables II, III, and IV.

The time for the onset of shivering appears to be inversely related to pressure. The chamber temperature ($T_{ch}$) before cooling started, was that recommended by Wilcock and Flook (7) at 51 ATA, while it was 1°C lower than the recommended value at 26 ATA. Shivering threshold occurred when the $T_{ch}$ was approximately 6°C lower at 26 ATA than at 51 ATA and still 11°C lower at surface, in air. Also, $T_s$ as well as $T_{re}$ and $T_m$ were considerably higher during the deepest dive, when shivering occurred.

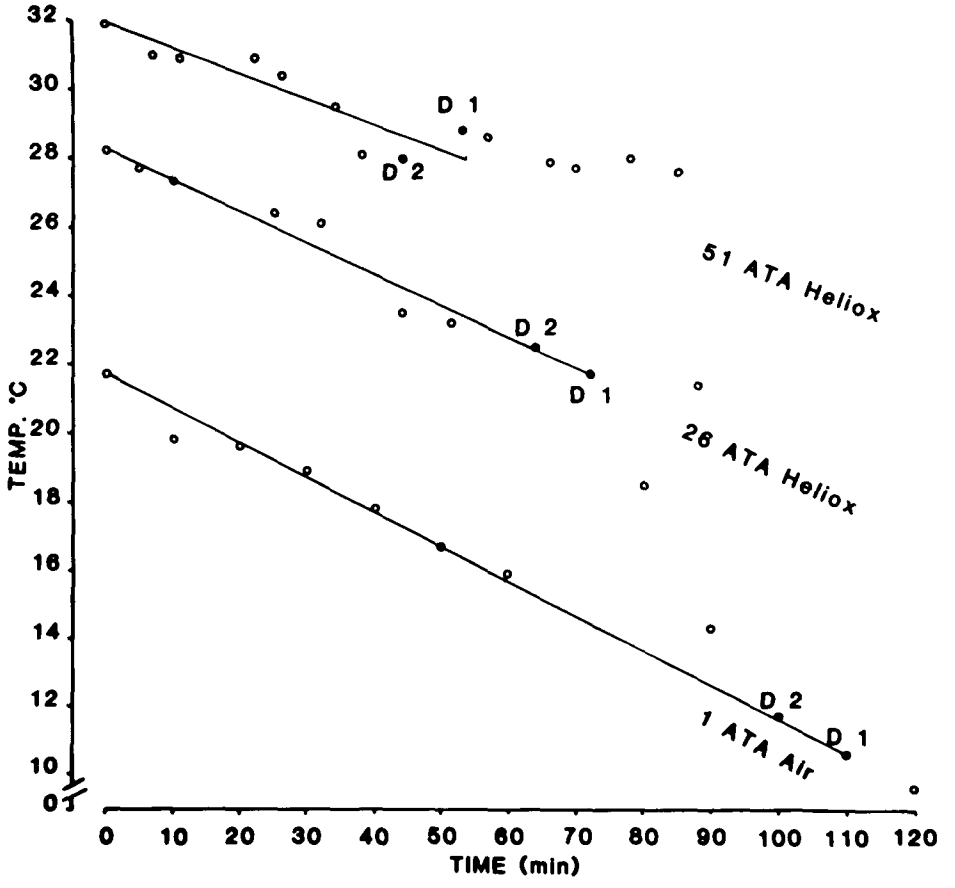



Fig. 1. Cooling profile of the chamber at three different pressures. ●: start of shivering; D1: *Diver No. 1*; D2: *Diver No. 2*.

At 51 ATA, shivering was reported in both divers after a decrease in $T_{ch}$, which was approximately half of that seen at 26 ATA, which again was only half of the decrease observed at surface. $T_s$ also showed the same tendency. In contrast to the decrease observed in $T_m$ at 1 and 26 ATA, $T_m$ had increased slightly at 51 ATA, even before the divers announced that shivering had started. Upon further exposure $T_m$ decreased, also at 51 ATA, although much less than what was seen at 26 ATA. $T_{re}$ was virtually unchanged at shivering threshold in both situations.

## DISCUSSION

Thermoregulation is considered to be a process in which the core temperature is compared to a "set" point (3). This set point is probably deter-

TABLE II

Absolute Temperature at Shivering Threshold

| Diver No. | Chamber Pressure (ATA) | Exposure Time to Start of Shivering (min) | Temperature (°C) | | | |
|---|---|---|---|---|---|---|
| | | | Chamber ($T_{ch}$) | Mean Skin ($T_s$) | Rectal ($T_{re}$) | Muscle ($T_m$) |
| 1 | 1 Air | 108 | 10.8 | 25.8 | 37.1 | 32.7 |
| | 26 Heliox | 72 | 21.7 | 27.1 | 36.8 | 31.5 |
| | 51 Heliox | 53 | 28.8 | 30.7 | 37.4 | 33.7 |
| 2 | 1 Air | 103 | 11.4 | 27.4 | 37.4 | 33.2 |
| | 26 Heliox | 64 | 22.5 | 27.7 | 37.6 | 33.9 |
| | 51 Heliox | 44 | 28.0 | 30.9 | 37.8 | 36.8 |

mined by biochemical and hormonal factors and may be varied, as happens when a person has a fever. Environmental temperature changes initiate regulatory processes like vasoconstriction and shivering.

Our data show that in these divers neither absolute nor relative environmental and diver temperatures seemed to initiate shivering. On the other hand, on the basis of these data rate of change of temperature cannot be discounted as a contributing factor.

One possible explanation for the data obtained in this study is that high environmental pressure and helium can influence the set point level. This is unlikely, as the core temperature was similar at different pressures. However, a change in the gain of the feed-back loop cannot be discounted. An increase in gain with increasing pressure could explain our data because it would lead to larger temperature oscillations. It is well known that divers under pressure have a very narrow range of comfortable temperatures, a fact that would agree with such a theory. Furthermore, the hyperirritability observed in man as well

TABLE III

Total Temperature Change at Shivering Threshold

| Diver No. | Chamber Pressure (ATA) | Temperature (°C) | | | |
|---|---|---|---|---|---|
| | | Chamber ($T_{ch}$) | Mean Skin ($T_s$) | Rectal ($T_{re}$) | Muscle ($T_m$) |
| 1 | 1 Air | −11.6 | −6.3 | −0.1 | −1.1 |
| | 26 Heliox | −5.6 | −4.8 | −0.1 | −2.4 |
| | 51 Heliox | −3.4 | −2.4 | −0.1 | +0.5 |
| 2 | 1 Air | −10.0 | −4.5 | −0.1 | −0.8 |
| | 26 Heliox | −5.7 | −4.9 | −0.1 | −3.0 |
| | 51 Heliox | −3.7 | −2.2 | −0.1 | +0.7 |

TABLE IV

Rate of Temperature Change at Different Environmental Pressures

| Diver No. | Chamber Pressure (ATA) | Rate of Temperature Change (°C/min) | | | |
|---|---|---|---|---|---|
| | | Chamber ($T_{ch}$) | Mean Skin ($T_s$) | Rectal ($T_{re}$) | Muscle ($T_m$) |
| 1 | 1 Air | −0.10 | −0.06 | −0.001 | −0.010 |
| | 26 Heliox | −0.08 | −0.07 | −0.001 | −0.033 |
| | 51 Heliox | −0.06 | −0.05 | −0.002 | +0.009 |
| 2 | 1 Air | −0.10 | −0.04 | −0.001 | −0.008 |
| | 26 Heliox | −0.09 | −0.08 | −0.002 | −0.047 |
| | 51 Heliox | −0.08 | −0.05 | +0.002 | +0.016 |

The rate of temperature change was small and did not show any consistent differences in the three situations.

as in animals at increasing pressure, i.e., the high pressure nervous syndrome, could also be compatible with such a hypothesis.

Piantadosi et al. (8) have shown that increased respiratory heat loss can initiate shivering at normal skin temperature. They found that the metabolic response was related to the rate of fall in rectal temperature. This effect could play a role in our subjects, although the changes observed are small.

The main effect of increased environmental pressure is an increased heat loss at the same temperature gradient. Our data seem to indicate that the body is capable of detecting the degree of heat loss and increasing metabolism before significant temperature changes occur.

This hypothesis, although admittedly based on few data, can be tested in hyperbaric environments and can perhaps give new insight into the mechanisms of temperature regulation in man.

## References

1. Hong S, Nadel E. Thermogenetic control during exercise in a cold environment. J Appl Physiol: Respir Environ Exercise Physiol 1979;47:1084–1089.
2. Martin S, Cooper KE. Factors which affect shivering in man during cold water immersion. Pfluegers Arch. 1981;391:81–83.
3. Benzinger TH. Heat regulation: Homeostasis of central temperature in man. Physiol Rev 1969;49:671–759.
4. Hayward JS, Eckerson JD, Collis ML. Thermoregulatory heat production in man: Prediction equation based on skin-core temperatures. J Appl Physiol: Respir Environ Exercise Physiol 1977;42:3377–384.
5. Veicsteinas A, Rennie DW. Thermal insulation and shivering threshold in Greek sponge divers. J Appl Physiol: Respir Environ Exercise Physiol 1982;52:845–850.
6. Ramanathan N. A new weighting system for mean surface temperature of the human body. J Appl Physiol 1964;19:531–533.
7. Wilcock SE, Flook V. Environmental and body temperatures of 52 divers in hyperbaric heliox. Undersea Biomed Res 1980;7:225–239.
8. Piantadosi CA, Thalmann ED, Spaur WH. Metabolic response to respiratory heat loss induced core cooling. J Appl Physiol: Respir Environ Exercise Physiol 1981;50:829–834.

# METABOLIC RATE OF DIVERS IN A SIMULATED "LOST BELL" SURVIVAL TEST

*J. Magnusson, I. Holmér, S. Elnäs, and R. W. Hamilton*

On several occasions diving bells dropped in deep, cold water have resulted in fatalities. The deaths have been attributed to failure of life support functions, which results in hypothermia and the accumulation of carbon dioxide ($CO_2$).

Cold plays a dual role here; the first is the obvious one of cooling the diver, but, in addition, at the temperatures in question the soda lime used to absorb $CO_2$ can lose most of its effectiveness.

The conditions in a stranded bell, if we assume no flooding or mechanical accident, are an atmosphere of helium and oxygen with generally 2 to 10% $O_2$ at the start, a cool-down rate that may reach 10°C within 5 h and 6°C in 16 h, and 2 or 3 divers exhaling $CO_2$ into a volume of about 2 $m^3$.

Any gas at high pressure, but particularly helium, causes rapid heat loss from the body. This loss is due to the high thermal conductivity of helium as well as the high convective heat transfer of dense gas. These losses occur from the surface of the body in the familiar way by convection and conduction, but especially from the breathing gas where the loss is due to convective transfer alone. With ambient temperature just a few degrees above freezing the high rate of heat transfer can be disabling in a few hours (1).

In one bell recovered after 17 h in the North Sea the divers were moribund and cold and were given up for dead and brought to the surface. In seeking a way to prevent future occurrences of this sort, both government authorities and scientific researchers considered whether it might be possible to protect a diver by means of passive insulation. A practical test was conducted at the Norwegian Underwater Technology Center (NUTEC) in January 1980 (2). In this test, *Polar Bear I*, several garments were evaluated and the passive insulation concept was demonstrated to be feasible. Traditional exposure suits

were shown to be ineffective, but two "systems" protected volunteer divers without significant body temperature loss for 10–11 h at 4–6°C in a helium-oxygen environment at 150 msw (metres of sea water; 1 msw ≡ 1/10 bar = 10 kPa). These systems consisted of heavy insulation for the body and "thermal regenerators" to prevent heat loss through the breathing gas. These breathing gas regenerators absorb $CO_2$; they capture heat from each exhaled breath and warm the next inspiration. The warmth and moisture of the exhaled breath also results in efficient $CO_2$ absorption, even at low ambient temperature (3).

A second *Polar Bear* experiment extended the test depth to 300 msw with results similar to the earlier experiment; by this time commercial manufacturers had refined the protective equipment (4,5), but there were still doubts as to whether these 10-h tests proved that survival for 24 h was possible. In retrospect, it appears that these doubts may have been based on standards of comfort and performance, not merely survival.

The next test was performed by the British Royal Navy in a real bell lowered with 3 divers to 250 msw in the sea (6). This test introduced another innovation, a net stretched across the bell interior to hold the divers off the cold metal and avoid blocking the entry hatch. The bell cooled down at its own rate in 6°C water; the *Polar Bear* experiments had started with the chamber already cooled down. Even with the slower cooling the test had to be stopped within 6 h because two divers had rectal temperature losses to below 36°C, and there was a buildup of $CO_2$. The buildup of $CO_2$ indicates that for some reason the $CO_2$ scrubber-regenerators were not working (either they leaked or were not used); the divers leaking $CO_2$ were likewise not being protected against respiratory heat loss.

Additional unmanned tests on the thermal regenerators were performed at NUTEC (7). A breathing machine provided warmed, humidified heliox with 4 kPa $CO_2$, at a chamber pressure of 200 msw. Breathing rates of 12.5 and 22.5 L/min were used. These are higher than one would expect from a person at rest.

The anticipated metabolic rate is rather critical to the design of the canister, because a higher flow requires larger tubing and hence a larger dead space. This might be totally inappropriate for the hypothermic diver, whose tidal volume will likely be quite low. One of the objectives of the test reported here was to determine metabolic rates under simulated survival conditions.

Another question had been that of whether the tests performed really confirmed that survival for 24 h was possible, especially in view of the British trial that had to be stopped. This period of 24 h had been selected as an expected rescue time for North Sea operations. A third *Polar Bear* at NUTEC ran for 24 h at 150 msw; this experiment used a cool-down curve like the British trial and was completely successful, with both divers completing the planned 24 h (8).

## The HMS BELOS Survival Test

The major objective of diving in the Swedish Navy is in support of submarine rescue. To this end the Navy operates a diving center, the rescue

submersible URF with diver lockout capability, and a submarine rescue vessel HMS BELOS, which is equipped with a commercial deep-diving system (9). Both vessels operate in always-cold Baltic waters up to 300 m deep. Both URF and the BELOS bell are equipped with protective equipment. Although the principles had been proven by the NUTEC trials, the investigators considered it was important to verify that the specific equipment in use would perform its function under Baltic conditions.

## METHODS

### Equipment

The experiment was carried out at the Naval Diving Center (MDC) as part of a longer experiment *Nisahex* (10). The BELOS diving bell was used. It is essentially spherical, 2.6 m in diameter; has a free volume of 2.8 $m^3$; and is covered with PVC foam insulation. It was mounted atop the hyperbaric chamber complex (*3* in Fig. 1) at the start of the experiment.

The survival equipment to be tested was the DUI Polar Bear system (Diving Unlimited, San Diego) as modified by MDC; two sets were included in the bell. The canister used was originally identified as the "12-hour" canister; it holds 1300 g of absorbent (Wimbourne, UK). The scrubber canister works in a "to-fro" mode. The diver exhales into the bed of $CO_2$ absorbent. He then inhales back through the absorbent bed, and the incoming breath picks up heat and moisture from the absorbent. This procedure acts not only to conserve heat in expired breath but also warms incoming gas with the reaction heat from the absorbent. It will continue to act as a passive thermal regenerator after the absorbent is spent. Carbon dioxide has two chances to be absorbed: on expiration and again on inspiration if there is a buildup in the ambient atmosphere.

Also included was a net made of 5-cm nylon strapping and designed to fit inside this bell (6). The center of the net is supported by the lifting block used to hoist a disabled diver.

Instead of the thermal trousers furnished with the system, special open-weave underwear and a woolly-bear dry-suit liner were substituted. Protective gear included gloves, hood, socks, jacket, sleeping bag, and waterproof plastic outercover. The kit also included a flashlight, alarm clock, water bottles, urine bags, and the combination $CO_2$ scrubber and thermal regenerator with a cap and oronasal mask. The canister is best kept outside the parka but inside the sleeping bag, to conserve heat but avoid creating an oxygen-deficient space.

During the test one diver was to stay awake on watch, the other could sleep. They alternated this duty each hour.

Before the test a "dry run" was made at normal temperature to rehearse the mechanical arrangements that had to be made. A 16-h test was deemed adequate if at the end of that time the divers could carry out certain procedures.

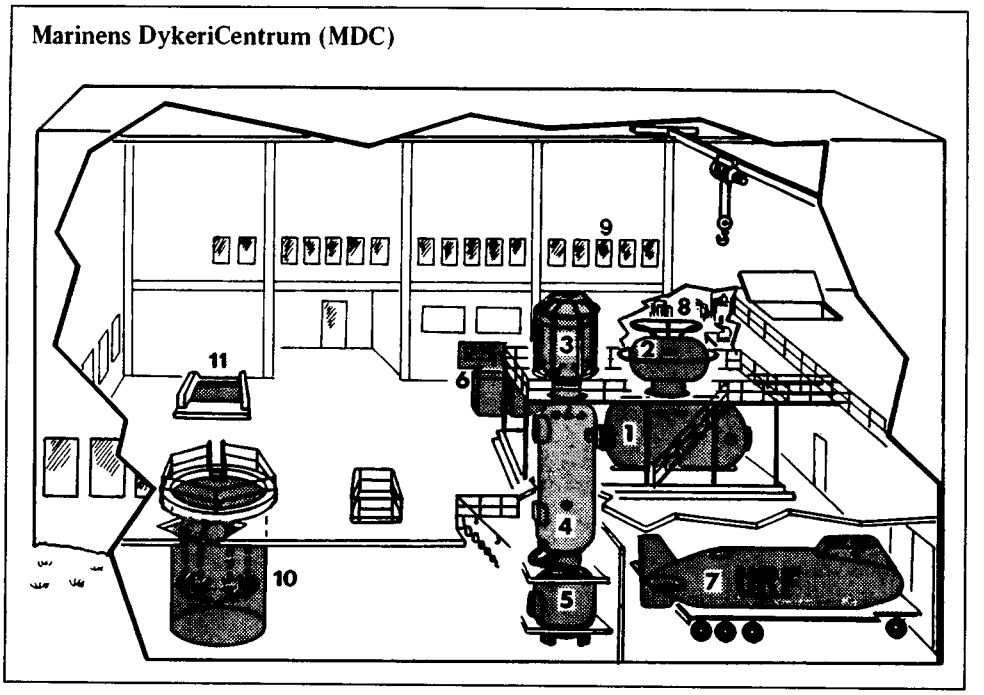



Fig. 1. Navy Diving Center facility (MDC). **1.**: Horizontal chamber system for dives to 400 m depth. **2**: Transfer capsule ("PTC") for URF. **3**: Diving bell from HMS BELOS. **4**: Vertical chamber, half filled with water. **5**: Lower, optionally wet, chamber designed for free-ascent training. **6**: Control panel. **7**: The URF in her garage. **8**: Laboratories. **9**: Computer and data room. **10**: Diving tank. **11**: Swimming tank with 4-knot current.

An additional preliminary test was performed by investigators to verify that the amount of gas on board would be adequate to reduce the bell atmosphere from the 1.5 atm used during bounce dives to the 0.8-1.0 atm recommended for the survival situation. This proved to be satisfactory; 44 $nm^3$ of 97% helium reduced the bell atmosphere $Po_2$ from 1.4 to 0.8 bar.

The cool-down curve of the unmanned bell was also determined in a preliminary test.

As a simulation of winter conditions the bell was stored overnight in the cold water tank and removed on 1 h before the dive began.

## Diver Subjects

The subjects were two experienced Navy divers; their descriptions are given in the report on *Nisahex* (10).

## Monitoring

The ECG of each diver was displayed on an oscilloscope and the heart rates were recorded. Rectal temperature was taken with a thermistor probe

having a small silicone ball 10 cm from the end to ensure correct placement. Skin temperature was monitored from the middle of the thigh, a location that tends to reflect average skin readings. A third spare probe (all were YSI 400–44) was available to each diver; because it was not needed as a replacement, it was used for checking skin temperature at various other places. These temperatures and heart rates were scanned automatically and recorded with an ABC–80 microcomputer equipped with an A-D converter and junction box. The computer took a reading each second for 40 s of each minute, then compiled an average for the minute. Every hour the data were stored on a disk.

Other temperatures logged manually included the middle of the bell and the breathing gas.

Gas samples were taken at a continuous flow rate of 1 slpm from several locations and fed to a Centronics mass spectrometer and recorded with an oscillograph. Oxygen and $CO_2$ were measured in the face mask on a breath-by-breath basis. The $O_2$ content in the sleeping bag was measured, as were $O_2$, $CO_2$, and helium at upper and lower parts of the bell atmosphere.

Communications with the divers was by headset, but after the "lost bell" procedure started the only communications were once per hour when the divers gave their condition from a choice list. The divers were monitored on TV and a video tape was made of the whole dive. Pressure was taken from a precision gauge. Separate breathing masks and emergency purge gases were available.

### Procedures

The experiment began with a simulated dive. After rapid compression to 150 msw, one diver made a short dive in the wet chamber (*4* in Fig. 1) and the divers entered the bell (*3*, Fig. 1). The bell was then moved to the test tank (*10*, Fig. 1), where it was immersed in 2°C water. Power was cut off, the bell covered to simulate darkness, and the divers began their lost bell procedures. They dried off, erected the net, donned their clothing and sleeping bags, installed all electrodes and probes, and began breathing on the regenerator canisters. These procedures took 1.3 h. At the start $P_{O_2}$ was 0.99 bar, and the initial pressure was 167.2 msw. No gas was added during the test.

The divers remained in their equipment for the planned 16 h, changing canisters as instructed. When the time was up the bell was returned to the chamber system, and the divers re-entered the living chamber to await other tests and decompression.

## RESULTS

The bell cooled according to *curve # 1* in Fig. 2, a low of 7°C, which was almost the same as in the unmanned test. As a result, cooling pressure

dropped in an almost parallel curve *(curve # 2*, Fig. 2). The water temperature remained stable at less than 3°C.

Oxygen content as measured in the center of the bell dropped slowly as it was consumed by the divers (Fig 3). The $O_2$ level in the sleeping bag was slightly lower than this throughout the test. The $CO_2$ level in the bell reached 0.8% sea level equivalent (SLE; 0.8 kPa) at the end of the test. The sharp rise after 12 h was due to a broken canister connection.

A breakthrough point of 2% sea level equivalent ($P_{CO_2}$ = 2 kPa) was arbitrarily selected as the point at which the canisters were to be changed. This point was reached at about 9 h into the test (Figs. 4 and 5). The breakthrough was measured just outside the canister and reflects the gas exhaled into the sleeping bag, not that inhaled by the diver. A much higher breakthrough point would be consistent with survival, but a conservative choice was made. The breathing gas had begun to cool down, and, in fact, the divers had requested a change just before it was directed by the supervisor. One reported a mild dyspnea. The divers did not have any trouble changing the canisters.

The second set of canisters was changed again after only 5 h. This change was due to a buildup of $CO_2$ in the sleeping bag of *Diver 2*, and was later found to be due to a broken connection on the canister.

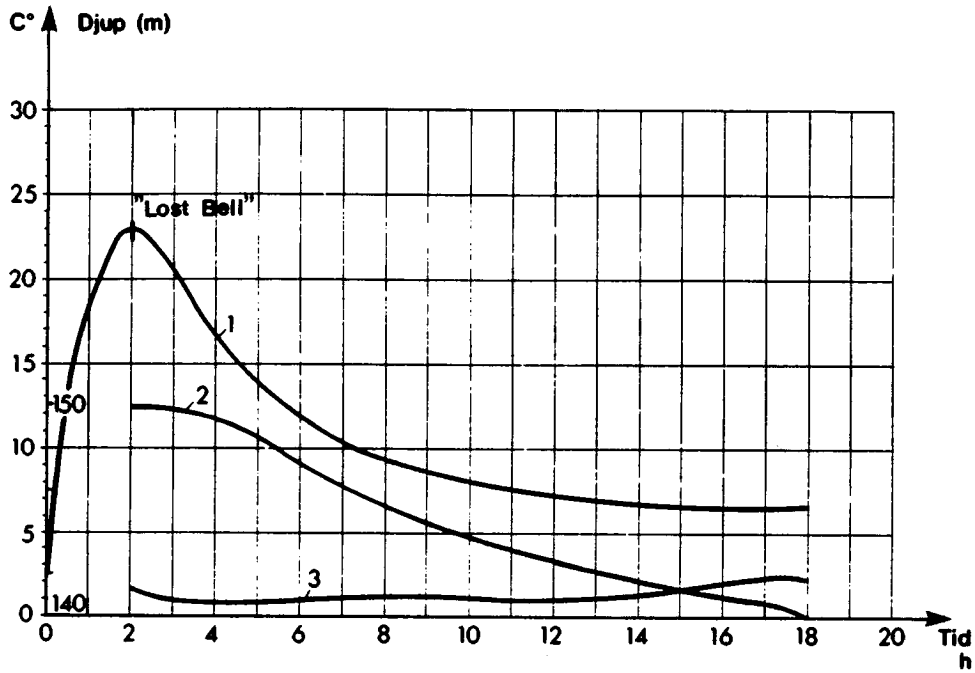



Fig. 2. Pressure and temperature in bell. *Curve #1* shows the gas temperature measured in the middle of the bell. *Curve #2* shows the pressure drop in the bell during the test (*inner scale, vertical axis*). *Curve #3* shows the temperature of the water in which the bell was immersed. Horizontal axis gives time in hours.

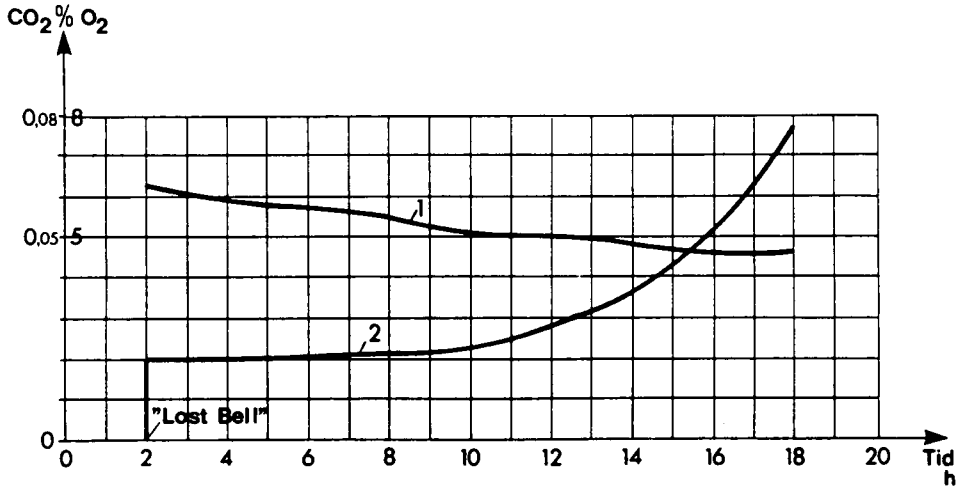



Fig. 3. $CO_2$ and $O_2$ concentrations in the bell. *Curve #1* shows oxygen concentration in percent. *Curve #2* shows concentration (fraction) of $CO_2$ analyzed at sea level. The peak $P_{CO_2}$ was about 1 kPa or 1% sea level equivalent, and was to a large extent the result of a leak.

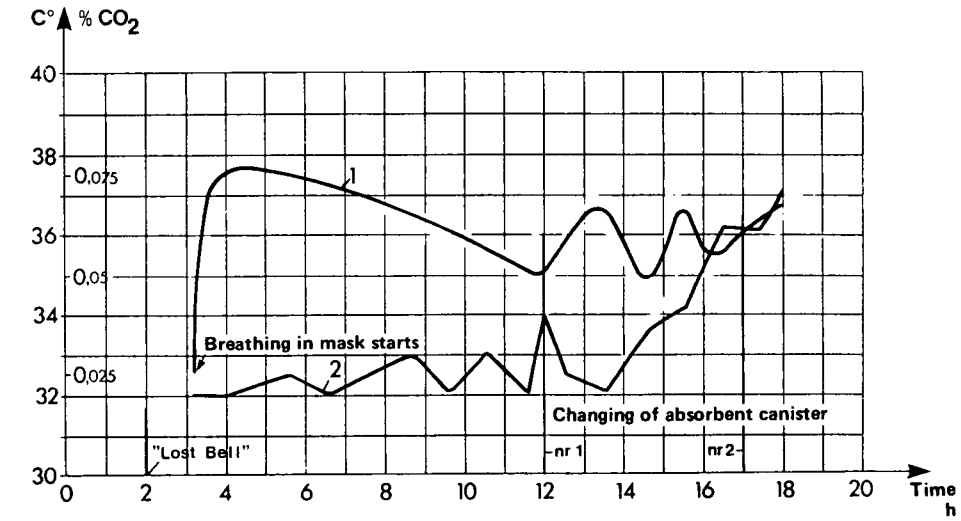



Fig. 4. Inhaled gas temperature and $CO_2$, *Diver 1*. *Curve #1* shows temperature of the gas inhaled by *Diver 1*. *Curve #2* shows the $CO_2$ concentration (analyzed at sea level) in his sleeping bag.

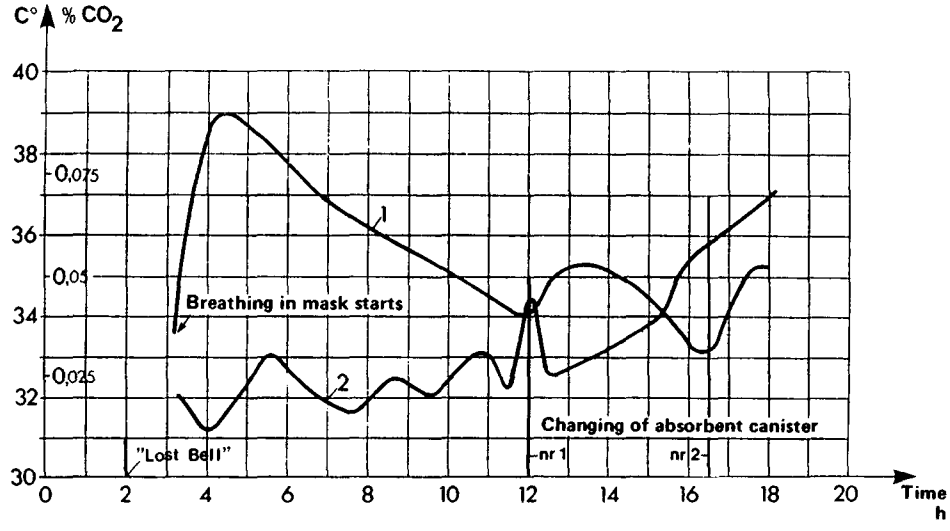



Fig. 5. Inhaled gas temperature and $CO_2$, *Diver 2*. *Curve #1* shows temperature of the gas inhaled by *Diver 2*. *Curve #2* shows the $CO_2$ concentration (analyzed at sea level) in his sleeping bag.

The divers used 0.3 L of water each and were able to use the urine bags without problems and without opening the sleeping bags.

In response to the questioning, the divers were both "comfortable" for the first 8 h, noted "cold feet" for the next 2–4 h, then were "a bit cold" for the balance of the test. *Diver 2* shivered briefly at 15 h. Equipment was scored "OK" throughout. After the test the divers went about their business of cleaning up, and the like, with no problems and no discomfort. Both divers said they would be willing to do the test again.

## Rectal and Skin Temperatures

The measured rectal and skin temperatures are shown in Figs. 6 and 7. The curves show the same pattern for both divers. Two cooling phases can be identified. During the first 2 h (*Phase 1*) the skin temperatures rose as the sleeping bags warmed up. At the same time the rectal temperatures decreased faster than during the latter part of the test (*Phase 2*). The bell was cooling rapidly, and the divers were unprotected.

The rectal temperature decrease during *Phase 1* was 0.33°C/h for *Diver 1* and 0.55°C/h for *Diver 2*. The equivalent decrease for the rest of the test (*Phase 2*) was 0.06°C/h and 0.11°C/h, respectively.

During *Phase 2* there was a gradual decrease of skin temperature, but this was not particularly pronounced at the measuring point that was used. As is

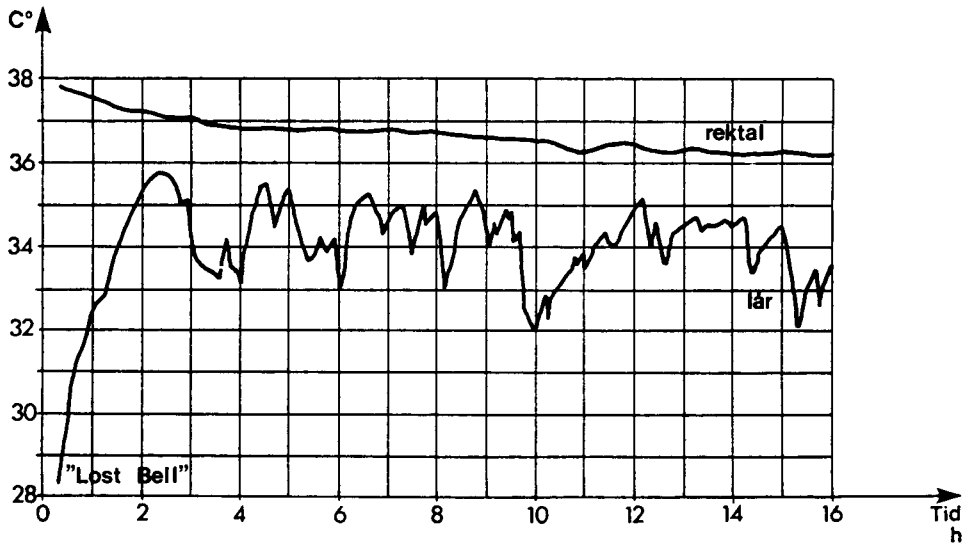



Fig. 6. Rectal and skin temperature, *Diver 1*. Rectal temperature (*rektal*) was measured with a thermistor inserted 10 cm. Thigh temperature (*lår*) was measured on the inside of the thigh and approximates average skin temperature.

shown by the figures, the thigh temperatures fluctuated during the whole tests but were on an average 2 or 1°C, respectively, below the rectal temperature.

For both divers a marked decrease in the skin temperature is shown as a result of changing of the absorbent canister.

The temperature of the back was measured by the spare temperature indicator during the 4th to 10th h. On this area the insulation is compressed due to the weight of the diver. The temperature was measured outside the underwear and may therefore have been slightly lower than the actual skin temperature. It fell from about 33 to 28°C for both divers during this period.

On two occasions, 11 and 16 h after the start of the test, the finger temperature was taken. The temperature was 31°C on both measurements of *Diver 1*, while the finger temperature of *Diver 2* was 30 and 27°C. Foot temperature was not measured but both divers started complaining about cold feet after about 9 h.

## Heart Rates

The heart frequency of *Diver 1* was approximately 70 beats/min in the beginning of the test. During the first half of the test the pulse varied between 50 and 65 beats/min; during the second half the pulse was about 50. *Diver 2* had a starting pulse of 90 beats/min, which decreased during the first 4 h to about 65. During the end of the test the pulse varied between 45 and 60.

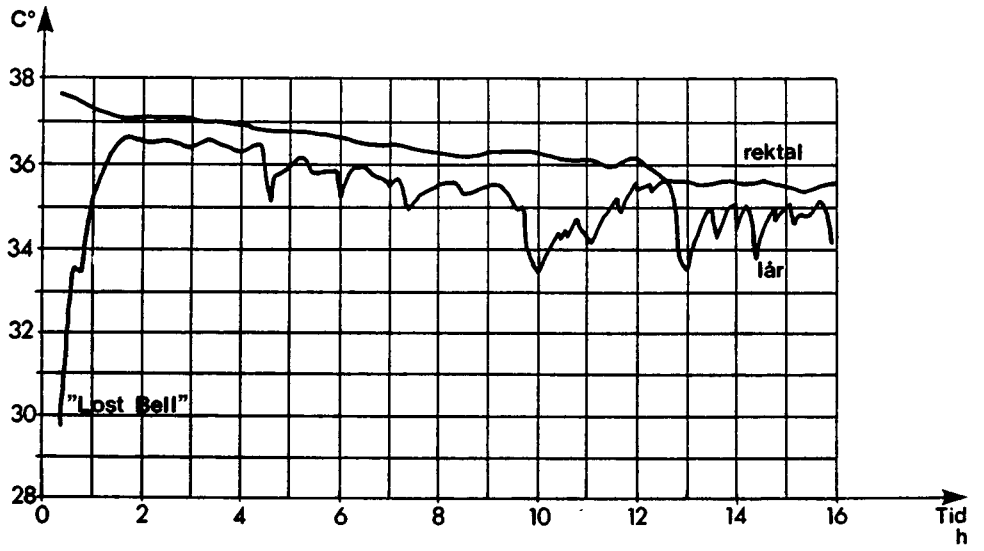



Fig. 7. Rectal and skin temperatures, *Diver 2*. *Upper curve* is rectal temperature (*rektal*); *lower curve* is skin temperature (*lår*), inner thigh. Dips in skin temperature are a result of movements during canister change.

The heart frequency indicates that there was slight activity during the early hours. In the latter half of the test the heart frequency stabilized at a rate that should correspond to resting. The energy output during this period should therefore correspond to the basic metabolism of each diver, or a produced heat of about 60 W/m$^2$.

Based on the steady, decreasing body temperature of both divers during the last 10 h of exposure, the continued decrease can be projected to a 24-h exposure. Given the same conditions (which includes an optimally functioning $CO_2$ scrubber/gas heater), this would mean a body temperature of 35.7°C for *Diver 1* and 34.5°C for *Diver 2*. With a body temperature of around 35°C and lower, both mental and physical performance gradually decrease. The measured levels are well above the limits connected with increased risk of unconsciousness and heart seizure.

So that temperatures toward the end of the test could be related to the diver's normal circadian shifts, rectal temperature was taken periodically through a predive night. *Diver 1* reached a minimum of 36.5°C at midnight and *Diver 2* 36.3°C at 0300. Thus, the low rectal temperatures recorded were only slightly below normal for that time of day.

## Oxygen Consumption

As another measure of the metabolic rate of the divers, we calculated the oxygen consumption of the team by considering the bell as a metabolic

chamber. No gas was added during the run, and 1 nL/min was drawn out for gas analysis. There were a few small leaks in the bell. When the bubbles from these leaks were trapped and measured, they showed a leak rate of only 20–30 nmL/min, so this was ignored.

For the last 14 h (*Phase 2*, discussed previously) the average oxygen consumption was 0.38 L/min for each diver. This figure is slightly higher than the basal rate for these two divers (280 and 250 mL/min) as calculated from their body size and is consistent with the observed low heart rate (11).

These results indicate that a diver with good thermal protection in cold hyperbaric helium can be expected to have a metabolic rate closer to resting levels than exercise levels.

## CONCLUSION

The two divers in the test, after a simulated wet dive, were able to use the thermal protection equipment to maintain themselves in good condition for 16 h.

The canisters tested lasted about 9 h; consequently, the procedures call for them to be changed at 7 h, and 4 canisters will be supplied for each diver to cover a 24-h period. After the equipment was adjusted, the metabolic rate of divers was maintained at a resting level. Diver body temperature dropped to about 1°C below its expected level for that time of night.

This test provides confidence that the equipment and procedures described will protect divers in the conditions of a stranded diving bell. When the equipment is used correctly and functions properly, divers can expect to be relatively comfortable for several hours and have cold feet and be moderately cold the next few hours. They should be able to function well enough to carry on with simple procedures and to assist in a rescue for at least 24 h.

## References

1. Schmidt T. Thermal considerations of survival in emergency hyperbaric situations. In: Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society Workshop. Bethesda: Undersea Medical Society, Inc., 1981:277–314.
2. Tønjum S, Hamilton RW, Brubakk AO, Peterson RE, Youngblood DA. Project *Polar Bear*: testing of diver thermal protection in a simulated "lost bell." Bergen: Norwegian Underwater Institute, 1980. (NUI rept 2–80).
3. Hamilton RW. Design of combination thermal regenerator and $CO_2$ absorption cannister for hyperbaric survival. In: Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society Workshop. Bethesda: Undersea Medical Society, Inc., 1981:359–372.
4. Tønjum S, Påsche A, Furevik D, Holand B, Brubakk AO, Olsen C. *Deep Ex 80*: Project II, Thermal studies in diving. Subproject: *Polar Bear II*: Test of survival systems for stranded bell divers at 31 bars. Bergen: Norwegian Underwater Institute, 1980 (NUI rept 40–80).
5. Long R. Development of performance standards of DUI's Polar Bear system. In: Kuehn, LA ed. Thermal constraints in diving. The twenty fourth Undersea Medical Society Workshop. Bethesda: Undersea Medical Society, Inc., 1981;373–390.

6. Hayes PA, Padbury EH, Atherton PJ. Astonaut trail. Section I: Passive systems. Validation of emergency procedures, the lost bell situation. Alverstoke, UK: AMTE Physiological Lab, 1981. (AMTE(E) R81 403).
7. Furevik D, Påsche A, Tønjum S. Endurance test of lung-powered scrubbers designed for hyperbaric rescue chambers and stranded diving bells. In: International conference Divetech '81. Workshop B4. London: Society for Underwater Technology, 1981.
8. Tønjum S, Påsche A, Onarheim J, Furevik D, Hayes P, Padbury H, Segadal K. *Polar Bear III*: Test of passive survival systems for 24 hours at 16 bars. Bergen: Norwegian Underwater Technology Center, 1982. (NUTEC rept 21–82)
9. Marinens DykeriCentrum. Report over the Swedish Navy Diving Center and test with personal survival equipment for deep divers in distressed diving bells (Lost Bell). Håsfjärden, Sweden: Navy Diving Center, 1982.
10. Muren AM, Adolfson J. Örnhagen H, Gennser M, Hamilton RW. *Nisahex*: Excursions with nitrox and trimix from deep nitrox saturation. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:000–000.
11. Consolazio CF, Johnson RE, Pecora LJ. Physiological measurements of metabolic functions in man. New York: McGraw Hill, 1963.

# THE IMPORTANCE OF STATIC AND DYNAMIC CHARACTERISTICS OF THERMORECEPTION TO THERMOGENIC RESPONSE IN THE HUMAN

*I. B. Mekjavic and J. B. Morrison*

Thermoregulatory effector mechanisms, initiated in response to peripheral or core thermal stresses, or both, have been explained on the basis of the *set-point concept* (1–5). The theory of set-point regulation, developed by Bazett (6) and Vendrik (7), suggests that the set-point temperature is an internal temperature at which the static firing characteristics of warm and cold central sensors are identical. As depicted in Fig. 1, the firing rate of central cold and warm sensors will vary with displacement of central temperature from the set-point temperature. A decrease in internal temperature will instigate thermogenic mechanisms and decrease heat loss mechanisms, while the opposite will ensue with increases in internal temperature.

If one compares the averaged static firing characteristics of cutaneous thermoreceptors (8–11) with that of cold and warm sensitive fibers in central and core regions, such as the spinal cord (12) and hypothalamus (13), it appears that the set-point temperature, as defined earlier, is lower for peripheral regions. This finding would indicate that different zones of thermoneutrality exist for different regions of the body.

Experimental evidence for the central set-point has been put forward by Benzinger (1,2). The close correlation between activity of central and peripheral thermosensitive structures and thermoregulatory mechanisms has been demonstrated by Boulant (13) and Hensel (14), respectively. From the comparison conducted by Hensel (14), depicted in Fig. 2, it appears that at a constant tympanic temperature, there is good agreement between the nature of the thermogenic response and static activity of cold receptors. The results indicate that peak thermogenesis would be anticipated at peripheral temperatures of approximately 25°C.

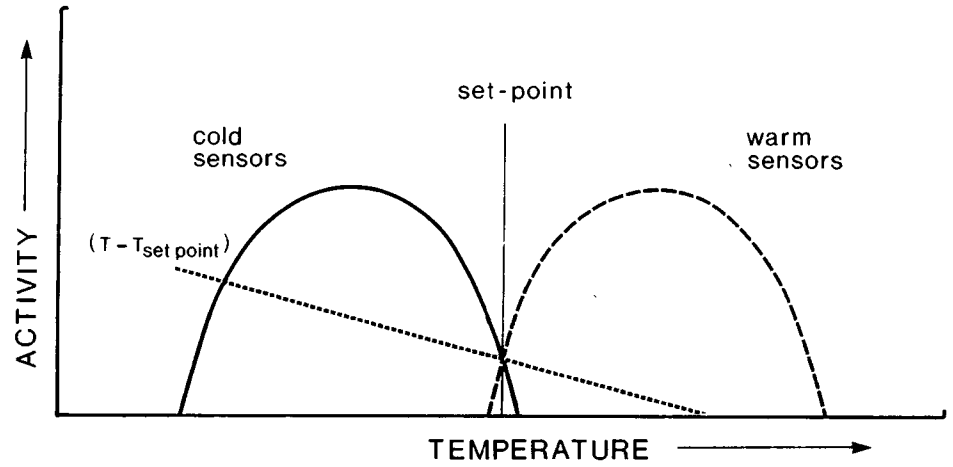



Fig. 1. General distribution of firing rate of cold and warm receptors as a function of local temperature. The set-point concept is defined as the temperature at which cold excitation is balanced by warm inhibition resulting in thermoneutrality. The linear response of ($T-T_{set}$) represents the output of a typical model predicting shivering thermogenesis (Table I).

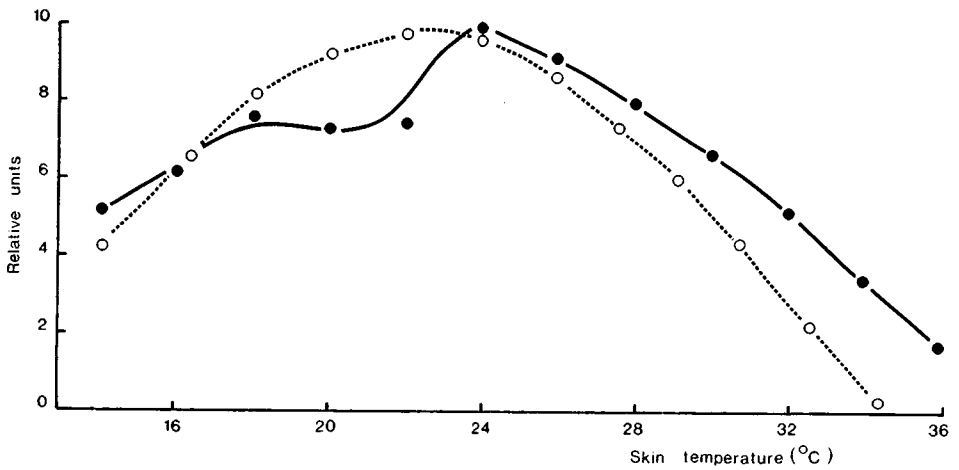



Fig. 2. Comparison of average static frequency of cold fibers in monkeys (*closed circles* from Dykes [32]) and metabolic rate in man (*open circles* from Benzinger [1]) as a function of skin temperature. Adapted from Hensel (14).

The close correlation of static thermoreceptor neural activity with thermogenesis suggests that the dynamic response characteristics of thermosensitive structures will also contribute to the thermogenic response. The dynamic response properties of cutaneous thermoreceptors have been demonstrated in detail by Zotterman (11), Kenshalo and Duclaux (10), and Duclaux and Kenshalo (8). Similar dynamic responses of cold receptors to cooling have been shown in central regions, in the medulla (15). It was observed from these studies that the magnitude of the transient response of thermoreceptors is dependent upon the magnitude of the temperature change imposed on the thermoreceptor, the rate of cooling, and the thermosensitivity of the receptor at the adaptive temperature.

The set-point concept has in recent years formed the basis of several stimulus-response equations (16–20). Most models suggest that thermogenesis may be predicted without consideration of cooling rates of core and peripheral regions. In most cases, we derive the gains and set points through regression analysis rather than incorporating neural characteristics of thermoreceptors.

In this study we have examined the predictive powers of several models and analyzed the results to identify the sources of error inherent in these models. An attempt is also made to elucidate the contribution of static and dynamic activity of thermoreceptors to the thermogenic response in man. A model based on these concepts of thermoregulation is developed and tested against experimental data.

## METHODS

The stimulus-response models chosen for evaluation were developed by Hayward et al. (16), Nadel et al. (17), Stolwijk and Hardy (18), and Timbal et al. (19,20). The original format of these expressions, as suggested by the authors, is shown in Table I.

We determined predictive ability of these models using empirical data obtained from a study by Morrison et al. (21), consisting of cooling 10

TABLE I

Stimulus-Response Models of Shivering Thermogenesis

| | |
|---|---|
| H1(16): | $MR\ (W/kg) = 0.0314^* (T_S - 42.4)(T_R - 41.4)$ |
| H2(16): | $MR\ (W/kg) = 0.0356^* (T_S - 41.8)(T_T - 41.0)$ |
| SH(18): | $\Delta MR\ (kcal/h) = 60^* (36.6 - T_T)(34.1 - T_S)$ |
| N(17): | $\Delta MR\ (kcal/h) = 36^* (36.5 - T_T)(32.2 - T_S) + 7^* (32.2 - T_S)$ |
| T(19): | $MR\ (W/m^2) = 41.31 - 57.77^* (dTs/dt) - 5.01^* (T_S - 34) + (894.15 - 23.79^* T_R)$ |

MR: metabolic rate; $T_S$: mean skin temperature; $T_R$: rectal temperature; $T_T$: tympanic temperature; $\Delta MR$: change of metabolic rate; $m^2$: square metre of surface area.

subjects in 10°C water followed by passive rewarming. The ability of these models to predict the empirical data was tested by analysis of the sum of squared residuals (SSR's). This measure is indicative of the magnitude of the error of prediction.

We attempted to minimize the error of prediction by determining personal coefficient and set-point values for each of the 10 subjects, through regression analysis (22). The benefits of this procedure were evaluated by determining whether there were significant reductions in the errors of prediction as indicated SSR's were obtained (23).

To ascertain whether inclusion of core and peripheral cooling rates would enhance the predictive power of these expressions, we restructured each independent variable to include a time-derivative component. The analysis of error (SSR) was then repeated.

A quantitative comparison of the errors of prediction for the various models does not identify the sources of error. Random errors plotted against time would be distributed about the abscissa. In the event that a time-dependent or a temperature-dependent function would be omitted from the expressions, however, the distribution of the residuals would not be random. We applied a graphical analysis of the residuals, as outlined by Draper and Smith (23), to examine the distribution of the residuals and to determine whether certain characteristics of central and peripheral temperatures were omitted in the models. The errors of prediction were plotted against time and also against the skin and core temperatures observed.

A further series of cold-water immersion trials was also conducted for investigation of the effect of progressively greater step changes of peripheral temperature on the metabolic transient occurring after immersion. Five subjects were immersed in baths of 20, 15, and 10°C on separate occasions. Values of skin temperatures (four sites) and oxygen consumption were sampled every minute. Immersions in cold water were conducted for 1 h or until core temperature, as indicated by rectal, tympanic, and esophageal temperatures, decreased to 35°C. The immersions were followed by passive rewarming in a sleeping bag.

A model incorporating the principle features of thermoreceptor responsiveness was then developed. In this model, predictions of thermogenesis were based on peripheral, core, and central thermosensitivity (24). Temperatures of these regions were weighted according to static and dynamic characteristics of cold and warm receptor activity. Thermogenesis was considered the result of excitatory and inhibitory interactions of thermogenic drives from these regions. We then solved the gain coefficients of the inhibitory and excitatory neural drives to thermogenesis using the experimental data obtained from the series of cold-water immersions. In this procedure, a least-squares regression technique was employed to provide a best fit of the model to the data (22).

## RESULTS

The models H1 and H2 of Hayward et al. (16) proved to be substantially better at predicting shivering thermogenesis than models T (19,20), N (17), and SH (18), as shown in Table II.

TABLE II

Comparison of Error of Prediction Generated by the Models Tested

| Models | Sum of the Squared Residuals | | |
|---|---|---|---|
| | Original Model | Personal Coefficients and Set Points | Time Derivative Terms Added |
| H1 | 1,699.6 | 277.7 | 145.5 |
| H2 | 1,409.6 | 237.2 | 174.0 |
| SH | 20,336.2 | 237.4 | 131.9 |
| N | 4,194.5 | 189.2 | 170.5 |
| T | 2,211.2 | 207.5 | — |

*n*: number of observations = 244. Predictions of shivering thermogenesis were obtained in units of W/kg for all models, i.e., SSR = $(W/kg)^2$.

Obtaining personal coefficients and set-point values dramatically reduced the magnitude of the SSR's. The reduction in predictive error was obtained, however, at the expense of large variations among subjects of the gain and set-point values. The values of these parameters (mean ± SD) for models H2 and N, derived through regression analyses, are presented in Table III. It is evident that the values exhibit substantial variation from those suggested by the authors. In some extreme cases, the set-point values were assigned negative values.

TABLE III

Mean Values and Standard Deviations of the Gains and Set Points Derived by Regression Analysis for Each of Ten Subjects

Modified Format of Models H2 and N :

H2 :

$$\overline{MR}\ (W/kg) = P1 \times (T_S - P2)\,(T_T - P3)$$

N :

$$\overline{\Delta MR}\ (W/kg) = P1 \times (P2 - T_T)\,(P3 - T_S) + P4 \times (P5 - T_S)$$

Values (mean ±SD) for Gains and Set Points

| Model | P1 | P2 | P3 | P4 | P5 |
|---|---|---|---|---|---|
| H2 | 0.1206±0.08 | 42.3±13.8 | 43.4±14.0 | N/A | N/A |
| N | 12.7±17 | 37.3±1.5 | 30.6±19 | 14.9±8.3 | 29.7±8.4 |

Predictions of thermogenesis were derived in units of W/kg. MR: metabolic rate; $T_S$: mean skin temperature; $T_T$: tympanic temperature; ΔMR: change of metabolic rate.

As seen from Table II, the addition of time-derivative terms further decreased the magnitude of the error of prediction and the variability of the SSR between models. Model T is excluded from this evaluation because it incorporates in its original format the rate of change of skin temperature in the prediction of shivering thermogenesis. Model SH appears to be the best predictor when time derivatives of the independent variables are added within the expressions.

A graphical analysis of the residuals indicated that all the original models evaluated exhibited a systematic error of prediction. The residual distribution was similar for all 10 subjects. There was some variability in the nature and magnitude of the distribution among the different models. A plot of the normalized error of prediction of models H1 and H2 for one subject, for the range of observed skin temperatures, is shown in Fig. 3. The residual distribution assumes a *bell shape,* with maximum errors of prediction occurring at skin temperatures of approximately 25°C. When the normalized errors were plotted over the range of central and core temperatures observed, minimum error of prediction was generated in the region of thermoneutrality. Above and below this temperature the residuals increased parabolically.

The results of the cold-water immersion series confirmed the existence of a distinct metabolic transient at the onset of immersion. It was evident that the

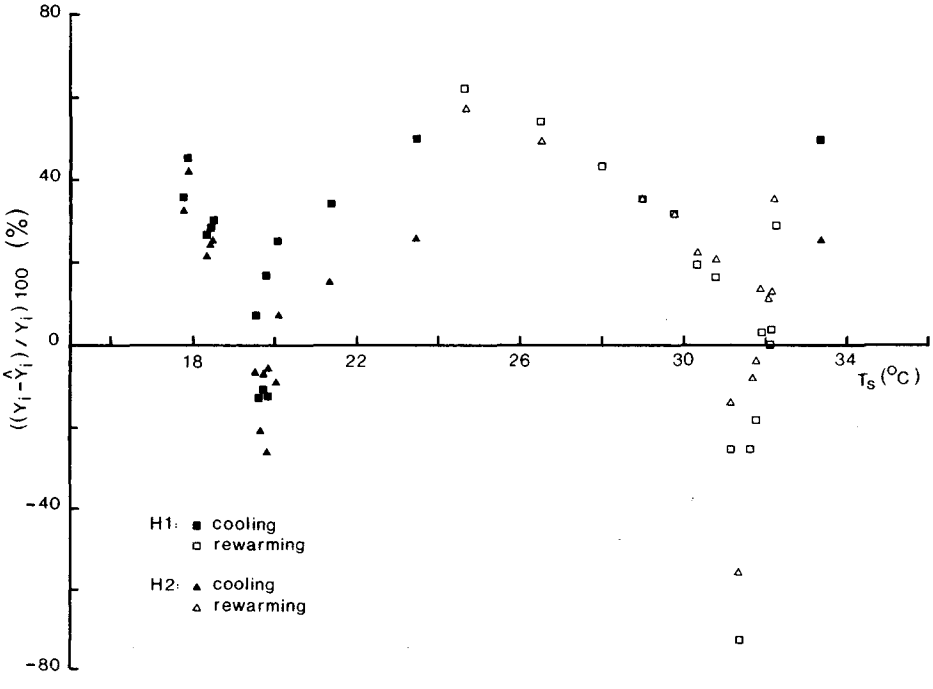



Fig. 3. Plot of normalized error of prediction of models H1 and H2 as a function of mean skin temperature.

metabolic transient was greater in magnitude with greater step changes in peripheral temperature. In addition, a considerable variability was found to exist between subjects with regard to the time of onset and the relative magnitude of the metabolic transient. On average, the peak of the metabolic overshoot occurred within 10 min of cold immersion. Examples of these response characteristics are shown in Table IV.

A second metabolic transient also occurred at the onset of warming. This transient resulted in metabolic rates during the first few minutes of rewarming that exceeded those observed during the immersion phase of the experiment. The magnitude of the metabolic overshoot was again greatest following the immersion in 10°C water. A typical example of these metabolic transients is shown in Fig. 4. It is notable that they occur when there are no substantial changes of central or core temperature. As seen from Fig. 4, the peak of the skin temperature transients does not coincide with the metabolic overshoot at onset of cooling and rewarming: a phase lag of several minutes exists between the temperature input and metabolic response.

In contrast to the models discussed previously, we found that the present model (M) was able to simulate the thermogenic transient at onset of immersion. The model also generated increased metabolic rates at the onset of rewarming. In some cases, however, the phase lag of the metabolic overshoot noted in the rewarming data tended to be greater than was predicted by the model. The predictions of model M were compared with models H2 and N through regression analysis. Model M, based on central and peripheral thermosensitivity, resulted in better overall predictions of shivering thermogenesis (MSE = 3.76) than obtained with models H2 (MSE = 5.3), and N (MSE = 4.34)—where MSE is mean square error in (watts/kilogram) and the number of cases is $n = 2129$.

TABLE IV

Metabolic Overshoot and Change of Skin Temperature at Onset of Immersion

| Bath Temperature (°C) | Subject | Maximum Rate of Change of Skin Temperature (°C/min) | Peak of Metabolic Overshoot: MR (mL $O_2$/kg/min) | Time of Peak MR from Onset of Immersion (min) |
|---|---|---|---|---|
| 10 | DT | 17.2 | 25.9 | 9 |
| | DS | 5.4 | 18.9 | 2 |
| 15 | DT | 17.13 | 23.9 | 11 |
| | DS | 10.36 | 14.3 | 1 |
| 20 | DT | 7.9 | 16.1 | 8 |
| | DS | 8.3 | 8.7 | 1 |

Resting metabolic rate: DT = 3.3 and DS = 2.8 (mL $O_2$/kg/min). Maximum rate of change of skin temperature occurred within the first minute of immersion.

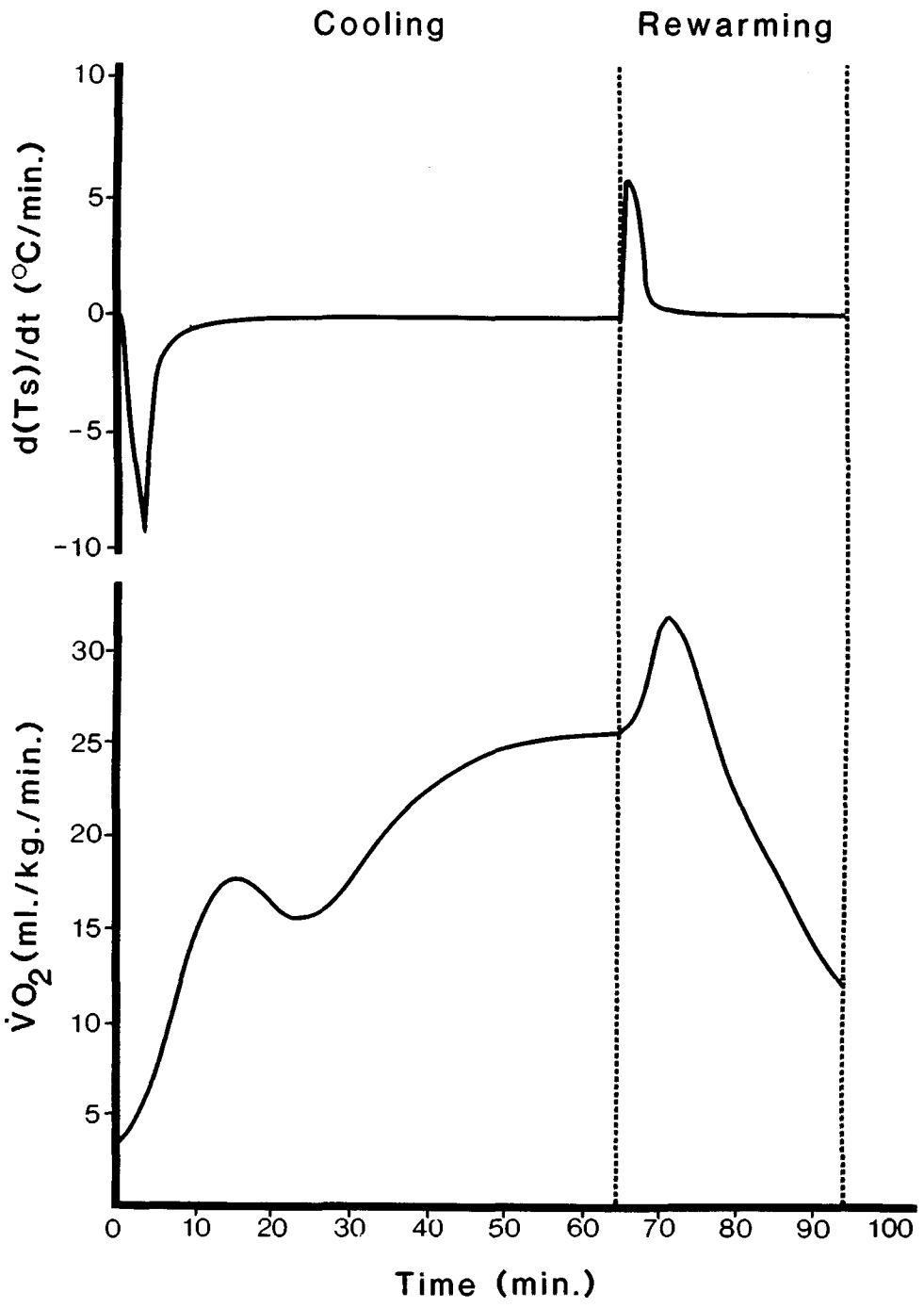



Fig. 4. The rate of change of mean skin temperature (dTs/dt) and oxygen consumption ($V_{O_2}$) during immersion in 10°C water and passive rewarming. Results of one male subject.

## DISCUSSION

The present study indicates that the stimulus-response models evaluated are not adequate in predicting shivering thermogenesis during cooling and rewarming of humans. By determining individual coefficients and set-points, and thus dramatically reducing the error of prediction, we took into account the biological variation among subjects. However, the regression analyses assigned to some parameters values that were several orders of magnitude different from those published by the original authors. We reduced the error of prediction in these instances at the expense of obtaining physiologically unreasonable set-point temperatures and gain coefficients.

Accepting the close correlation between static response characteristics of cold receptors and thermogenesis as suggested by Hensel (14), we believe it is apparent that present models do not simulate this relationship. For example, in Fig. 1 relative predictions of thermogenesis of model H2 are plotted against generalized thermoreceptor activity over the physiological range. Only the peripheral contributions to metabolic rate are considered. Model H2 was chosen for this comparison because it offered the best predictions of thermogenesis and is also representative of the general format of the expressions evaluated. Figure 1 shows that the present models predict linear increases in shivering thermogenesis with displacement of either core or peripheral temperatures. If one assumes thermogenesis is proportional to the characteristics of cold sensors, then the errors of prediction for a range of temperatures will assume a distribution equal to the difference of the responses of predicted thermogenesis and the cold sensor activity. The analysis of residuals (23) shown in Fig. 3 tends to confirm this hypothesis. The peak and range of the residual distribution compare favorably with those of the averaged static response curve of cutaneous cold receptors shown in Fig. 2 (14). This suggests that peripheral, core, and central temperature terms in present models should include a weighting factor that incorporates the static characteristics of thermosensitive structures in these regions.

The static linear relationships of expressions H, N, SH, and T are also not capable of predicting the thermogenic transients at onset of cooling and rewarming. It is demonstrated in Fig. 4 that the peak of the thermogenic transient occurs with a lag of several minutes after the peak of the peripheral temperature transient. During the transient phase, skin temperature passes from a level of thermoneutrality through a region of maximum thermosensitivity towards the new adaptive temperature. It is not possible to explain these observations solely on the basis of static responsiveness of cold receptors. Greater step changes in temperature and increased cooling rates imposed on cold receptors will induce dynamic responses in discharge frequency of greater magnitudes. Hence, immersions in 20, 15, and 10°C water will instigate greater dynamic responses from cutaneous cold receptors. This finding was reflected in the experimental data by greater magnitudes of the metabolic overshoot in response to immersion in water at lower temperatures (Table II).

The analyses of predictive models reported in this paper indicate that predictions may be enhanced by incorporating the nonlinear response characteristics of thermosensitive neural structures, which have been demonstrated to be closely related to thermoregulatory responses. The inadequacies of the new model presented arise from the assumptions and simplifications which have been necessary. In particular, there is a lack of experimental evidence of the interactions of excitatory and inhibitory neural drives to thermogenesis and of the time constants of metabolic response. It is considered that the benefits of model M are not so much the substantially improved predictions, but the approach undertaken in the simulation. By further testing and adapting the static neural characteristics and transient neural responses incorporated within this model, we should find it possible further to improve both physiological accuracy and predictive power of thermoregulatory models.

The evaluation of predictive expressions of thermogenesis was not meant as a criticism of the modelling attempts. These models are the most commonly used and referred to in thermoregulatory modelling. Models H1, H2, SH, and N were chosen because they represent the mode of prediction of metabolic heat production in various regions of the body by complex thermodynamic models (25–31). These thermodynamic models evaluate heat production and transfer within the body and overall heat loss to the ambient. The models usually account for the multilayered structure of the body segments and allow for increased respiratory evaporative heat loss and insulation offered by wet or dry suits, as would be encountered in simulation of diving conditions.

It would be difficult to identify the inadequacies of prediction of metabolic heat production when one utilizes complete thermodynamic models. Hence, the present analysis was constrained to evaluation of the predictions of thermogenesis only. It is anticipated, in light of present findings, that these complex models incorporate similar errors of prediction, as they do not account for the characteristics of thermosensitive neural structures in the body.

## References

1. Benzinger TH. Heat regulation: Homeostasis of central temperature in man. Physiol Rev 1969;49:671–759.
2. Benzinger TH. Role of thermoreceptors in thermoregulation. In: Zotterman Y, ed. Sensory functions of the skin in primates. With special reference to man. Oxford: Pergamon Press, 1976.
3. Bligh T. Temperature regulation in mammals and other vertebrates. Amsterdam: North-Holland Publishing Co., 1973.
4. Hammel HT. Regulation of internal body temperature. Ann Rev Physiol 1968;30:641–710.
5. Hammel HT, Jackson DC, Stolwijk JAJ, Hardy JD, Stromme SB. Temperature regulation by hypothalamic proportional control with an adjustable set point. J Appl Physiol: Respir Environ Exercise Physiol 1963;18:1146–1154.
6. Bazett HC. The regulation of body temperatures. In: Newburgh LH, ed. Physiology of heat regulation and the science of clothing. Philadelphia: Saunders, 1949.
7. Vendrik AJH. The regulation of body temperature in man. Ned Tijdschr Geneesk 1959;103:240–244.
8. Duclaux R, Kenshalo DR. Response characteristics of warm receptors in the monkey. J Neurophysiol 1980;43:1–15.

9. Hensel H, Boman KKA. Afferent impulses in cutaneous sensory nerves in human subjects. J Neurophysiol 1960;23:564–578.
10. Kenshalo DR, Duclaux R. Response characteristics of cutaneous cold receptors in the monkey. J Neurophysiol 1977;40:319–332.
11. Zotterman Y. Special senses: Thermal receptors. Ann Rev Physiol 1953;15:357–372.
12. Simon E, Iriki M. Sensory transmission of spinal heat and cold sensitivity in ascending spinal neurons. Pfluegers Arch 1971;328:103–120.
13. Boulant JA. Hypothalamic mechanisms in thermoregulation. Fed Proc 1981;40:2843–2850.
14. Hensel H. Correlations of neural activity and thermal sensation in man. In: Zotterman Y, ed. Sensory functions of the skin in primates. With special reference to man. Oxford: Pergamon Press, 1976.
15. Poulos DA, Molt JT. Response of central trigeminal neurons to cutaneous thermal stimulation. In: Zotterman Y, ed. Sensory functions of the skin in primates. With special reference to man. Oxford: Pergamon Press, 1976.
16. Hayward JS, Eckerson JD, Collis ML. Thermoregulatory heat production in man: prediction equation based on skin and core temperature. J Appl Physiol: Respir Environ Exercise Physiol 1977;42:377–384.
17. Nadel ER, Horvath SM, Dawson CA, Tucker AT. Sensitivity to central and peripheral thermal stimulation in man. J Appl Physiol: Respir Environ Exercise Physiol 1970;29:603–609
18. Stolwijk JAJ, Hardy JD. Temperature regulation in man—a theoretical study. Pfluegers Arch 1966;291:129–162.
19. Timbal J, Boutelier C, Loncle M, Bonges L. Comparison of shivering in man exposed to cold in water and in air. Pfluegers Arch 1976;365:243–248.
20. Timbal J, Loncle M, Boutelier C. Mathematical model of man's tolerance to cold using morphological factors. Aviat Space Environ Med 1976;47:958–964.
21. Morrison JB, Conn ML, Hayes PA. Influence of respiratory heat transfer on thermogenesis and heat storage after cold immersion. Clin Sci 1982;63:127–135.
22. Dixon WJ, Brown MB, Engelman L, Frane JW, Hill MA, Jennrich RI, Toporek JD. BMDP statistical software. Berkeley: University of California Press, 1981.
23. Draper NR, Smith H. Applied regression analysis. New York: John Wiley and Sons, 1966.
24. Mekjavic IB. A model of shivering thermogenesis based on the characteristics of central and peripheral thermoreception. Ph.D. thesis. Burnaby: Simon Fraser University, 1983.
25. Montgommery LD. A model of heat transfer in immersed man. Ann Biomed Eng 1974;2:19–46.
26. Montgommery LD. Analytic model for assessing the thermal performance of SCUBA divers. J Hydronautics 1974;8:108–115.
27. Stolwijk JAJ. Mathematical model of thermoregulation. In: Hardy JD, Gagge AP, Stolwijk JAJ, eds. Physiological and behavioural temperature regulation. Springfield: Charles C Thomas, 1970.
28. Stolwijk JAJ. A mathematical model of physiological temperature regulation in man. NASA Contractor Report. (NASA CR rpt No. 1855)
29. Wissler EH. Steady state temperature distribution in man. J Appl Physiol: Respir Environ Exercise Physiol 1961:16:734–740.
30. Wissler EH. A mathematical model for the human thermal system. Bull Math Biophys 1964;26:147–166.
31. Wissler EH. The use of finite difference techniques in simulating the human thermal system. In: Hardy JD, Gagge AP, Stolwijk JAJ, eds. Physiological and behavioural temperature regulation. Springfield: Charles C Thomas, 1970.
32. Dykes EW. Coding of steady and transient temperatures by cutaneous "cold" fibers serving the hand of monkeys. Brain Res 1975;98:485–500.

# STATUS OF COLD ACCLIMATIZATION IN CONTEMPORARY KOREAN WOMEN DIVERS WEARING WET SUITS

*Y. S. Park, D. W. Rennie, and S. K. Hong*

For centuries Korean women divers dived for sea food wearing only cotton swimming suits. The cold stress during their diving work in winter was greater than that of any other human group known (1). When studied in the 60's, their thermoregulatory functions were different from those of nondivers. For instance, a) their basal metabolic rate (BMR) was increased significantly during winter when sea water temperature decreases to 10°C (2); b) the resting oxygen consumption increased slightly in response to exogenous norepinephrine in winter (i.e., apparent nonshivering thermogenesis) (3); c) the shivering threshold was much higher than in nondivers of comparable subcutaneous fat thickness throughout the year (4,5); d) the maximal tissue insulation for a given subcutaneous fat thickness was considerably higher than in nondivers throughout the year (4,5); e) during whole-body immersion in cold water the heat flux through extremities for a given blood flow was lower than in nondivers throughout the year (6); and f) the finger skin temperature and blood flow during hand immersion in 6°C water were lower than in nondivers (7). These alterations in thermoregulatory functions of women divers were taken as evidence for cold acclimatization (1).

If cold acclimatization is indeed developed through repeated exposures to severe cold-water stress, it should disappear when the cold stress is removed. Since 1977, their diving union has allowed Korean women divers to wear wet suits to avoid cold-water discomfort and this practice was soon adopted universally. Field studies of these contemporary wet-suit divers (8) have indicated that they indeed are no longer exposed to the severe cold-water stress to which previous cotton-suit divers were subjected daily (Table I). Therefore,

TABLE I

Thermal Cost of Daily Diving Work in Korean Women Divers

| | 1960* (Cotton Suits) | | 1980† (Wet Suits) | |
|---|---|---|---|---|
| | Summer | Winter | Summer | Winter |
| Number of Work Shifts | 3 | 1–2 | 1 | 1 |
| Duration of a Work Shifts (min) | 70 | 16 | 180 | 120 |
| Final Rectal Temperature (°C) | 35 | 35 | 37.2 | 36.7 |
| Total Extra Heat Loss (kcal) | 1000 | 500–1000 | 260 | 370 |

*From Hong (1) and Kang et al.(9). †From Kang et al.(8).

we undertook the present longitudinal study over a 3-year period (1980–82) to reassess the status of cold acclimatization in these contemporary divers (10).

## METHODS

### Subjects

Thirty each of divers and nondiving housewives (control) were recruited at random from a similar socioeconomic level in the village of Hae Woon Dae, Busan, Korea. After thorough physical examinations, 18 each of divers and nondivers who showed no apparent cardiopulmonary diseases were selected. On the average, they were 40 (27–49) years old: 155.4 (145–167.2) cm in height; and 55.4 (39–71) kg in weight.

### Basal Metabolic Rate

In 18 each of divers and nondivers we determined the BMR in spring (April, 1980), summer (August, 1980) and winter (January–February, 1981) by standard protocols (11) using a 9-liter Collins spirometer.

### Shivering Threshold and Maximal Tissue Insulation

We determined these BMR's in 11 each of divers and nondivers over a 3-year period (summer and winter of 1980, 1981, and 1982), using the water bath method employed by Rennie et al. (5). Subjects were clothed in swim suits and sat on a stool immersed in water with only their heads out. The bath water was continuously circulated by means of two pumps of 70 L $min^{-1}$ capacity and its temperature was regulated to within 0.01°C at any selected level with a thermistor thermostat. The rectal temperature was measured at 10-min intervals with a telethermometer. The oxygen consumption was measured

for 10 min at 20-min intervals with a 9-liter Collins spirometer. The shivering threshold was defined in terms of the critical water temperature ($T_{cw}$), which represents the lowest water temperature an individual could tolerate for 3 h without shivering. Thus, a lower $T_{cw}$ is synonymous with an elevated shivering threshold. We computed tissue insulation (I) for 30-min intervals using the following formula: $I = (T_R - T_W)/\dot{H}_S$, where $T_R$ = the rectal temperature; $T_W$ = the water temperature; and $\dot{H}_S$ = the skin heat loss ($Kcal \cdot h^{-1} \cdot m^{-2}$). The skin heat loss was assumed to equal metabolic rate ($\dot{V}_{O_2} \times 4.83$) minus respiratory heat loss (8% of the metabolic rate). In those cases where the $T_R$ declined, the decrease in body heat store for the 30-min intervals ($\Delta T_R \times 0.83 \times 0.6 \times$ body weight) was added to metabolic rate. Maximal insulation ($I_{max}$) was taken to be the highest I value observed during the steady-state conditions of the third hour. Because both $T_{cw}$ and $I_{max}$ are directly related to the mean subcutaneous fat thickness of the body (SFT), subjects were arbitrarily selected to span a large range of SFT (5–15 mm range). We measured the SFT according to Allen et al. (12) by measuring skinfold thickness at 10 different body sites with a Lange caliper.

### Finger Blood Flow in Cold Water

The finger-flow flow during hand immersion in 6°C water was measured in 4–6 divers and 4–7 nondivers in 1980 (summer); 1981 (winter and summer); and 1982 (winter) by finger plethysmography as employed in the previous study (7). In each subject, pressure cuffs and a mercury-in-rubber gauge were fixed over the left upper arm and the middle finger. A cuff was also placed over the right upper arm for the measurement of blood pressure by sphygmomanometry. For the measurement of finger blood flow, the pressure cuff on the left upper arm was first inflated above systolic pressure to stop the arm blood flow and to standardize the residual blood volume in the arm. Immediately thereafter the venous occlusion cuff located at the base of the proximal phalange of the middle finger was inflated to a level similar to the diastolic pressure, and then the change in finger volume induced by releasing the arm cuff was measured by a mercury-in-rubber gauge located in the middle of the distal phalange and recorded by an Offner oscillograph. The finger blood flow was computed on the basis of the initial slope of this circumference/time curve and expressed in $mL \cdot min^{-1} \cdot 100\ mL^{-1}$. Once the blood pressure and the blood flow were stabilized the left hand was immersed in 6°C water and the measurements were continued for the following 60 min.

## RESULTS

### Basal Metabolic Rate

Figure 1 summarizes average values of BMR of divers and nondivers. The values of BMR are expressed as percent deviation from DuBois standard.

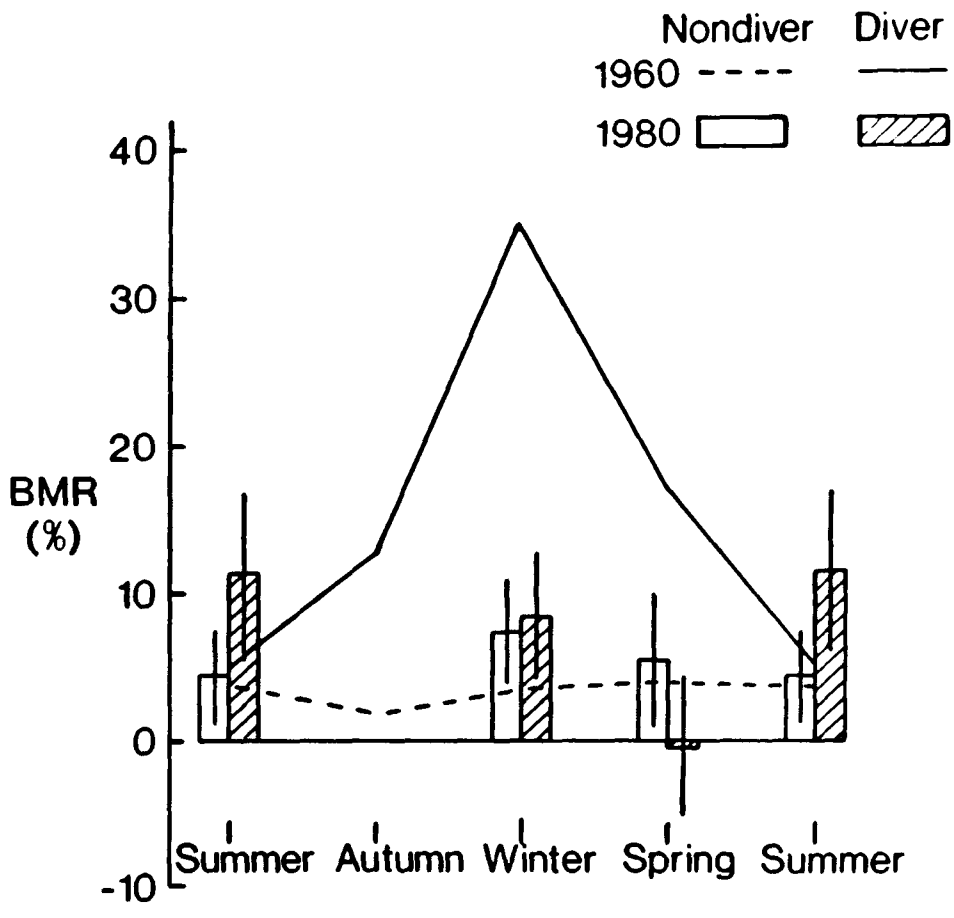



Fig.1. Seasonal changes in basal metabolic rate (% deviation from DuBois standard). Values for 1960 series are from Ref. 2. Values for 1980 series represent means ±SE of 18 subjects.

For comparison, values obtained in previous studies (2) are also included. In the previous study (1960), the BMR of divers showed a marked seasonal variation, showing a maximum in winter and a minimum in summer, whereas the BMR of nondivers was constant throughout the year. The BMR was approximately 30% higher in the diver than in the nondiver in winter but it was comparable in both groups in summer. In the present study (1980), the BMR of divers did not show any seasonal fluctuations, and the value was not significantly different from that of nondivers in any season. This indicates that the reversible increase in BMR of divers during the cold season had disappeared by 1980, i.e., within 3 years of wet-suit diving.

**Shivering Threshold and Maximal Tissue Insulation**

Figure 2 depicts the critical water temperature ($T_{cw}$) as a function of the mean subcutaneous fat thickness (SFT) in divers and nondivers. Overall, the

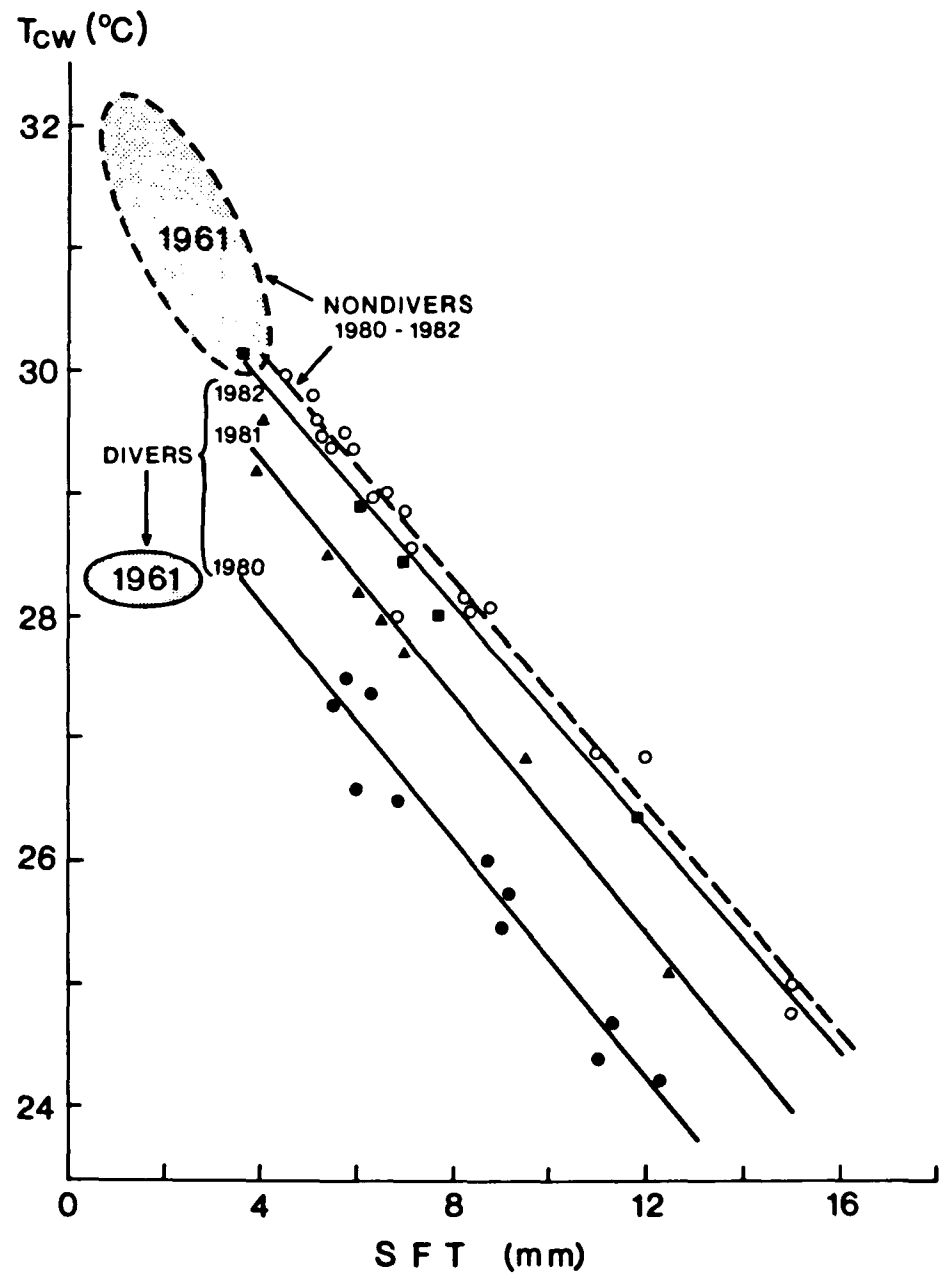



Fig.2. Critical water temperature ($T_{cw}$) as a function of the mean subcutaneous fat thickness (SFT) in divers (*solid symbols*) and nondivers (*open symbols*). Because the regression line for nondivers did not not change with the season, all the data were pooled to construct a combined regression line ($T_{cw} = -0.464$ SFT + 31.99, $r = -0.986$, $P<0.01$). Equations of regression lines for divers are: $T_{cw} = -0.496$ SFT + 30.11 ($r = 0.974$, $P<0.01$) in 1980; $T_{cw} = -0.490$ SFT + 31.24 ($r = 0.994$, $P<0.01$) in 1981; $T_{cw} = -0.448$ SFT + 31.59 ($r = 0.997$, $P<0.01$) in 1982. Values for 1961 series are from Ref. 4. (Figure from Park et al. [10] with permission from the American Physiological Society)

$T_{cw}$ decreased linearly as the SFT increased, with the identical slope of approximately $-0.46°C \cdot mm^{-1}$ in both divers and nondivers. The $T_{cw}$/SFT relationship in nondivers was identical in the 1980, 1981, and 1982 series without displaying any seasonal variations. The $T_{cw}$/SFT relationship in divers, however, changed markedly over the 3-year period. Until 1981 the $T_{cw}$ of divers was still lower than that of nondivers of comparable SFT: the difference was 2°C in 1980 and 1°C in 1981; this difference in $T_{cw}$ decreased further to less than 0.2°C in 1982. In 1961, the $T_{cw}$ of divers was 2–4° lower than that of nondivers. Thus, the high shivering threshold previously observed in Korean divers reverted completely to the control level after 5 years of wet-suit diving.

Figure 3 represents the relationship between $T_{cw}$ (or $T_S$) and the core temperature ($T_R$) measured during the third hour of immersion (i.e., at the

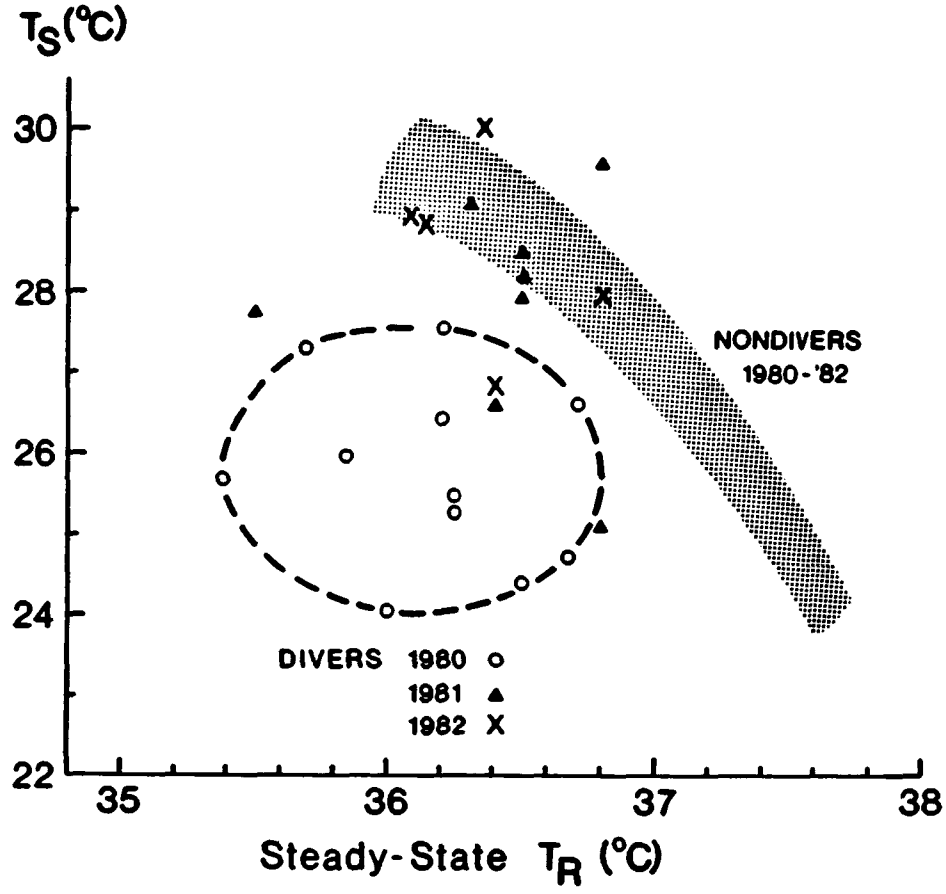



Fig. 3. Relationship between the skin temperature ($T_S$, i.e., $T_{cw}$) and the steady-state rectal temperature ($T_R$) at the third hour of immersion in water of critical temperature. *Dashed-line ellipse* includes all of the divers in 1980.

shivering threshold). The relationship in nondivers followed the same general pattern in 1980, 1981, and 1982, thus data were pooled and the upper and lower limits of data points were depicted. Overall, the higher the $T_{cw}$, the lower the rectal temperature. That is, there was an inverse relationship between the skin temperature and the core temperature at the shivering threshold. The relationship in divers was quite different from that of nondivers until 1982. In general, at the same $T_{cw}$ their steady-state rectal temperature was much lower than that of nondivers, an indication that at the same cold-water stress divers begin to shiver at a lower core temperature as compared with nondivers. This trend was much less evident in 1981 than in 1980. In fact, in 5 out of 8 divers of the 1981 series, the $T_{cw}/T_R$ relationship was similar to that of nondivers. By 1982 most divers (4 out of 5) had the same $T_{cw}/T_R$ relationship as nondivers. Thus the mechanism underlying the elevated shivering threshold does not operate any longer in most modern divers.

Figure 4 illustrates the maximal tissue insulation ($I_{max}$) as a function of the SFT. As expected, the $I_{max}$ was directly and linearly related to the SFT in both divers and nondivers. Regression lines for the 1980, 1981, and 1982 series were not significantly different from each other in both groups. Moreover, the combined regression lines for nondivers ($I_{max} = 0.012$ SFT + 0.094, $r = 0.849$, $P<0.01$) and divers ($I_{max} = 0.011$ SFT + 0.109, $r = 0.878$, $P<0.01$) were not significantly different. In other words, the $I_{max}$ of divers was not different from that of nondivers if it is corrected for the subcutaneous fat thickness. This is in contrast to the previous data obtained in the 60's (*dashed lines* in Fig. 4), which showed considerably higher $I_{max}$ in divers than in nondivers of comparable SFT.

## Vascular Responses to Hand Immersion in Cold Water

Figure 5 depicts the average time course of the change in finger blood flow during hand immersion in 6°C water. The finger blood flow decreased precipitously immediately following the immersion of hand and then increased within 5 min as the result of cold-induced vasodilation. In nondivers, this blood flow change was not significantly different between the season and the year, thus the overall mean (±SE) of all measurements is depicted in the graph (*stippled area*). In 1980 (August), divers maintained a significantly lower finger blood flow during hand immersion compared with nondivers, as observed in a previous study (Fig. 5 *inset*). The average finger blood flow of divers during hand immersion (14 $mL \cdot min^{-1} \cdot 100\ mL^{-1}$) was approximately 33% lower than that of nondivers, an indication that divers developed a significantly higher degree of vasoconstriction of finger blood vessels. In 1981 (February and August) and 1982 (February), the blood flow was not apparently different between divers and nondivers. This finding indicates that the local vascular acclimatization observed in the previous divers has completely disappeared in contemporary divers since the beginning of 1981.

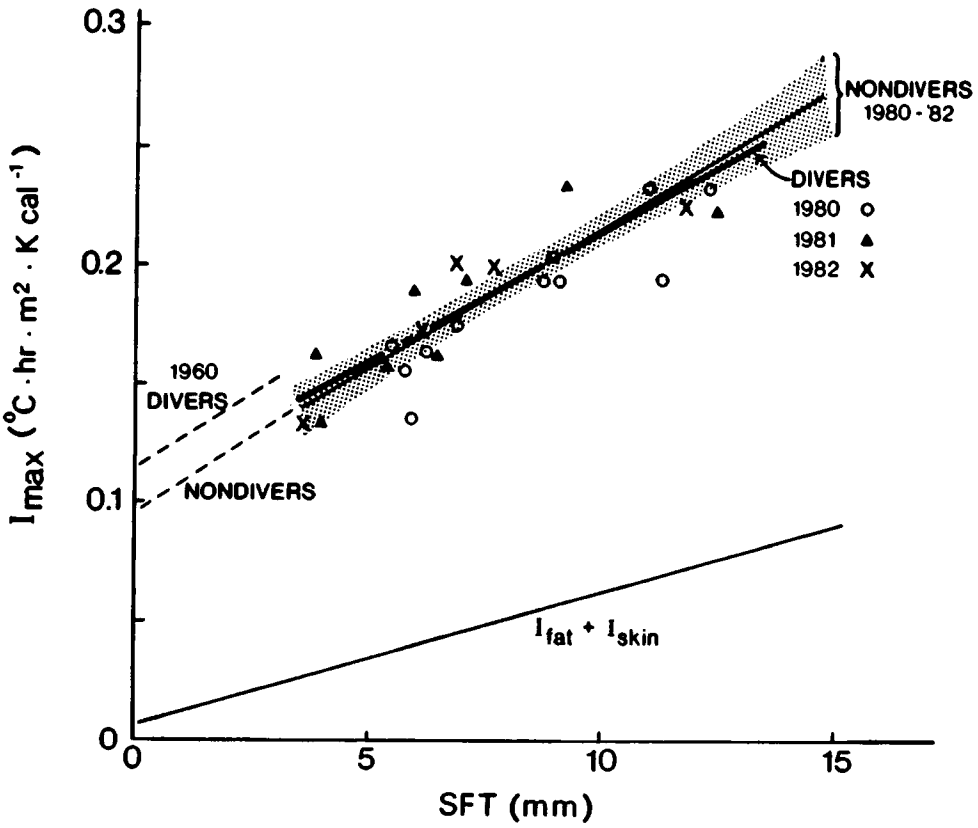



Fig. 4. Maximal body insulation ($I_{max}$) as a function of the mean subcutaneous fat thickness (SFT). Because the regression lines for divers in 1980, 1981, and 1982 series were not different from each other, a combined regression line of all series ($I_{max}$ = 0.011 SFT + 0.109, $r$ = 0.878, $P$<0.01) was constructed. The regression line for nondivers did not change with the season, thus a pooled regression line for all data ($I_{max}$ = 0.012 SFT + 0.094, $r$ = 0.849, $P$<0.01) was constructed. *Stippled area* represents ±1 SE. *Dashed lines* representing 1960 subjects are from Ref. 1. *Thin solid line* representing $I_{fat}$ + $I_{skin}$ is calculated from insulations of human fat (0.31 clo/cm) and skin (0.14 clo/cm) reported in Ref. 18.

## DISCUSSION

The results of the present longitudinal study indicate that various types of cold acclimatization phenomena documented in previous Korean divers, who wore cotton suits during diving, have gradually disappeared since divers started wearing wet suits in 1977. Figure 6 summarizes the time course of deacclimatization for each of several thermoregulatory functions. The reversible increase in BMR during the cold season and the ability to maintain a high

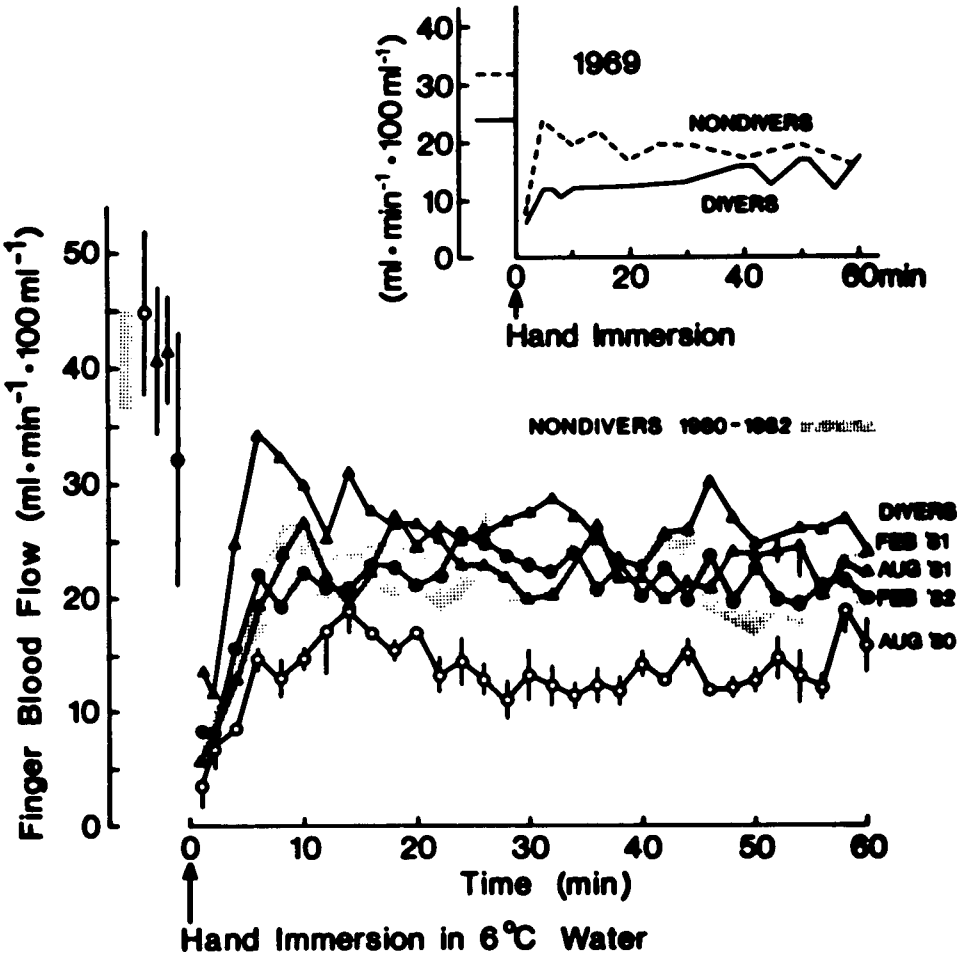



Fig.5. Changes in finger blood flow during immersion of the left hand in 6°C water. Each *solid curve* and *vertical bar* represents mean ± SE of 4–6 divers; *stippled area* represents the overall mean ± SE of 20 measurements in nondivers during the period of 1980 to 1982. *Inset:* finger blood flow measured in a previous study (7).

tissue insulation in cold water had disappeared by 1980, i.e., within 3 years of wet-suit diving. The mechanisms of shivering suppression and the greater vasoconstriction of finger blood vessels during cold-water immersion were sustained until the third year of wet-suit diving, but disappeared during the subsequent 2 years. One reason for the latter finding may be related to the fact that the contemporary divers wear only cotton gloves throughout the year and hence their hands are still subjected to cold-water stress.

This gradual return to normal physiological responses to cold reinforces the original conclusion that cold acclimatization as manifested by earlier

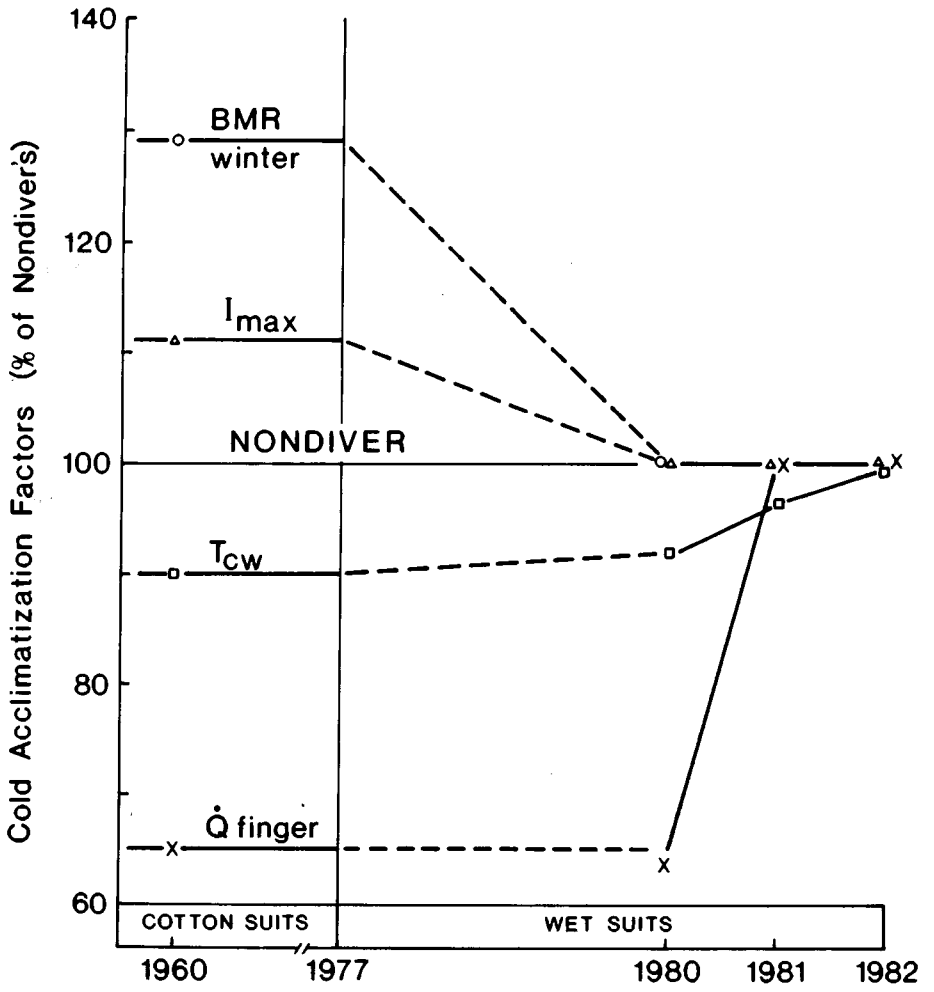



Fig. 6. Relative values of various thermoregulatory functions in previous (1960) an contemporary (1980) divers. (From Park et al. [10] with permission from The American Physiological Society)

studies (1) did in fact exist before adoption of wet suits. A winter-high and summer-low type of seasonal variation of BMR has also been documented among Japanese (13), though the magnitude was much smaller than that in previous Korean divers. Interestingly, this variation of Japanese BMR has been gradually diminished as the ratio of fat to carbohydrate (F/C ratio) in their diet has increased (13). Comparison of food survey data of contemporary divers (8) and previous divers (9) indicates that the F/C ratio of Korean divers (0.097 in 1962 vs. 0.104 in 1982) changed little over the last 20 years. Thus, the lack of seasonal variations in BMR of the contemporary Korean divers

cannot be attributed to dietary changes. This, in turn, suggests that the elevated BMR in previous divers during the cold seasons was a manifestation of metabolic acclimatization to cold, as observed in animal studies (14).

The shivering mechanism of previous divers was suppressed as indicated by the lower $T_{cw}$ in divers than in nondivers (Fig. 2). Because shivering accelerates body heat loss from unprotected individuals, such an attenuation of shivering among divers has been interpreted as an acclimatization process economizing body heat balance of divers in cold water (5). The mechanism underlying the shivering attenuation is not clearly understood. According to commonly accepted theory, the shivering mechanism is activated by peripheral cold receptors and inhibited by central (anterior hypothalamic) warm receptors. The interaction between the two receptor activities is such that shivering is not induced by cutaneous cold receptor impulses unless the cranial temperature is lower than a critical temperature, the *set point*, which is itself a function of skin temperature. The lower the skin temperature, the higher the set point at which shivering occurs, as shown by Cabanac et al. in studies on highly anesthetized dogs (15). We can interpret the $T_{cw}/T_R$ relationship of our control subjects (Fig. 3) quite reasonably in the light of this hypothesis. The higher the $T_{cw}$ of a nondiver, the lower her steady-state $T_R$. Such a relationship was not evident in divers until their shivering threshold returned to the nondiver level in 1982. When their shivering threshold was higher than nondiver's (such as in 1980) at the same cold stress to the skin, divers began to shiver at a lower core temperature than nondivers. Whether this was due to suppression of cutaneous cold receptor sensitivity, or to alterations of the central set-point mechanism is not clear. It is, however, interesting to mention that during cold-water immersion, divers usually complained of internal chilling, not external chilling, whereas nondivers complained of external chilling. This may imply that the cold receptor sensitivity (or at least the perception of skin cooling) was suppressed in divers. In fact, it has been observed that the rate of cutaneous cold receptor discharge (both static and dynamic) is lower in long-term cold-acclimatized animals than in warm-acclimatized animals (16).

The maximal tissue insulation($I_{max}$) attainable by previous Korean divers was much higher than that by nondivers (Fig. 4). The overall tissue insulation consisted of insulations provided by unperfused skin, cutaneous fat, and muscle layers in series. The fat and skin insulations remain unchanged in cold water, regardless of whether the subject exercises or not, but the muscle insulation decreases in proportion to exercise intensity, and the total insulation approaches very closely to the level of fat plus skin insulation in severe exercise (17,18). Because the physical insulations of fat and skin may not be different between divers and nondivers of the same SFT, the relatively high $I_{max}$ in previous divers must be due to a relatively high muscle insulation (i.e., more intensive vasoconstriction in muscle layer, or an increased thickness of muscle shell). Such an insulative acclimatization in the muscle layer disappeared in contemporary divers even faster than the mechanism of shivering attenuation (Fig. 6).

## Acknowledgment

This report is based on the investigation carried out as a project under the U.S.-Republic of Korea Cooperative Science Program supported by grants from NSF (INT–79–18378); KOSEF (1980–1981); and USPHS (HL–14414) in collaboration with Drs. I. S. Lee, Y. D. Park, D. S. Yeon, K. S. Paik, D. H. Kang, D. J. Suh, S. H. Lee, and S. Y. Hong. The authors gratefully acknowledge all subjects for their splendid cooperation. Thanks are also extended to Miss Sung Hee Choe and Mrs. Elizabeth Miranda for typing the manuscript.

## References

1. Hong SK. Comparison of diving and nondiving women in Korea. Fed Proc 1963;22:831–833.
2. Kang BS, Song SH, Suh CS, Hong SK. Changes in body temperature and basal metabolic rate of the ama. J Appl Physiol 1963;18:483–488.
3. Kang BS, Han DS, Paik KS, Park YS, Kim JK, Kim CS, Rennie DW, Hong SK. Calorigenic action of norepinephrine in the Korean women divers. J Appl Physiol 1970;29:6–9.
4. Rennie DW. Thermal insulation of Korean diving women and nondivers in water. In: Rahn H, ed. Physiology of breath-hold diving and the ama of Japan. Washington, DC: Natl Acad Sci-Natl Res Counc 1965:315–324.
5. Rennie DW, Covino BG, Howell BH, Song SH, Kang BS, Hong SK. Physical insulation of Korean diving women. J Appl Physiol 1962;17:961–966.
6. Hong SK, Lee CK, Kim JK, Song SH, Rennie DW. Peripheral blood flow and heat flux of Korean women divers. Fed Proc 1969;28:1143–1148.
7. Paik KS, Kang BS, Han DS, Rennie DW, Hong SK. Vascular responses of Korean ama to hand immersion in cold water. Appl Physiol 1972;32:446–450.
8. Kang DH, Park YS, Park YD, Lee IS, Yeon DS, Lee SH, Hong SY, Rennie DW, Hong SK. Energetics of wet suit diving in Korean women divers. J Appl Physiol: Respir Environ Exercise Physiol 1983;54:1702–1707
9. Kang DH, Kim PK, Kang BS, Song SH, Hong SK. Energy metabolism and body temperature in the ama. J Appl Physiol 1965;20:40–50.
10. Park YS, Rennie DW, Lee IS, Park YD, Paik KS, Kang DH, Suh DJ, Lee SH, Hang SY, Hong SK. Time course of deacclimatization to cold water immersion in Korean women divers. J Appl Physol: Respir Environ Exercise Physiol 1983;54:1708–1716.
11. Consolazio CF, Johnson RE, Pecora LJ. Physiological measurements of metabolic functions in man. New York: McGraw Hill, 1963.
12. Allen TH, Peng MP, Chen KP, Ruang TF, Chang C, Fang HS. Prediction of total adiposity from skin folds and the curvilinear relationship between external and internal adiposity. Metabolism 1956;5:346–352.
13. Yurugi R, Yoshimura H. Seasonal variation of basal metabolism in Japanese. In: Yoshimura H, Kobayashi S, eds. Physiological adaptability and nutritional status of the Japanese. Tokyo: University of Tokyo Press, 1975:45–59.
14. Burton AC, Edholm OG. Man in a cold environment. Physiological and pathological effects of exposure to low temperatures. New York, London: Hafner, 1969:102–199.
15. Cabanac M, Chatonnet J, Philipot R. Les conditions de temperatures cerebrale et cutanee moyennes pour l'apparition du frisson thermique chez le chien. Compt Rend 1965;260:680–683.
16. Hensel H. Neural processes in long-term thermal adaptation. Fed Proc 1981;40:2830–2834.
17. Rennie DW, Park YS, Veicsteinas A, Pendergast D. Metabolic and circulatory adaptation to cold water stress. In: Cerretelli D, Whipp B, eds. Exercise bioenergetics and gas exchange. Amsterdam: Elsevier/ North-Holland, 1980:315–321.
18. Veicsteinas A, Ferretti CT, Rennie DW. Superficial shell insulation in resting and exercising men in cold water. J Appl Physiol: Respir Environ Exercise Physiol 1982;52:1557–1564.

# COLD EXPOSURE IN HELIOX ENVIRONMENT AT 16 BARS FOR 24 HOURS

*S. Tønjum, A. Påsche, J. Onarheim, P. Hayes, and H. Padbury*

Conditions in a stranded bell in the North Sea are mainly defined by pressure and temperature. Pressure may range between 7 and 20 bars, and temperature can drop as low as 4–6°C.

Representatives of the diving industry indicate that rescue might take as long as 24 h under typical North Sea conditions. Therefore, divers need a protection system enabling survival for at least that long, while they are waiting to be rescued.

Life support in a stranded bell requires oxygen to breathe, removal of carbon dioxide ($CO_2$), and protection against excessive heat loss. Sufficient oxygen for an extended period without surface supply is built into the life-support system of the bell and is usually not a problem. The build-up of $CO_2$ can be prevented if the divers breathe through a $CO_2$-absorbent material. The heat produced by this biochemical $CO_2$ reaction can be used to heat the breathing gas, and, accordingly, prevent respiratory heat loss. Thermal protection of the stranded divers' bodies can be achieved by use of heavy insulative clothing. Based on the results of two former tests at 16 and 31 bars in a cold heliox environment in which both tests lasted for approximately 10 h, investigators at the Norwegian Underwater Technology Center (NUTEC) concluded (1,2) that by use of only passive thermal protection, survival for 24 h in a stranded diving bell would be possible. However, in 1981 investigators at the Admiralty Marine Training Establishment (Physiological Laboratory) (AMTE[PL]) in conjunction with investigators from the Royal Navy (3) validated three different passive-survival systems at 26 bars under realistic conditions in a diving bell, but had to abort the test after 6 h because of a critical drop in body core temperature in two of the test divers.

Because the earlier test had been aborted, it was decided that another test was necessary. The same gas-cooling profile was used as for the AMTE(PL) test for the first 6 h, and a cooling profile extrapolated from that curve was used for the following hours of the test.

## MATERIALS AND METHODS

The experiment was performed at 16 bars in the hyperbaric chamber complex at NUTEC. One of the living chambers had been modified, so that it was easy to cool and heat. A canvas sheet was stretched inside the chamber, approximately 80 cm above the deck plates, and the test subjects lay on this in a supine position for the whole test period.

Before the dive, the two divers who took part in this experiment had been through a test which gave information of their response to gradual cooling. They were immersed in water at 32°C, and the water was slowly cooled to 28°C over the next 130 min. During the immersion period, the skin and rectal temperatures were measured. Mean skin temperatures were almost the same for both subjects, whereas the rectal temperature remained approximately constant at a slightly decreased level, for one subject. The rectal temperature for the other subject fell slowly to a subnormal level.

Both test subjects were scuba divers with limited diving experience, one was a medical doctor and the other a physiologist. The physical characteristics of the two subjects are given in Table I.

A limited food intake during the test period was established and consisted of chocolate, biscuits, and high-energy bars (not candy). Approximately 1 L of cold water was available to drink. It was planned that the subjects would stay in the survival systems as long as possible, a maximum of 24 h, or until their core temperatures dropped to 35.5°C.

The core temperatures of the divers were assessed directly by thermistor rectal probes. Skin temperatures were measured by six thermistors and, in addition, three heat-flow discs were used. These devices measured skin temperatures as well as heat loss in watts per square metre of body surface. Inspiratory gas temperatures were measured inside the oronasal masks, and

TABLE I

Physical Characteristics of Two Subjects

| Age (yr) | Height (cm) | Weight (kg) | Surface Area ($m^2$) |
|---|---|---|---|
| 36 | 178 | 79 | 1.97 |
| 40 | 185 | 73 | 1.96 |

temperatures were also measured in the $CO_2$-absorbent canisters in addition to several temperature registrations from the experimental chamber.

Two survival systems were tested. Both are passive systems and designed specifically for the purpose of providing protection for a diver in a stranded-bell situation. Both systems consist of high quality sleeping bars and combined thermal regenerators and $CO_2$-absorbent canisters. In addition, both systems had individual thermal protection clothing, i.e., a hooded survival vest without sleeves, underwear, boots, and mattress.

## RESULTS

The test was performed for 24 h as planned. The temperature at the bottom of the experimental chamber was 6.2°C at the termination of the test. Thirty minutes before the test was terminated, the heat for the experimental chamber was turned on. Both subjects were able to leave their survival systems without assistance, but complained about some temporary dizziness. They disconnected all the monitoring equipment themselves before they left the experimental chambers. When they entered the warm living chamber, they had no need to go into the sleeping bags, which were prepared for rewarming procedures. The rectal temperatures of the two test subjects never dropped below 36°C (Fig. 1), but had a tendency to fall during the night, increasing again in the morning. At the end of the test there was a small drop in the rectal temperatures of both divers, which were 36.2 and 36.6°C at the time of termination.

The *middle curves* in Fig. 1 show the subjects' rectal temperatures during the test. The *curve* with the *open circles* represents data from the subject with the highest heat loss per square metre body surface.

The *bottom curves* show the inspired gas temperatures measured in the subjects' oronasal masks. The *curve* with the *open circles* again is from the subject with the highest heat loss per square metre body surface.

Mean skin temperatures were based upon readings from chest, thigh, front calf, and upper arm. During the 5–6 h of the test, one subject had a much higher mean skin temperature than the other. This obviously is because this diver put on the complete survival system immediately when the test started and felt uncomfortably warm during the first few hours, while the other subject was not in his "complete system" until 2–3 h later. However, the subject who had the highest mean skin temperature for the first half of the test had the lowest skin temperature for the second half of the test. His survival system had no pants or boots; the other system had them. Toe temperature measured as low as 13°C for the test subject in the system without pants or boots; toe temperature never measured below 27°C for the subject testing the other system.

The inspiratory gas temperatures (Fig. 1), measured in the oronasal masks, averaged higher in one subject's mask than in the other. The subject with the highest temperature complained several times that the gas temperature

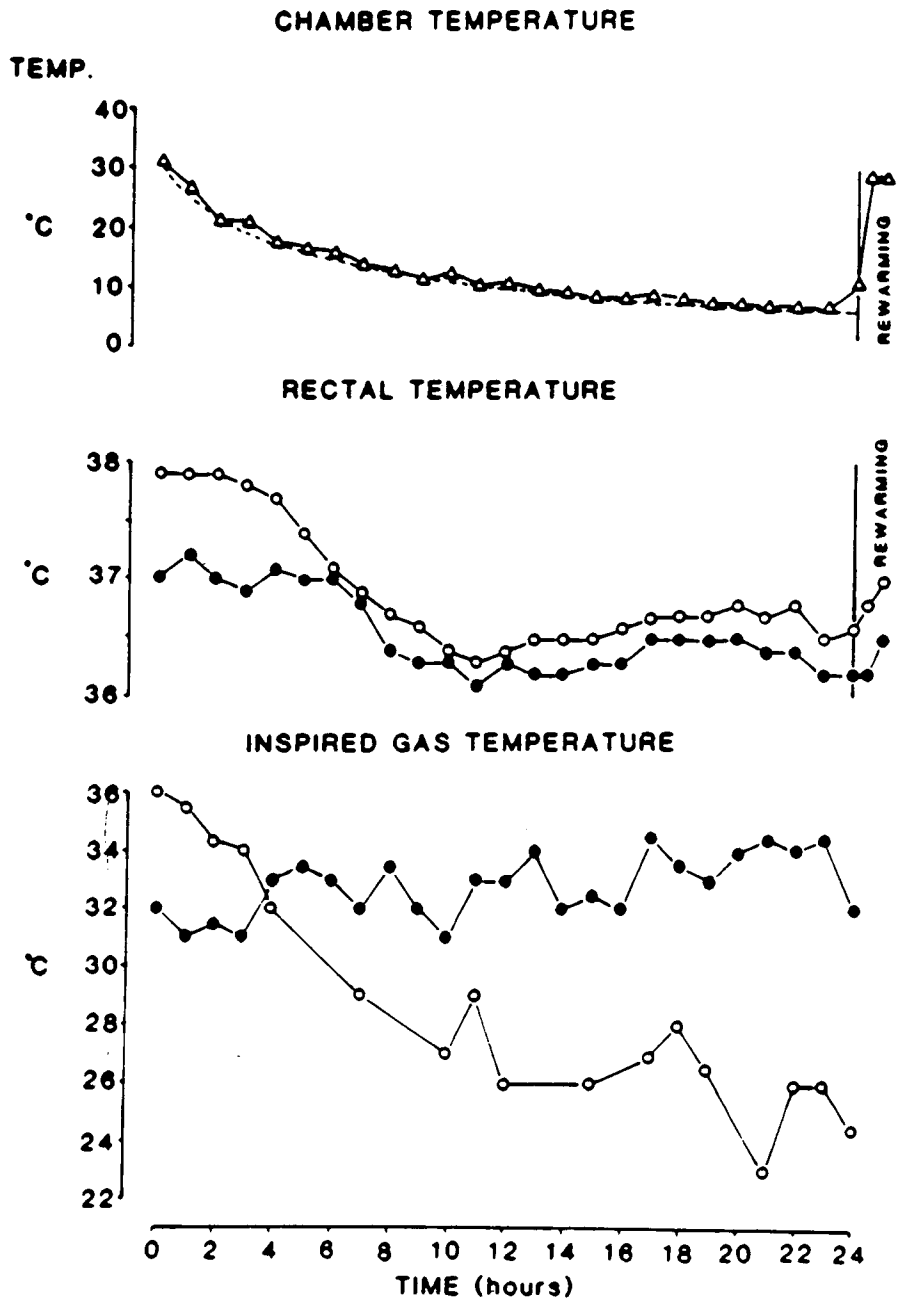



Fig. 1. The *top curves* show chamber temperature, both the temperature cooling profile extrapolated from the AMTE(PL) test and the temperature cooling profile used in the 24-h test. The *middle curves* show rectal temperatures for the subjects during the test. The *curve* with the *open circles* is from the subject with the highest heat loss per $m^2$ body surface. The *bottom curves* show the inspired gas temperatures measured in the subjects' oronasal masks. The *curve* with *open circles* is from the subject with the highest heat loss per $m^2$ body surface.

felt too hot and that he therefore either had to go off the mask for some minutes or breathe without keeping the oronasal mask tight to the face. The temperature in this mask was relatively stable during the whole test and varied between 31.5 and 34.5°C. The temperature in the other mask was as high as 36°C in the beginning of the test, but dropped to 27°C during the following 10 h. For the rest of the test it varied 24 and 28°C. The subject testing this system, however, never complained about cold breathing gas. The heat-flow measurements were made from thigh, chest, and back; 48 recordings were taken from each of the mentioned places in both divers. The measurements represent heat loss in watts per square metre ($W/m^2$) of body surface. Both subjects had the greatest heat loss from their backs, with mean values of 81 $W/m^2$ and 69 $W/m^2$, respectively. Both had the lowest heat loss from their chests, with mean values of 32 $W/m^2$ and 31 $W/m^2$, respectively. The mean value of the heat loss from the thigh was the same in both subjects: 45 $W/m^2$.

## DISCUSSION

Data concerning the gas cooling profile of a manned stranded diving bell in cold water are limited. The AMTE(PL) test (3) of survival systems gave valid data for the first 6 h of the gas cooling profile in a manned bell without heat supply in 7–8°C water, and a pressure equivalent to 250 msw. To complete a 24-h cooling profile, extrapolation was necessary. It is, of course, possible that the cooling of a stranded bell could be faster than that used in this test. The humidity in the test chamber was lower (60–75%) than it would have been in a real "lost bell" situation, where 100% is to be expected.

During the test one subject had a higher mean skin temperature than the other; in addition, for more than half of the second part of the test (the coldest part), this subject had higher inspiratory breathing gas temperatures than the other. The same subject had a smaller average heat loss (48 $W/m^2$) than the other (53 $W/m^2$). In spite of this, the subject with the highest heat loss had the lowest average inspiratory breathing gas temperature, the lowest mean skin temperature, and the highest rectal temperature during the whole test.

These findings should indicate that the subject with the greatest heat loss was testing a survival system with poorer passive thermal protection than the other. Additionally, there is indication that individual morphological characteristics are present and of great importance in such tests: their physical characteristics (Table I) show a significant difference. The shorter subject is the one who had the greatest heat loss during the test, yet in spite of that had a higher rectal temperature than the other. In the predive test when both divers were immersed in 32–28°C water for 130 min the same subject had a higher rectal temperature than the other, while both skin temperatures were similar. The night-day fluctuations of rectal temperatures of both subjects in normal hyperbaric temperatures were similar, however, and within the range that is normal at the surface (36.4–37.4°C). These observations support the view that heat production (metabolism) is individual for each subject and that core

temperature depends on the balance between heat prodution and heat loss. No observations were made that should indicate that hyperbaric heliox environment is different from surface conditions, especially when one takes into account that neither of the subjects was shivering during the test period.

## References

1. Brubakk AO, Tønjum S, Holand B, Peterson RE, Hamilton RW, Morild E, Onarheim J. Heat loss and tolerance time during cold exposure in heliox atmosphere at 16 ATA. Undersea Biomed Res 1982;9:81–90.
2. Tønjum S, Påsche A, Furevik D, Holand B, Brubakk A, Olsen C. *Polar Bear II*: Test of survival systems for stranded bell divers at 31 bars. Bergen: Norwegian Underwater Institute, 1980. (NUI Report No. 40–1980)
3. Hayes PA, Padbury EH, Atherton PJ. Validation of emergency procedures—The lost bell situation. In: International conference DIVETECH' 81, Workshop B: Aids to underwater operations, Session 4. London: Society for Underwater Technology, 1981.

# IMPAIRED PERFORMANCE FROM PROLONGED MILD BODY COOLING

*P. Webb*

Divers who seem adequately protected against cold water may nevertheless lose heat progressively over many hours, not feeling especially cold or shivering. Laboratory experiments with "long slow cooling" (1), or with "progressive symptomless hypothermia" (2), have been prompted by observations of similar cooling during underwater naval operations (3) and in divers working in North Sea oil fields (4). Slow cumulative heat losses produce internal temperatures in the range of 35 to 36.5°C, a level of hypothermia usually considered safe, based on experiences with rapid cooling of unprotected men immersed in cold water.

But is this mild hypothermia safe? Vaughan (3) reported cognitive performance changes (response blocking, perseveration) in men navigating an underwater sled for 6 h in cold water, but in a later study (5) he concluded that such performance losses were probably due to the distraction of cold discomfort, in agreement with Baddeley et al. (6), and Davis et al. (7). But recently, Coleshaw et al. (8) circumvented the distraction issue by first cooling subjects in cold water, then immersing them in warm water so that they felt comfortable, after which, while the subjects were still mildly hypothermic, there was slowing of reasoning and an inability to retain facts that had been memorized while hypothermic.

To further investigate cognitive performance degradation during mild hypothermia, we have employed three performance measures during 6.5 to 7 h of long slow cooling. There was no distraction caused by cold discomfort, since the subjects were gently cooled in a suit calorimeter, while the effects of fatigue and of circadian rhythms were accounted for in the experimental design. There was unequivocal degradation in performance in one test, and

one subject in three performed poorly on a second test at the end of the cooling period. The third test was insensitive to mild hypothermia.

## METHODS

### Performance Tests

Leonard's 5-choice serial reaction test (9) was chosen because it is widely used, is sensitive to environmental stress, and measures overall reaction time, which includes signal recognition, decision making, and motor response. The subject sits before a display of five signal lights arranged in a pentagon; lights are lit one by one in a random pattern, and the subject responds by touching a metal target disc located next to each light. The stylus used for touching the target disc acts to complete an electrical circuit, which extinguishes the light and allows the next signal to appear. An interval of 1.3 s is allowed for a response, and a gap is scored if none is made. From an earlier experience with the test apparatus as originally described, when subjects maintained steady scores despite sleep loss, we decided to make the test more difficult by separating the pentagonal array of five lights from the matching array of target discs so that they were not in the same visual field, and by making the test self-paced. As soon as any target disc was touched with the stylus, the light was extinguished and a new light was lit. Thus, there were no gaps to score, only errors and total responses. The test was administered for exactly 5 min, so that 300 s divided by the number of responses gave reaction time. Fifteen training sessions produced plateaus in the learning curves, and periodic repetition showed that performances without stress were steady with acceptably small standard deviations (Fig. 1). When the calorimeter suit (to be discussed) was worn, there was a small negative effect on both errors and reaction time, which became stable after four or five sessions.

The second test was a twin task meant to measure reserve performance capacity, patterned after one described by Brown (10). Subjects sat before a television set and played a video game (Night Driver, Atari), which simulated steering a rapidly moving car along a curving road, avoiding oncoming traffic and other obstacles. At the same time, while not neglecting the difficult driving task, the subject listened to a series of four-digit numbers and repeated them backwards as he had time. The test lasted 1.5 min and was scored by both the number of backward repetitions attempted and the number incorrectly given. There was little evidence of a learning curve for the demanding driving task; each subject quickly established a characteristic performance level (scored by the video game) and held that level quite steadily throughout training and experiments. There was no evidence of a learning curve for the secondary task of repeating numbers backwards.

The third test was another video game (Air Combat Maneuvering, Atari), which had been recommended by Jones et al. (11) in their test battery for subjects under environmental stress. On the television screen a player's mov-

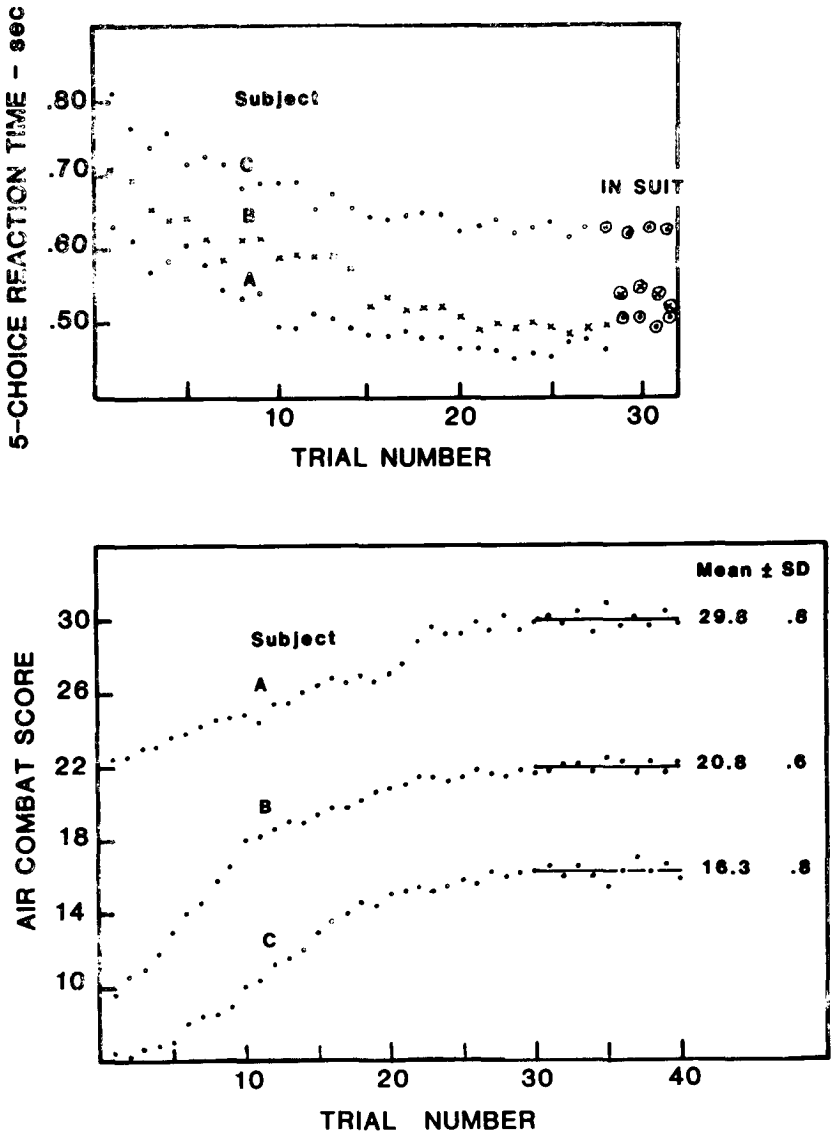



Fig. 1. Learning curves for the three subjects in two performance tests.

ing airplane tried to shoot down the moving target plane; each game lasted 2.25 min, and the number of hits was scored automatically. Ten games were played per session. Learning curves plateaued for our subjects in 25 to 30 test sessions, and remained stable thereafter (Fig. 1). We observed that after training, numerous refresher sessions (the game was often played just for fun), and the sessions during control experiments, subjects had learned the game so

well that it became almost automatic. Each of the three subjects had his own characteristic score; the youngest had the highest score level, the oldest the lowest. The youngest and best-scoring subject was the first to discern the repetitive flight pattern of the game and was able to anticipate the moves of both target and subject airplanes.

Training sessions for all three performance tests were conducted twice daily for 4 weeks before the first control experiments in the suit were carried out. For the next year there were sporadic experiments and periodic training sessions to maintain scores. The main group of cooling and experiments was done during 1 month, some 14 months after the initial training. During all this time the test scores for our subjects without stress remained remarkably stable.

During experiments with the suit calorimeter, the three tests were always given in the same sequence: first, air combat; then 5-choice reaction time; and lastly, the twin task. The three tests took about 40 min to complete.

## Slow Cooling

Cooling experiments were done while the subjects wore a suit calorimeter (12), which measured heat loss from the body and was controlled to either maintain thermal balance or to produce the desired slow cooling. The calorimeter contained a tube suit, a snug-fitting suit of underwear carrying a network of small plastic tubes over the skin, through which water circulated at controlled flow rates and temperatures. Three layers of insulating garments were worn over the tube suit to provide thermal isolation from the environment. Rectal temperature and six skin temperatures were monitored. In addition, the subject wore a light plastic full-face mask, which was used like a ventilated hood to provide a continuous measure of $O_2$ consumption and $CO_2$ production, from which metabolic rate was calculated (13).

The temperature of the water entering the suit was initially adjusted for thermal comfort, and readjusted occasionally to maintain both thermal comfort and thermal balance, wherein metabolic heat production was matched by total heat loss. The range of comfortable inlet temperatures was 30 ± 0.5°C.

To provide slow cooling over many hours, we reduced inlet water temperature by about 1°C, then over the next several hours reduced it progressively to about 27°C. Subjects felt cool but not cold, and skin temperatures fell typically from around 33 to 30.5°C. If the lowering of water temperature was too rapid the subject's metabolic rate rose abruptly, a signal that shivering would soon begin, and the inlet temperature was raised a little to avoid shivering. Later, we could lower it again to maintain a net heat outflow.

Cooling rates were in the range of 0.25 to 0.43 kcal/min. These were net cooling rates, which were continuously computed from the difference between metabolic heat production and total heat loss. Total heat losses were the sum of: heat loss to the tube suit, which was the temperature difference between water entering and leaving the suit times the mass flow rate; evaporative heat loss from weight change corrected for intakes and outputs including the masses

of oxygen and carbon dioxide; the convective heat loss to the current of air traversing the ventilated mask; and the small heat leak across the suit insulation, known from calibrations to be proportional to the gradient between mean skin temperature and environmental temperature.

## Subjects

Three subjects volunteered from the laboratory staff. Two, *Subjects B* and *C*, were trained scuba divers. All were in good health and physically active, although not trained athletes. Medical histories, physical examinations, blood chemistries, hemograms, and resting and exercise electrocardiograms were all negative. All three men were completely familiar with the calorimeter, both as subjects in previous experiments and as operators of the equipment. Subjects are further characterized in Table I.

Procedures and protocols were approved by the laboratory's Institutional Review Board, and subjects signed informed consent forms.

## Protocols

Three different sets of experiments were done, two without cooling for control measurements of performance, and one with long slow cooling.

The first set of experiments had each subject wear the suit calorimeter for 36 h, beginning after supper one evening and continuing through that night, all the next day, and through the second night. Thermal comfort and thermal balance were maintained throughout, and food intake matched energy expenditure. The three performance tests were administered four times, 4 h apart, at 0900, 1300, 1700, and 2100. Our purpose was to see if performance varied with time of day, as circadian rhythms vary.

The second set was an 8-h period in the suit for each man, starting at about 0800, again without cooling, but this time the performance testing was done four times 2 h apart, on the same schedule used in the cooling experi-

TABLE I

Description of Subjects

| Subject | Age | Height | Weight | Body Fat | Estimated $VO_{2\,max}$ |
|---|---|---|---|---|---|
| | (yr) | (cm) | (kg) | (%) | (mL/kg-min) |
| A | 26 | 183 | 80 | 16 | 44 |
| B | 49 | 181 | 69 | 14 | 41 |
| C | 58 | 172 | 88 | 28 | 39 |

ments. Our object was to see if fatigue or boredom would affect the test results.

The third and final set of experiments were those with long slow cooling. There were six of these, since each subject did the protocol twice. Starting at 0800, thermal comfort was maintained in the suit for 1 to 1.5 h, then cooling started. Cooling was continued until 8 h had elapsed, that is, for 6.5 to 7 h. The performance test battery was administered from the beginning of the 2nd, 4th, 6th, and 8th h (Fig. 2).

## RESULTS

A typical set of physiological data is shown in Fig. 3. Note the effect of lowering the inlet water temperature on rectal temperature, skin temperature, and net heat loss. The average value for heat loss from long slow cooling was 134 kcal in 6 experiments, with a range of 98 to 166 kcal.

The average fall in rectal temperature from the cooling was 0.6°C, with a range of 0.2 to 0.9°C.

Physiological data are summarized in Table II.

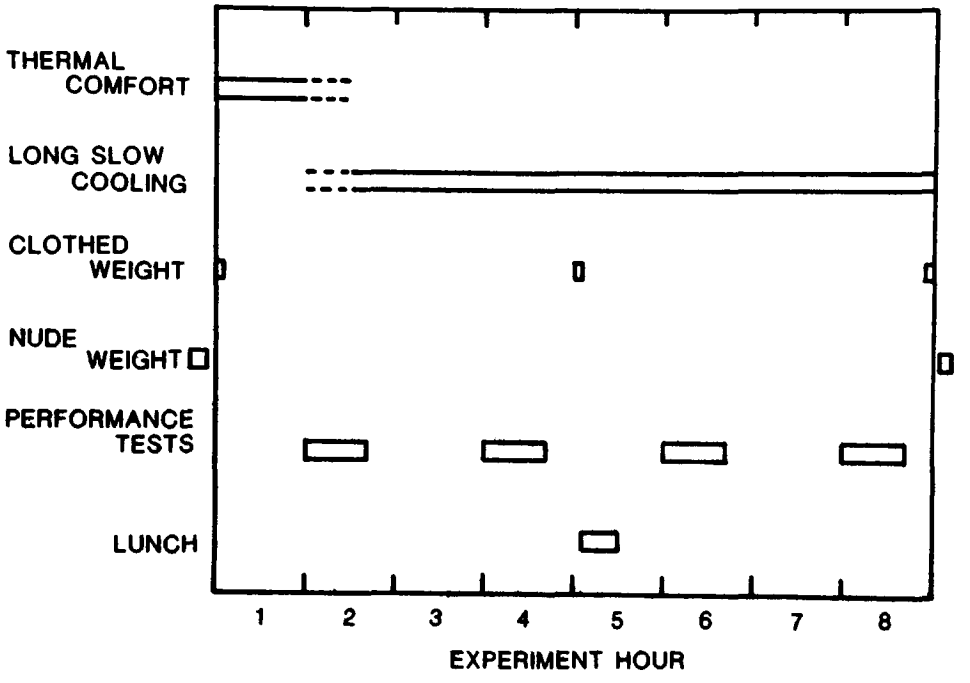



Fig. 2. Schedule of events during the 8-h cooling experiments.

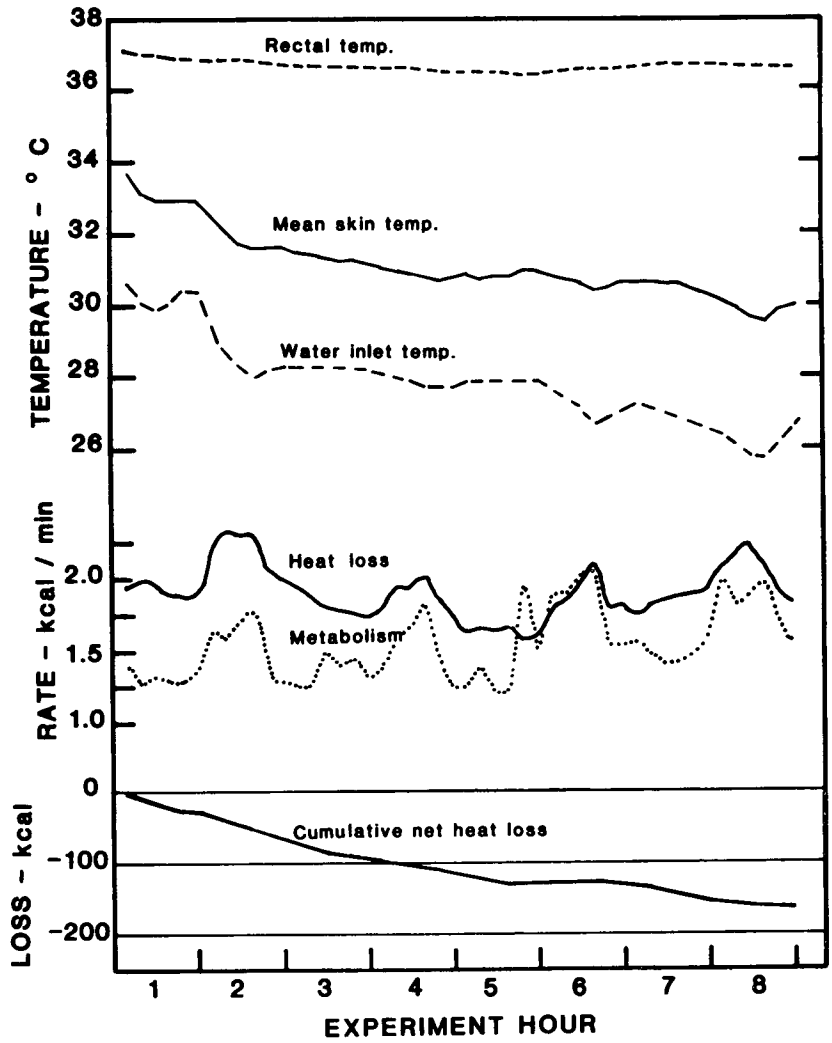



Fig. 3. Typical responses of a subject during long, slow cooling. Note the rises in metabolic rate during the 4th, 5th–6th, and 8th h. These preshivering episodes were signals to raise inlet water temperature slightly.

## Performance

Control experiments for 36 h and for 8 h showed little change in performance scores, so that the effects of circadian rhythms and of fatigue and boredom were negligible. There was a small effect of the bulky suit on the 5-choice reaction time test (Fig. 1); the control values in the suit were used as a base of comparison for the cooling experiments.

TABLE II

Physiological Effects of Long Slow Cooling

| Subject | Net Heat Loss | Change in $T_{re}$ | Mean Skin Initial | Temperature Final |
|---|---|---|---|---|
| | (kcal) | (°C) | (°C) | (°C) |
| A | 98 | −0.2 | 32.6 | 30.5 |
| | 166 | −0.5 | 33.0 | 30.5 |
| B | 138 | −0.8 | 32.8 | 31.5 |
| | 161 | −0.9 | 32.4 | 30.5 |
| C | 123 | −0.7 | 32.0 | 30.4 |
| | 115 | −0.5 | 32.2 | 30.0 |

$T_{re}$: rectal temperature.

Results of the 5-choice reaction time test showed performance impairment in the final hour of cooling in all three subjects. Scores held at the control levels during the 2nd, 4th, and 6th experiment hours (the first 5 h of cooling); but, in the last testing session there was both slowing of overall reaction time and an increase in the number of errors (Fig. 4). The lengthening of reaction

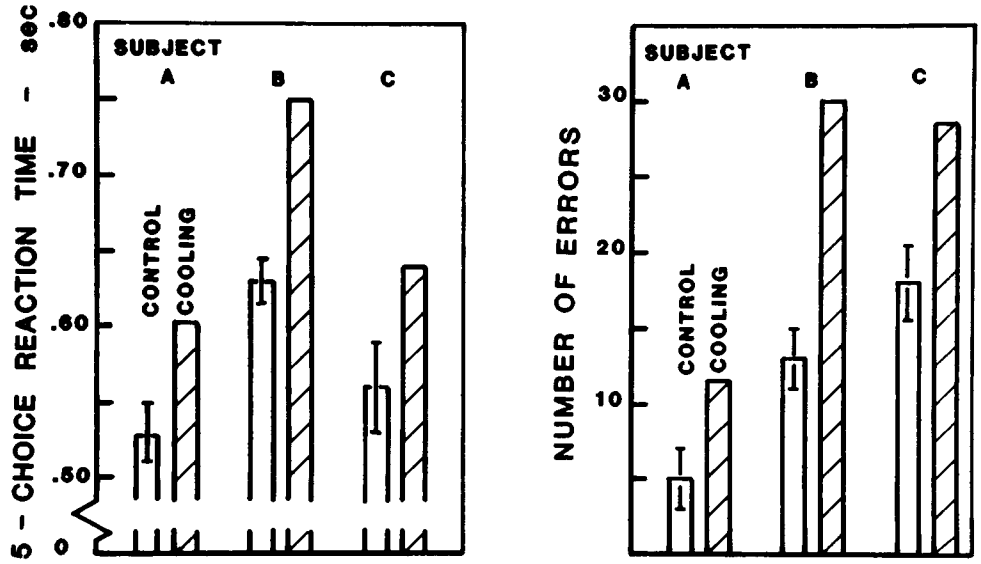



Fig. 4. Impairment of performance in the 5-choice reaction time test at the end of slow cooling. The *clear bars* are control means with standard deviations shown as vertical bars. *Lined bars* are the mean of the two scores for each subject in the final hour of the two cooling experiments.

time averaged 0.09 s above a control average of 0.57 s, or 16%. Errors increased from a control average of 12 errors in 527 responses during 5 min to 23 errors in 454 responses. Thus, the error rate increased from 2 to 5%.

The twin task gave equivocal results. Although all three subjects maintained their driving scores in the primary task throughout cooling, as heat loss accumulated *Subject B* attempted fewer trials of the secondary task (repeating four-digit numbers backwards), and he made more errors. In the last hour of cooling this man attempted only 16 and 17 number responses in the two cooling experiments, compared to his control average of 22 ± 3 (SD) trials, and his errors increased from a control level of 1.1 ± 1.0 to 6 and 7 errors during the last hour of cooling. However, *Subjects A* and *C* attempted as many trials and made as few errors as they had during control experiments.

The air combat task proved to be insensitive to cooling. All three subjects maintained their usual scores throughout the long slow cooling experiments.

## DISCUSSION

There is little doubt that progressive, symptomless hypothermia from long slow cooling occurs in diving. The question is, does this condition have subtle but important consequences? The laboratory simulation reported here appears to produce the same physiological state, since skin and rectal temperatures were like those reported from underwater operations by Vaughan (3) and from the North Sea by Keatinge et al. (4). So, performance changes like those we observed might occur in real dives. In diving operations greater heat losses and faster cooling rates than those we report may well occur, yet still be symptomless, i.e., with little shivering or cold distress.

The clear finding of performance degradation in our study adds to the evidence that mild hypothermia changes cognitive behavior. Additional evidence includes reports that divers often do not remember what they did during the late stages of an underwater stay, which is consistent with the partial amnesia reported by Coleshaw et al. (8); Vaughan (3,5) observed that underwater swimmers forgot a well-learned task and made judgmental errors during mild hypothermia. It is tempting to speculate that some unexplained diving accidents may be caused by performance degradation of this hard-to-measure variety.

In view of the importance of maintaining peak performance in divers, further investigation is warranted of higher order (cognitive) performance, such as memory and decision making, during mild hypothermia of slow onset.

## Acknowledgment

This study was supported under Office of Naval Research Contract N00014–80–C–0193 with funds provided by the Naval Medical Research and Development Command.

## References


1. Webb P. Troutman SJ Jr, Annis JF. Heat loss and body temperature change during measured cooling. Undersea Biomed Res 1979:6(Suppl):27.
2. Hayward MG, Keating WR. Progressive symptomless hypothermia in water: possible cause of diving accidents. Br Med J 1979;i:1182.
3. Vaughan WS Jr. Diver temperature and performance changes during long-duration, cold water exposure. Undersea Biomed Res 1975;2:75–88.
4. Keatinge WR, Hayward MG, McIver NKI. Hypothermia during saturation diving in the North Sea. Br Med J 1980;280:291.
5. Vaughan WS Jr. Distraction effect of cold water on performance of higher-order tasks. Undersea Biomed Res 1977;4:103–116.
6. Baddeley AD, Cuccaro WJ, Egstrom GH, Weltman G, Willis MA. Cognitive efficiency of divers working in cold water. Hum Factors 1975;17:446–454.
7. Davis FM, Baddeley AD, Hancock TR. Diver performance: the effect of cold. Undersea Biomed Res 1975;2:195–213.
8. Coleshaw SRK, van Someren RNM, Wolff AH, Davis HM, Keatinge WR. Impairment of memory registration and speed of reasoning caused by mild depression of body core temperature. J Appl Physiol:Respir Environ Exercise Physiol 1983;55:27–31.
9. Leonard JA, 5-choice serial reaction apparatus Cambridge: Applied Psychology Research Unit, Medical Research Council, 1959. (Report No. 326/59)
10. Brown ID. Measurement of control skills, vigilance, and performance on a subsidiary task during 12 hours of car driving. Ergonomics 1967;10:665–673.
11. Jones MB, Kennedy RS, Bittner AC. A video game for performance testing. Am J Psychol 1981;94:143–152.
12. Webb P, Annis JF, Troutman SJ Jr. Human calorimetry with a water-cooled garment. J Appl Physiol 1972;32:412–418.
13. Troutman SJ Jr, Webb P. Instrument for continuous measurement of $O_2$ consumption and $CO_2$ production of men in hyperbaric chambers. J Biomech Eng 1978;100:1–6.

# PREDICTED SURVIVABILITY OF "LOST BELL" ACCIDENTS UNDER VARIOUS CONDITIONS

*E. H. Wissler*

Provision of adequate thermal protection for occupants of a bell accidentally stranded on the ocean floor is an important aspect of saturation diving. This need was forcefully demonstrated by two early accidents that proved to be fatal for unprotected divers, followed by a third accident in which protected divers survived until an underwater rescue could be effected (1,2). Currently, protection is provided by a heavy antiexposure bag and a $CO_2$ absorption canister that delivers breathing gas warmed by the exothermic absorption reaction. The adequacy of such systems for specific conditions has been demonstrated both in the laboratory (3–5) and in the open sea (1). Nevertheless, experience with such systems is still very limited, and legitimate questions can be raised about their adequacy under other conditions. The purposes of this paper are threefold: a) to describe a theoretical model that can be used to predict human thermal behavior under conditions typical of those surrounding a lost bell accident, b) to show that the model yields results consistent with experimental observations, and c) to analyze the effect of important parameters on the probability of survival under various conditions.

Experimental evaluation of hyperbaric survival systems began in January 1980, when four systems were tested in 4–6°C, 16-bar heliox at the Norwegian Underwater Institute (NUI) (3). Only the system that had been assembled as a prototype hyperbaric system came close to providing adequate protection. However, these results were useful in developing two similar commercial systems tested at NUI in November, 1980 (4). At that time it was established that both systems were capable of maintaining divers for 10½ h in 6–8°C, 31-bar heliox without a significant drop in core temperature. Although the second test seemed to indicate that these systems provided sufficient protection for 24-h survival, a subsequent open-sea test conducted in June 1981 by the

Admiralty Marine Technology Establishment (Physiological Laboratory) (AMTE[PL]) had to be terminated after only 6 h because core temperatures in 2 of the 3 divers had fallen to 35.5°C (6). This test was conducted at a pressure of 26 bars with an initial bell temperature of 30°C; the temperature within the bell had fallen to 15°C by the end of the test. Due to questions raised by the open-sea trial, another study was carried out at the Norwegian Underwater Technology Center (NUTEC) in January 1982 (5). This test established that both commercially produced systems provide sufficient thermal protection for 24-h survival in 16-bar heliox when the temperature approximates that of the AMTE(PL) open-sea trial; the final temperature was 7–8°C.

During this period of system development and testing, a theoretical computer model was also developed to analyze various aspects of the problem (7,8). The experimental and theoretical efforts were complementary in the sense that good agreement between computed and measured values served to validate the theoretical model, which could then be used to predict behavior under conditions other than those studied experimentally. This paper summarizes the results of the theoretical study.

The principal feature of the model is the energy balance which states that when there are no external sources of heat available, divers must generate metabolically all of the heat that is transferred to the sea. If there is sufficient thermal resistance between the divers and the sea, they will be able to maintain body temperatures within an acceptable range; otherwise, generalized cooling will occur. The following factors, each of which potentially affects in a significant way the final conclusions, must be taken into consideration:

1) Excessive loss of heat becomes life-threatening to the divers. Vasomotor action and enhanced heat generation owing to shivering serve to reduce the rate of bodily heat loss.

2) Heat transfer from the body can be reduced by increasing the thermal resistance of the diver's garment.

3) The gas space that surrounds the divers can provide an appreciable thermal resistance between the external surface of the garment and the interior surface of the bell. However, natural convection and heat transfer by radiation tend to reduce the resistance of the gas space.

4) If the bell is warm when contact with the surface is lost, several hours will pass before the walls cool to nearly the temperature of the sea. Even after the wall cools, it still provides some thermal resistance. Rigid foam applied to the exterior of the bell can significantly prolong the cooling time and enhance the thermal resistance of the wall.

5) Although it is generally small, there will be some thermal resistance at the bell-sea interface.

The manner in which each of these factors is incorporated into the model will be discussed briefly before results of the "lost bell" simulations are presented.

In this analysis, divers are simulated by a human thermal model (7,9) in which man is subdivided into 15 cylindrical elements representing the head, thorax, abdomen, and proximal, medial, and distal segments of each arm and

leg. Each element is composed of radial layers having appropriate physical and physiological properties to simulate bone, muscle, viscera, fat, and skin. The model assigns dimensions and physical properties, allowing input of individual body size and skinfold thickness. The various elements and layers are linked by the circulatory system, which includes a central thoracic pool and arterial flow through a sequence of elements; in each cylinder some blood is routed to the capillaries of various layers, while the remainder continues to the next element. Venous return may be via deep or superficial veins.

Metabolic heat, which is generated at an appropriate rate in each layer, is transmitted from warmer to cooler regions by conduction between adjacent layers and by circulatory convection among layers and elements. It is assumed that capillary blood reaches local thermal equilibrium with the perfused tissue. Exercise or shivering, or both, raise metabolism in the muscle layers of involved segments. Active thermoregulation is represented by a series of feedback loops; tissue temperatures at selected sites are compared to appropriate set-points, and error signals are centrally integrated to drive effector systems, which include vasomotion, sweat secretion, and shivering. A unique feature of this model is that radial distribution of venous return varies with temperature; in a warm environment, flow is superficial, but in cold, venous blood enters deep veins next to arteries, where countercurrent exchange provides significant conservation of heat. This feature was found necessary for realistic results in the simulation of thermal exposures ranging from heat stress through the comfort zone to severe cold.

A crucial problem in modeling hypothermia is evaluation of realistic shivering levels. Heat production owing to shivering peaks at 4 to 5 times the resting level; submaximal rates depend on both hypothalmic and skin temperatures. However, a near maximal rate cannot be maintained indefinitely because attenuation owing to fatigue occurs during prolonged exposure to severe cold. After the literature pertaining to endurance times for various tasks had been reviewed, it was decided that this could be an important factor in one's ability to survive cold exposure for more than 10 h. Admittedly, fatigue is a very complex phenomenon, but a dominant factor under many circumstances appears to be depletion of glycogen stores in muscle. When this occurs, one can develop a relatively simple expression for endurance times for various forms of exercise. The fatigue equation that was incorporated into the model is consistent with Beckman and Reeves' observation (10) that U.S. Navy personnel who were immersed for 8–10 h in 75°C water became hypoglycemic and developed severe, incapacitating muscle cramps.

A very important aspect of this study was verification that the human thermal model responds realistically to cold stress. This verification was accomplished by comparison of computed and measured values of central temperature, skin temperatures, metabolic rate, and thermal fluxes for a variety of cases. These results have been presented elsewhere (11,12) and will not be repeated here. Suffice it to say that, in general, the comparisons were quite satisfactory for conditions ranging from nude immersion in 10°C water to exposure to 31-bar, 17°C heliox. Hence, it is reasonable to assume that the

accuracy of the human thermal model is sufficient for the purposes of this analysis.

In addition to analyzing correctly human thermal behavior, one must consider those physical factors that define environmental stress. The obvious one is temperature, but the thermal properties of the survival bag, thermal resistance of the surrounding gas, and thermal inertia and resistance of the bell wall are also important. Because the thermal resistances of the survival bag and the gas space are strongly dependent on the physical properties of the bell gas, these properties must be known. A short tabulation of relevant values for heliox and two trimix mixtures is presented in Table I.

The form of the thermal energy balance used for regions within the body is also applicable to regions occupied by clothing, and one can simulate a garment or survival bag by simply adding shells outside of the body to represent various layers of material. One can use analytical techniques and data summarized in a recent series of papers (13,14) to estimate the effect of gas properties on the thermal resistance of a garment. In regard to the commercially available survival bags tested in the *Polar Bear III* study, reference values for a particular situation (16-bar trimix) can be derived from thermal flux measurements made on the chest, back, and thigh. These data indicate that the thermal resistance lies in the range of 2.0 to 2.5 clo, where, by definition, 1 clo is equivalent to a resistance of 0.155 $m^2 \times$ °C/watt (W). The gas property having the greatest influence on thermal resistance is the conductivity, which does not depend strongly on pressure. However, the conductivity does depend on composition, decreasing approximately 20% with the addition of 10 mole percent nitrogen to heliox. Such a decrease in thermal

TABLE I

Physical Properties of Various Heliox and Trimix Mixtures at 300°K

| $P$ | Mole Fractions | | | $\overline{MW}$ | $\rho$ | $C_p$ | k | $\mu$ |
|---|---|---|---|---|---|---|---|---|
| | | | | | gm/L | cal/gm·°C | cal/cm·s·°C | micropoise |
| ATA | $X_{N_2}$ | $X_{He}$ | $X_{O_2}$ | | | | | |
| 31 | 0.00 | 0.984 | 0.016 | 4.449 | 5.61 | 1.13 | $3.55 \times 10^{-4}$ | 201.4 |
| 31 | 0.05 | 0.934 | 0.016 | 5.649 | 7.12 | 0.92 | $3.10 \times 10^{-4}$ | 206.5 |
| 31 | 0.10 | 0.884 | 0.016 | 6.850 | 8.63 | 0.76 | $2.90 \times 10^{-4}$ | 209.3 |
| 36 | 0.00 | 0.986 | 0.014 | 4.392 | 6.42 | 1.14 | $3.57 \times 10^{-4}$ | 201.3 |
| 36 | 0.05 | 0.936 | 0.014 | 5.593 | 8.18 | 0.92 | $3.22 \times 10^{-4}$ | 206.6 |
| 36 | 0.10 | 0.886 | 0.014 | 6.794 | 9.94 | 0.77 | $2.92 \times 10^{-4}$ | 206.6 |
| 41 | 0.00 | 0.988 | 0.012 | 4.336 | 7.23 | 1.16 | $3.59 \times 10^{-4}$ | 201.2 |
| 41 | 0.05 | 0.938 | 0.012 | 5.537 | 9.26 | 0.92 | $3.25 \times 10^{-4}$ | 206.7 |
| 41 | 0.10 | 0.888 | 0.012 | 6.738 | 11.22 | 0.77 | $2.95 \times 10^{-4}$ | 209.8 |
| 46 | 0.00 | 0.989 | 0.011 | 4.308 | 8.05 | 1.16 | $3.61 \times 10^{-4}$ | 201.1 |
| 46 | 0.05 | 0.939 | 0.011 | 5.509 | 10.29 | 0.93 | $3.26 \times 10^{-4}$ | 206.9 |
| 46 | 0.10 | 0.889 | 0.011 | 6.710 | 12.54 | 0.78 | $2.96 \times 10^{-4}$ | 210.2 |

conductivity certainly increases the resistance of a survival suit, but not by the same fraction; an increase of 10% in thermal resistance is more reasonable.

In addition to the thermal resistance of the garment, one must consider the resistance across the gas space separating the divers from the cold wall to the bell. Although helium has a relatively high thermal conductivity, the considerable thickness of the gas layer would yield an appreciable resistance if the gas were motionless. However, this resistance is always limited by convection, which occurs to some extent. Even when the divers are quiet and blowers are not operating, free convection is driven by the temperature gradient that exists across the gas space. This is a complex phenomenon, especially for irregular geometries, but a wealth of empirical information exists in the technical literature. All experimental studies (15) support the notion that the rate of heat transfer for free convection varies as $\Delta T^{1.25}$ for small $\Delta T$ and as $\Delta T^{1.33}$ for larger $\Delta T$'s, depending on whether the flow is laminar or turbulent. ($\Delta T$ = difference between the outer garment and inner wall temperatures.) The proportionality factor, *KHTC*, in the expression,

$$q = KHTC\ \Delta T^{1.25},$$

which relates thermal flux, q, to $\Delta T$ depends on the geometry of the system and the thermal conductivity, density, specific heat, viscosity, and coefficient of thermal expansion of the gas. To obtain an estimate for *KHTC*, one most model the geometry of the system as two eccentric spheres, which is a system that has been studied rather thoroughly in the laboratory (16,17). A representative value of *KHTC* for a small bell at a pressure of 21 bars is 9.1 $W/(m^2 \times {}^\circ C^{1.228})$. If one assumes that the temperature difference between the outer surface of the survival bag and the inner surface of the bell wall is 5°C, the thermal resistance of the gas space becomes 0.43 clo for this case. Hence, the gas space provides a thermal resistance that is appreciable, but not sufficient to obviate the need for other protection.

It is interesting to consider the variation of *KHTC* with other parameters, such as pressure, composition, and geometry. Comparison of results for various cases reveals that *KHTC* increases approximately as the square root of pressure. Hence, doubling the depth reduces the thermal resistance of the gas space by 30%. The dependence on composition is such that adding a small percentage of nitrogen to heliox does not cause a large change in *KHTC*. For eccentric spheres, one can vary the geometry both by changing the ratio of the diameters of the spheres and by varying the vertical position of the inner sphere. Although it appears to be counterintuitive, increasing the outer radius while holding the inner radius constant increases *KHTC*. For an inner radius of 0.06 m, increasing the outer radius from 1.25 m to 2.00 m increases *KHTC* from 6.67 to 10.1 $W/(m^2 \times {}^\circ C^{1.228})$ when the gas is 16-bar trimix and the spheres are concentric. Lowering the position of the inner sphere also increases *KHTC* by as much as 20 to 30%. The worst case is probably represented by a large welding habitat in which the divers are near the floor; *KHTC* is approximately 20.5 $W/(m^2 \times {}^\circ C^{1.228})$. Hence, the thermal resistance for this case is only 0.21 clo.

Because the natural convection is driven by the same temperature difference that causes heat transfer, the process is nonlinear, and the thermal resistance of the gas space decreases as the temperature difference increases. This leads to the interesting situation that the thermal resistance of the gas space increases as the resistance of the survival bag increases because a well-insulated bag has a low external surface temperature. Conversely, the plight of an inadequately protected diver will be exacerbated by enhanced natural convection.

In addition to the factors mentioned above, one must consider the effect that changing pressure or gas composition has on the rate of heat loss through the respiratory tract. It is well known that because gas density increases with increasing pressure, the rate of respiratory heat loss increases proportionately for a given temperature of inspired gas. If nitrogen is added to heliox, the density increases significantly, but the specific heat decreases such that the density-specific heat product remains nearly constant. Hence, the rate of respiratory heat loss should be no more severe for trimix than for heliox.

Heat transfer within the wall of the PTC (including any rigid foam applied to the exterior of the bell) is important for two reasons: it determines the initial rate of cooling within the bell, and it makes a non-negligible contribution to the total thermal resistance. In the model, transient-state heat conduction equations are solved for the steel and foam regions, with appropriate boundary and initial conditions. At the interior surface of the shell, the thermal flux is assumed to be uniform and equal to the rate of heat transfer across the gas space divided by the area of the wall.

The first case analyzed corresponds to the NUTEC *Polar Bear III* study: two divers in 16-bar trimix (2.3% $O_2$, 5% $N_2$, and 93% helium) with the temperature decreasing exponentially from an initial value of 30°C to a final value of 7°C (5). The divers were protected by two different commercially available survival bags, which were assigned a thermal resistance of 2.0 clo in the simulation. Resistances on the arms and legs were increased to account for the reduction in exposed surface provided by a sleeping bag. The effect of breathing gas heating was simulated by assuming that 75% of the sensible heat content (over ambient conditions) of expired gas was returned to the divers as warm inspired gas. For computation of ambient conditions, the bell was assumed to have an inside diameter of 1.7 m, steel walls 20 mm thick, and insulation on the outside with a 22-mm thick layer of foam. Heat loss through penetrations, windows, and the hatch was assumed to reduce the effective thermal insulation of the foam to one-fourth of its original value. For this system, a sea temperature of 5°C yields temperatures within the bell similar to those employed in the NUTEC study.

The results of this simulation are shown in Fig. 1 together with measured values (5). Plotted in the *upper panel* are the measured chamber temperature and the computed inside wall temperature. Although the computed wall temperature decreases more rapidly than the measured gas temperature, that is reasonable and the discrepancy does not appear to be significant.

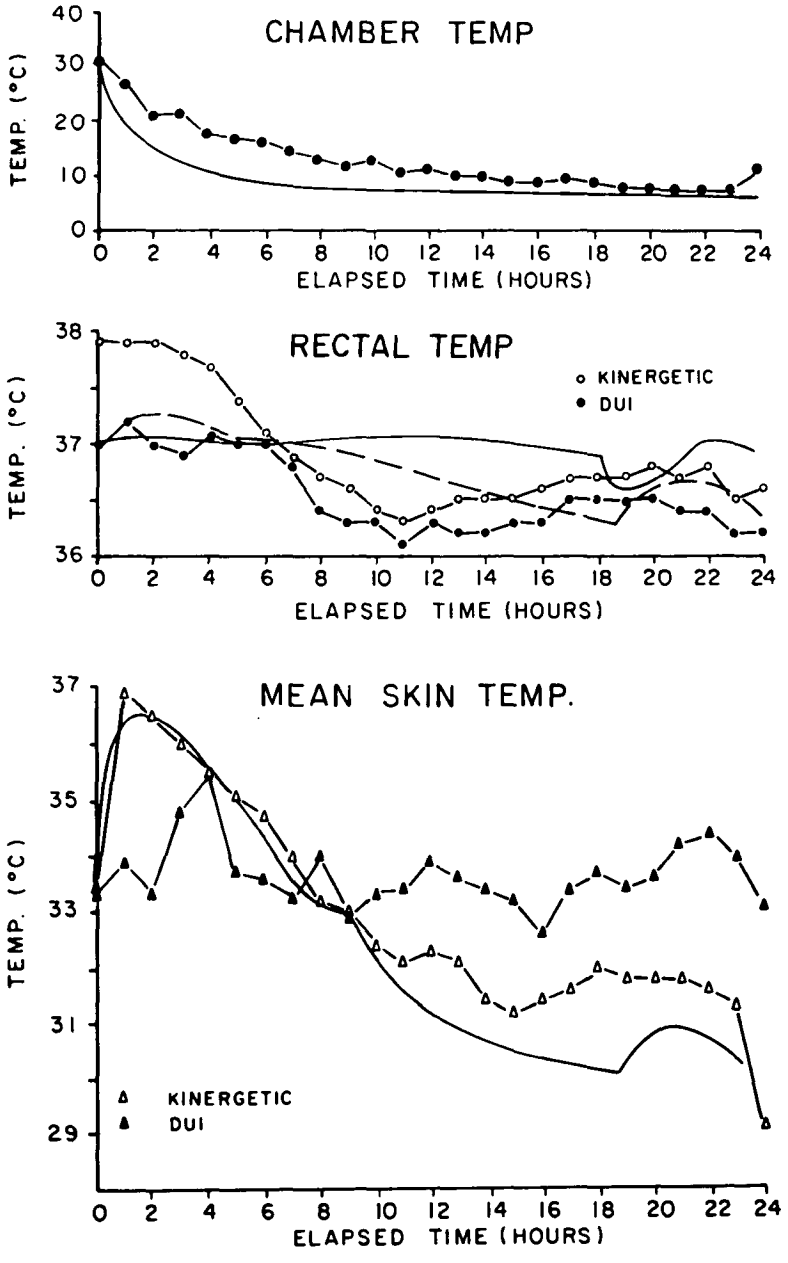



Fig. 1. Comparison of computed temperatures with values measured during the *Polar Bear III* study (5). *Solid curves* show the computed wall, rectal and mean skin temperatures; *Broken curves* in the *middle panel* is the computed arterial temperature. Conditions are: pressure = 16 bars; gas composition = 2.3% $O_2$, 5% $N_2$, and 93% helium.

The *middle panel* displays measured rectal temperatures for both divers and computed arterial and rectal temperatures. Of the two computed central temperatures the arterial temperature is better defined, because the rectal temperature is known to be influenced by many factors not easily incorporated into the model. In spite of this unavoidable ambiguity in definition, the computed rectal temperature is displayed because previous studies have shown that computed values agree reasonably well with measured values for a variety of cold exposures. As shown in Fig. 1, agreement between computed and measured central temperatures is quite satisfactory. The measured values decreases approximately 0.5°C during the night, which is normal and not caused by thermal stress, while the computed arterial temperature remains nearly constant and the rectal temperature drifts slowly downward. Because the model makes no allowance for diurnal variations, the discrepancy between computed and measured values is understandable and does not invalidate the computed results.

The transient variations in computed central temperatures beginning 18 h into the test are attributable to shivering, which occurs between 18 and 21½ h. A maximum intensity of 50 W is reached at 19 h, followed by a steady decline to zero. Because both test subjects reported brief periods of shivering toward the end of the exposure, it appears that the model describes this aspect of the response quite well: it correctly predicts that cooling during the first 18 h is just sufficient to reach the shivering threshold. Because the divers are well insulated, increasing the rate of heat production by shivering is effective in increasing body temperature, although transient decreases in central temperature do occur as the perfusion rate in cold muscle increases owing to increased oxygen demand.

There is also good agreement between computed and measured mean skin temperatures. It was assumed in the simulation that the divers enter their survival bags at time zero, which was the procedure followed by only one of the test subjects; the other one did not enter the bag fully until several hours into the test. According to the results shown in the *bottom panel* of Fig. 1, the model correctly predicts that employing the full survival system before the chamber cools leads to elevated skin temperatures and discomfort from overheating.

In addition to skin temperatures, heat flux measurements were recorded at three sites: chest, back, and thigh. Mean values were approximately 31 $W/m^2$ on the chest, 75 $W/m^2$ on the back, and 45 $W/m^2$ on the thigh. When computed values of 71 $W/m^2$ on the upper torso and 61 $W/m^2$ on the thigh (at time = 14 h) are compared with the measured values, the agreement is seen to be reasonable. It should be noted that the low thermal flux measured on the chest could have been influenced by the warm canister located near the measurement site, and may not be truly indicative of the mean heat flux on the trunk.

The last comparison to be made involves the temperature of inspired gas. Throughout most of the exposure, the computed value was 28°C, while the measured values fell in the range of 31.5–34.5°C for one system, and 24–28°C

for the other. Hence, heat loss through the respiratory tract was modeled quite accurately.

To summarize, the model appears to provide an accurate description of experimental observations obtained in a 24-h survival test conducted at 16 bars. Agreement between computed and measured central temperatures, mean skin temperatures, inspired gas temperatures, and thermal fluxes was quite satisfactory. Furthermore, the model correctly predicted that light shivering would occur during the last quarter of the 24-h exposure. Such close correspondence between computed and measured results strongly supports the contention that the theoretical analysis is essentially complete and correct, and can be used to predict behavior under conditions not too different from those employed in the laboratory study. Additional discussion of other conditions and the effect of changing various parameters is presented in a previously published paper (8).

In evaluating the protection provided by currently available systems under various conditions, one must consider the effect of pressure, geometry, and quality of the survival bag. As discussed earlier, increasing the pressure decreases the thermal resistance of the gas space surrounding the divers and increases the rate of respiratory heat loss. Because a survival bag in good condition provides a thermal insulation of at least 2 clo, a reduction of gas space insulation from 0.4 to 0.25 clo should not seriously affect the overall performance of the system. Similarly, the effect of pressure on respiratory heat loss depends on the performance of the breathing gas heater. If the temperature of inspired gas can be maintained above 27°C and the ventilation rate remains below 10 L/min, the rate of respiratory heat loss in 36-bar heliox will not exceed 30 W. Although the rate of heat loss is acceptable, one can double the rate with relatively small changes in operating conditions, such as increasing the ventilation rate of 15 L/min and reducing the inspired gas temperature of 24°C. Hence, satisfactory performance of the $CO_2$ scrubber/heater is absolutely essential for survival at deep depths. One of the scrubbers used in the *Polar Bear III* test supplied gas at 31.5–34.5°C for 24 h, but for the other scrubber, the temperature fell into the range of 24–27°C (5). Although this caused no difficulty at 16 bars, it could be troublesome at 36 bars.

Another factor that must be considered is the geometry of the system; i.e., the size and shape of the bell or habitat. The British open-sea test that provided the model for the NUTEC temperature profile and for other analytic studies employed a relatively small, externally insulated bell, but it is also possible that divers could be trapped in a larger, uninsulated welding habitat. In the latter case, the thermal resistance provided by the gas space will be lower than in a bell, and the walls will cool rapidly to a temperature only slightly above that of the sea. Because a habitat at 36 bars appears to represent one of the most hazardous locations in which divers could be trapped, it is worth considering in detail.

In a series of habitat simulations, *KHTC* was assigned a value of 18.2 $W/(m^2 \times °C^{0.228})$, and the insulation on the outside of the habitat was assumed to be minimal, which caused the inside wall temperature to fall to 6°C within

the first hour. Under these conditions, divers do not have much time available for activating the survival system.

Divers who have the full 2-clo survival bags should be adequately protected in this environment. If the inspired gas temperature is 27°C, light shivering will begin after 5 h and continue at a rate of approximately 50 W throughout the exposure. Because this level of shivering can be maintained nearly indefinitely, fatigue should not limit the divers' ability to survive. Approximately 20% of the heat loss occurs through the respiratory tract and the remainder occurs from the skin.

Because light shivering would be required to maintain even the "fully protected" diver in a 36-bar habitat, it is important to estimate the margin of safety provided by a 2-clo survival bag. To obtain such an estimate, one can repeat the computations using successively lower values of thermal insulation until a value is obtained that is inadequate for 24-h survival. From this procedure, it was determined that a 1.5-clo survival bag provides marginal protection. Shivering would begin after only 2½ h and increase during the next 3 h to a level of 85 W, which is sufficient to prevent further loss of heat. As long as a metabolic rate of 190 W can be maintained, survival is possible, but fatigue may eventually limit the diver's ability to shiver. The best estimate currently available suggests that fatigue will become important toward the end of a 24-h exposure.

For completeness, simulations were also performed for a 1-clo survival bag. The results of these computations indicate that shivering could be expected to begin soon after the 1st h of exposure. At the end of the 2nd h, the level of shivering would be approximately 75 W, increasing to 130 W after 5 h. Although this level of metabolic heat production is sufficient to stabilize bodily temperatures, it cannot be maintained beyond the 14th h, and a rapid decline occurs at that time.

Because it is well known that the rate of cooling during immersion in cold water is strongly influenced by subcutaneous fat, a simulation was also conducted for a diver who has less fat than the average person used in most of the simulations. Reducing the mean skinfold thickness from 13.0 to 9.5 mm had very little effect on the diver's ability to survive a lost bell accident. The reason for this behavior is that the thermal resistance of 6.5 mm of subcutaneous fat is only 0.2 clo, which is small compared to the 1.0 clo provided by the survival bag. Hence, the effect of subcutaneous fat is not nearly as pronounced in this case as it is during immersion in cold water, where the external resistance is very small.

The analysis and results presented lead to several conclusions, which are of value in interpreting and extending the results of experimental studies. Foremost among these is the conclusion that a fully effective system of the kind commercially available today should provide adequate protection for 24 h survival in an isolated bell or habitat when the depth is no greater than 350 m. At moderate depths, say less than 200 m, these systems provide an appreciable margin of safety for divers in a small, well-insulated bell. As the depth

increases and the bell becomes larger, the margin of safety decreases until very little remains for divers in an uninsulated welding habitat at 350 m.

Survival systems consist of two parts, the survival bag and the breathing gas scrubber/heater. If the survival bag is kept dry, one can be reasonably certain that its thermal resistance will be at least 2 clo at all depths, and the rate of heat loss through the skin should not exceed 130 W. In addition to removing $CO_2$, the scrubber must supply breathing gas at a temperature not lower than 27°C, which limits the rate of respiratory heat loss to approximately 30 W. Scrubbers used in the *Polar Bear II* and *III* tests satisfied this requirement at 31 and 16 bars, respectively. Furthermore, temperatures measured inside of the canisters were in the 36–45°C range, which indicates that heat released when $CO_2$ reacts with hydroxide is sufficient to produce satisfactory inspired gas temperatures. Even though it is reasonable to expect the scrubber/heater systems to operate satisfactorily at 36 bars, it must be remembered that they are not as reliable as survival bags and the possibility of malfunction always exists. During normal operation, the rate of respiratory heat loss is only 20% of the total, but that fraction increases greatly when the breathing gas is not adequately warmed. Because the margin of safety in a habitat at 36 bars is small, it is essential that the scrubbers be maintained in good condition and used properly. When that is done, divers should be able to survive for 24 h under conditions likely to be encountered within the foreseeable future.

## Acknowledgment

Development of the model used in this analysis was supported by the U.S. Naval Medical Research and Development Command and by the U.S. Air Force School of Aerospace Medicine.

## References

1. Anonymous. Diving bell crew on thistle field rescued in 'wet' transfer operation. Ocean Industry, March 1981:83–84.
2. Arnoux G. Rescue of the stranded divers of the STENA Seaspread by divers of the Uncle John—21 January, 1981. In: International Conference Divetech '81. The way ahead in diving technology, Session B4. London: Society for Underwater Technology, 1981.
3. Tønjum S, Hamilton RW, Brubakk AO, Peterson RA, Youngblood DA. Project *Polar Bear*: testing of diver thermal protection in a simulated "lost bell." Bergen: Norwegian Underwater Institute, 1980. (NUI report 2–80)
4. Tønjum S, Påsche A, Furevik D, Holand B, Brubakk A, Olsen C. Deep-Ex 80, Project II, Thermal studies in diving: Test of survival systems for stranded bell divers at 31 bars. Bergen: Norwegian Underwater Institute, 1981. (NUI Report No. 40–80)
5. Tønjum S, Påsche A, Onarheim J, Furevik D, Hayes P, Padbury H, Segadal K. *Polar Bear III*: Test of passive survival systems for 24 hours at 16 bars. Bergen: Norwegian Underwater Technology Center, 1982. (NUI Report No. 21–82)

6. Hayes PA, Padbury EH, Atherton JP. Validation of emergency procedures—the lost bell situation. In: International conference Divetech 81. The way ahead in diving technology, Session B4. London: Society for Underwater Technology, 1981.
7. Wissler EH. A mathematical model of the human thermal system with reference to diving. In: Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society Workshop. Bethesda: Undersea Medical Society, Inc., 1981:187–212.
8. Wissler EH. An analysis of the lost bell problem. In: Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society Workshop. Bethesda: Undersea Medical Society, Inc., 1981:315–335.
9. Wissler EH. Mathematical model of the human thermal system. Bull Math Biophys 1965;26:147–166.
10. Beckman EL, Reeves E. Physiological implications as to survival during immersion in water at 75°F. Aerosp Med 1966;11:1136–1142.
11. Nunneley SA, Wissler EH. Immersion hypothermia: validation of computer model and prediction of cooling rates. In: Kuehn LA, ed. Thermal constraints in diving. The twenty fourth Undersea Medical Society Workshop. Bethesda: Undersea Medical Society, Inc., 1981:213–252.
12. Tønjum S, Påsche A, Wissler EH. Body cooling rate in hyperbaric heliox. Paper presented at the European Undersea Biomedical Society annual meeting, Cambridge, July 1981.
13. Nuckols ML. Thermal considerations in the design of divers' suits. In: Johnson CE, Nuckols ML, Clow PA, eds. Hyperbaric diving systems and thermal protection. New York: American Society of Mechanical Engineers, 1978.
14. Wattenberger JF, Breckenridge JR. Dry suit insulation characteristics under hyperbaric conditions. In: Johnson CE, Nuckols ML, Clow PA, eds. Hyperbaric diving systems and thermal protection. New York: American Society of Mechanical Engineers, 1978.
15. Jakob M, Hawkins GA. Elements of heat transfer, 3rd ed. New York: John Wiley and Sons, 1958.
16. Bishop EH, Mack LR, Scanlan JA. Heat transfer by natural convection between concentric spheres. Int J Heat Mass Trans 1966;9:649–662.
17. Weber N, Powe RE, Bishop EH, Scanlan JA. Heat transfer by natural convection between vertically eccentric spheres. J Heat Trans 1973;95:47–52.

# INVITED REVIEW: THERMAL EFFECTS OF THE HYPERBARIC ENVIRONMENT

*L. A. Kuehn*

In the following review, I will endeavour to describe the progress attained in the understanding of the physiological requirements of cold water divers since the review presented by Paul Webb to this society at the time of the Seventh Underwater Physiology Symposium (1). That review, plus the two earlier Undersea Medical Society Workshop proceedings pertinent to the topic (2,3), described the state-of-knowledge of this aspect of underwater physiology attained during the last decade.

Basically, there have been few new aspects of physiology identified in the last 3 years that have not been established or foreseen from earlier work; however, the absence of knowledge and data on operational cold water diving that had plagued earlier investigators has largely disappeared. We now have a better grasp of the stress of cold water diving and a much better technological base from which to implement cold diver protection.

In particular, realistic limits are now in place for diver exposure to cold water, cold hyperbaric gas, or cold air. Mysteries of physiology such as shivering and its import for diver protection are now being unravelled. New diver technology has been developed, in particular for the acute problem of life-support systems for the "lost bell" diver, a problem that has acted as a focus for the energies of many investigators over the last 3 years.

Modellers, too, have refined and extended their work to produce mathematical structures that can predict limits to diver exposure or protection that would be difficult or impossible to measure by experiment, particularly for diver hypothermia pertinent to core temperatures below 35°C. This work has been particularly helpful in the understanding of the lost bell diver problem.

Yet there remains much to be done. New technological breakthroughs may be on the horizon, increasing markedly the safety and comfort of the

cold-exposed diver. Certain aspects of prophylactic and therapeutic diving thermal practices are capable of being refined to aid significantly in diver efficiency. These and other topics will be explored in this paper.

## GENERAL PHYSIOLOGY OF COLD WATER EXPOSURE

### Temperature-Heat Flow Relationships

Despite considerable investigation during the last decade, the relationship between body heat loss and body temperature change in cold water exposure has not yet been definitively elucidated. As Paul Webb and his colleagues have shown (4,5), the relationship appears to be dependent on the rate of body cooling in cold water. Loss of a given amount of heat at a rapid rate by a diver causes a greater decrease in rectal temperature and more shivering than is the case for loss of the same quantity of heat over a longer time; the result is little rectal temperature decrease, no shivering, and only minor complaints of mild subjective cold stress. Although the physiological decrement associated with either lengthy or brief exposure remains to be determined, there has not yet been any explanation of this documented effect of rate of cooling. Indeed, there is even great uncertainty as to the mechanism of input from skin and deep body thermal receptors to the hypothalamic temperature controller that is responsible for maintenance of thermoneutrality in comfortable environments (6).

The general relationship between characteristics of individual body size/obeseness and cooling rate have been well established (7). For the same body mass and height, variation of body fat has been shown (8) to be associated with a large variation in rectal temperature decreases and heat losses in cold water; generally, the fatter the individual, the less the physiological response in cold water. For the same body fat percentage, variation of body mass and height has a large effect in rectal temperature and heat loss; but, generally, the larger the individual, the less the physiological response in cold water, also.

Considerable individual variation in rates of cooling can exist, however, in people of identical skinfold thickness and sex. Such individual variation has confounded many hyperbaric thermal experiments, particularly when these involve only a few subjects because of space or cost constraints (9,10). Even within a particular individual, considerable thermal diversity exists over the body. Recent experiments (11) on the distribution of heat flow over the body immersed in cold water showed that skinfold thickness is of minor importance in the foot, hand, lower arm, upper arm, thigh, and calf, but of major importance in the trunk, neck, and head regions. Heat flow and skin temperatures are highest in the neck and head and least in the foot.

### Cold Habituation/Adaptation

Although body fat has long been attributed to being the major factor in the success of long-distance swimmers in very cold water, new studies (12)

have shown that metabolic heat production or the peripheral vascular response may be even more important. People accustomed to cold water immersion become very efficient in use of the shiver response and only shiver at a fraction (say 20%) of their $V_{O_{2max}}$. Habituation to cold water is also considered to be an important factor but little is known of how it is acquired and maintained. Regular daily diving in cold water may lead to habituation in approximately 1 week. It appears to be an acquired peripheral vascular physiological response in that it depends specifically on the task involved.

Regular use of wet suits by commercial divers may prevent cold adaption from developing, as has been reported recently (13). People, such as the Korean Ama, reported earlier to have acquired significant cold acclimatization to cold water, have lost much of their physiological protection with the use of such technology. Studies conducted on these people recently (14) show that wearing of wet suits routinely for 3 or 4 years completely reverses metabolic and insulation acclimatization with partial reversal of hypothermic adaption and no effect at all on local cold-induced vasodilation. A more recent report on this work presented in this *Proceedings* (15) describes further decay of the physiological responses after 5 years of wearing wet suits, with disappearance of the local cold-induced vasodilation. Such concerns for acclimatization and habituation are important in that these phenomena may alter physiological responses in complex ways, reducing responses to the cold stress, but also placing the diver at greater risk in terms of self-appraisal of his physiological status.

## Symptomless Hypothermia

Concern in this context has been expressed during the last 3 years to progressive symptomless hypothermia in cold hyperbaric operations. In a questionnaire in one study of North Sea divers (16), information on 114 incidents showed that various degrees of unconsciousness or mental incompetence were involved, although respiratory difficulties, unsteadiness, and weakness were also common. Other observers (17) have raised the concern that symptomless hypothermia may have been a factor in many of these incidents. Such a malady would not be detected in routine hot-water diving in which regulation of water temperature flowing to the suits is determined by diver comfort. Insensible respiratory heat loss would be the main factor in such occurrences. Various studies (18) have shown that subjective thermal comfort is related to fall in core temperature, yet with high respiratory heat losses and the consequent inception of hypothermia, a diver's assessment of comfort is not always related to physiological temperature change. This is true not only for diving (19) but for other cold water activities as well, such as immersion survival (20) and canoeing (21). In diving, rigorous attention to automatic regulation of respiratory heating and hot-water-suit entry temperatures would alleviate this problem.

## Normobaric Cold Water Respiratory Distress

Several studies have taken place during the last 3 years to elucidate the physiology of sudden immersion in cold water, especially the sudden respiratory distress that is predominant in the first few minutes of immersion. Kurss et al. (22) showed that vital capacity is reduced in head-out immersion because of intrathoracic pooling, secondary to warming and vasodilation in peripheral tissues. Reduction of vital capacity was shown to be due to hydrostatic pressure and cold exposure. Malkinson et al. (23) demonstrated a significant difference between males and females in pulmonary ventilation over the first 4 min of cold water immersion but not in warm water. A prestress sauna exposure attenuated the ventilatory response for both groups and no significant differences were observed between males and females after this exposure. The rates of change of deep skin temperature of males and females due to cold stress were similar.

Isometric exercise produces hyperventilation in subjects as does immersion in cold water. Dahms et al. (24) studied the combination of these two effects in subjects and found that an unexpected fall in the ratio of the two effects occurred with decreasing water temperature.

Other effects have been noted. Diminution or loss of sensitivity of peripheral chemoreceptors may be largely responsible for failure of ventilatory control of body core temperatures below 22°C during general hypothermia (25). Cold water causes breath-holding time in man to be reduced, presumably due to the cold stimulation over-riding the dive reflex (26). Mysteries remain. Keatinge and Hayward (27) reported an unusual death in which a young man collapsed with no pulse but continuing respiration after a cold water swim of only a few seconds; death occurred despite resuscitation attempts. Their experiments showed the possibility of provoking ventricular ectopic beats by facial immersion during trunk immersion.

## Cold Diving Limits

As to recommended limits for cold exposure of divers, these were extensively refined in the last decade; yet, a question remains as to whether there should be one set of limits or a scale of limits, since any one set must perforce be set conservatively to apply to the majority of the population that is to be affected by them.

Selection of divers with particular skinfolds, or size, or body shape, or previous history, may be important means whereby diver hazard and risk are significantly reduced in operational cold stress environments.

## Hyperbaric Chamber Thermal Stress

The problem of inexplicable weight loss by divers in hyperbaric environments remains. Studied extensively by Webb (4), this phenomenon occurs during comfortable deep hyperbaric chamber exposures and represents a net

imbalance of approximately 900 kcal per man-day of food intake that does not show up either as energy loss or as increased body heat storage. One explanation is that an increased heat loss occurs from the divers with no decrease in body temperature or increase in metabolism. Another observation is that energy metabolism under hyperbaric conditions may not be completely measurable by known techniques.

Thermal requirements of chamber divers were extensively examined in the last 4 years in several international experiments. Classical thermal experiments were undertaken by the Japanese investigators Shiraki and Tomiyasu (28–31) at both shallow and deep depths. The partitional calorimetric studies at 4 ATA heliox (28) at various environmental temperatures showed increases in convective and radiative losses and a decrease in evaporation, as expected. With subjective thermoneutrality setting the ambient temperature at 32°C, vasoconstriction was still present. Several interesting diuretic effects were noted, namely, a disappearance of diuresis with increases in environmental temperature and an inverse relationship between urine flow and mean skin temperature. Extensive thermal measurements in deeper studies to 300 msw (29,30) showed that forehead temperature, unlike other body areas, showed no clear correlation with depth and mean skin temperature. Heat losses from the skin were minimal at comfortable temperatures. When the pressure increased, insensible water loss through respiration decreased slightly but loss through the skin decreased very sharply.

Extensive data were also collected in several Norwegian and British experiments. Work done by the former (9,10,32) demonstrated the importance of subject morphology in affecting heat loss rates as well as the dangers inherent in subjects' assessment of thermal state. Respiratory heat losses were clearly the major factor limiting survival and endurance in cold hyperbaric chambers (33). Significant amounts of data were collected by Wilcock and Flook (34) on 52 divers to depths of 300 msw, which led to the determination of equations predicting the changes of body and chamber temperatures with depth.

Although another Japanese study (31) showed that no change in rate of resting metabolism took place during a dive to 31 ATA, this was not observed in a deeper dive to 51 ATA (35). Sheehan and Brauer (36) showed, in animal studies, that in the euthermal interval temperature distribution within an animal is very different at high pressures from that in low-temperature normobaric environments. The hypothalamic responsiveness increases with depth. Investigations on the hyperbaric limitations of mammalian physiology were performed on cats by Imbert (37) who showed that in 80-h exposures to depths of 750–1000 msw, with respiratory heat losses greater than 20% of the heat balance, normal core temperatures could not be maintained. This work was extended (38) to show that in the 900- to 1000-msw range, core temperature of cats tended to drop despite heat input and elevated environmental temperatures. The hypotheses for these phenomena are: a) irregulation of nervous mechanisms involved in thermoregulation under conditions of the high pressure nervous syndrome (HPNS); b) insufficient thermogenesis due to limita-

tions of respiratory gas exchange or a vigorous metablism; and c) excessive heat loss brought about by the thermally conductive heliox, aggravated by hyperbaric pressures. Thermal conditions, then, may limit man's ultimate hyperbaric exposure, as does HPNS.

This research group has continued their study of the thermal physiology of cats, but the correct hypothesis of their results has not yet been established.

## Hyperbaric Respiratory Gas Thermal Physiology

The existing standards for minimum inspired gas temperature for divers have been significantly improved. Physical models of the human respiratory tract exist (39), which provide detailed characterization of the heat and mass transfer mechanisms as well as the effects of environmental pressures, gas composition, and respiratory rates on the body cooling capacity of respiratory airways. New limits have been proposed by U.S. Navy investigators (40,41), based on experiments that showed that divers breathing cold oxyhelium mixtures at great depths did not respond metabolically to high respiratory heat losses: they suffered prompt rectal temperature decreases linearly related to the product of inspired gas density and specific heat, with very little shivering. Breathing of cold hyperbaric heliox can rapidly lower core temperature, even in the presence of warm hyperbaric chamber conditions.

The proposed new standards were based on the concept that a rectal temperature change of 0.25°C/h can be tolerated hyperbarically for a cumulative rectal temperature decrease of 1°C. Some concern has since been expressed that these new U.S. Navy limits are pertinent primarily to subjects at rest in a hyperbaric chamber environment and that more conservative limits may have to be developed for the working, helmeted cold-water diver. Concern has also been expressed (42) for the asthma-like symptoms that occur in certain divers who possess no previous history of asthma, pulmonary disease, or smoking.

Another set of minimum inspired-gas temperature standards has been recommended by English investigators (43,44) to a respiratory heat loss rate of only 200 watts (W), instead of the previous accepted value of 350 W. They recognized that the major benefit to respiratory gas heating is the prevention of respiratory heat loss. Since thermal limitations are placed on supply of very hot gas to the respiratory tract because of the potential for burning of the tract, these thermal intake limits, restricting inspired gas temperatures to 45°C, only provide for a heat input rate of approximately 50 W to a diver. However, because of the prevention of respiratory gas loss, provision of even such a small amount of respiratory gas heating markedly improves prolongation of diver work and performance at great depths, although concern still exists as to the capability of the present level of respiratory gas heating technology to provide even these modest heating requirements.

Control of hot water flow and temperature to a suit or a breathing gas heater is difficult. Such data as do exist show that inhalation rewarming is a valid procedure for administering some form of first aid to hyperbaric hypo-

thermic divers inside diving chambers or large bells. This procedure may prevent further deterioration of a diver when hot-water-immersion rewarming techniques are not possible or are contraindicated. Mucus formation in the respiratory tract under application of these new limits for respiratory gas thermal heating is not thought to be a problem. It is not yet known if formation of respiratory mucus under cold hyperbaric conditions is a thermal effect or a rate-of-heat-loss effect (i.e., temperature-dependent or heat-loss-dependent).

It is important, before leaving this section, to correct a misconception that has developed in the diving literature concerning the excessive heat loss concomitant with breathing hyperbaric helium. It is true that a hyperbaric helium atmosphere does remove heat faster from a diver's skin by convection than does an air or nitrogen-based atmosphere. This is due to the higher thermal conductivity of helium; however, this situation does not pertain to hyperbaric respiratory gas heat loss as Riegel and Schmidt have shown recently (45,46). Respiratory gas from a diver's lungs is exhaled at a temperature nearly equivalent to that of his core: the heating of the gas is accomplished deep in the diver's lungs. The volume that the diver inhales with each breath does not change greatly with depth so that hyperbaric respiratory heat loss is approximately proportional to (gas density) (specific heat). It turns out that for the hyperbaric pressures of interest, the respiratory heat loss with oxynitrogen is approximately 40% higher than for oxyhelium. Along with argon, helium requires the least heat to increase the temperature of a given volume. Were it not for the narcotic effects of air, oxyhelium would be recommended at all depths as a respiratory gas in terms of thermal efficacy. Schmidt (46) has recommended its use in the air diving range where diver heating capacity is limited and where the beneficial difference noted above may become significant to diver performance and safety.

## Shivering

During the early stages of hypothermia, intense shivering commences shortly after the onset of body cooling: the result is a marked increase of metabolic rate of up to five or six times in the muscles involved, with a concomitant production of heat. Burse and Stroschein (47) have shown that shivering progressively increases metabolic rate of even exercising subjects partially submerged in cold water.

Shivering is thought to be an extended manifestation of the physiological action tremor that is inherent in all body muscle groups, in frequency bands between 5 and 12 Hz. It is not a new physiological response brought into being when the hypothalamus is appropriately triggered (48,49). Muscle groups affected do not fire in phase but, even in subjects who do not appear to shiver, there is synchronized electromyographic activity in various muscle groups. As this progresses under continual stimulation, a tensing occurs in the muscles preparatory to visually noticeable shivering. Tremor frequency significantly increases with cold exposure, but a significant drop in frequency

precedes the actual onset of shivering (50). The control of shiver is not considered to be entirely governed by a central hypothalamic pacemaker but in part by spinal cord generators operating at subvertebral or subhypothalamic levels.

A significant shift in shivering frequency takes place in the legs of cold-water-immersion victims on standing up, due to the activation of hyperactive stretch receptors after a period of relative inactivation in the cold water; therefore, shiver in cold air exposure differs from that in cold water exposures. Mental concentration, voluntary isometric tensing, and habituation or experience all have been shown to cause an amelioration of shivering in cold water (51–53).

The temperature of inspired air appears to have influence on the amplitude of shiver. Temperature sensors in the mouth or respiratory tract may signal shivering by spinal cord reflex loops whenever inspired gas is cold. Warm, moist inspired air tends to reduce shivering and it has been reported (54) that such inspiration at 1 atm led to reduction of shivering by amounts as large as 25 or 33%. When the mouth is anesthetized, inspiration of warm air at 1 atm fails to reduce shivering. In contrast, breathing of cold hyperbaric oxyhelium by subjects with warm peripheral body temperatures does not provoke shivering in certain subjects, even though a pronounced rectal temperature decrease is effected by the respiratory heat loss (55).

With prolonged shivering, the affected person is not able to move. This has been considered to be an effect of fatigue; however, it may be due instead to an alteration of electromyographic timing, causing a "poverty of movement." Fatigue in shivering will occur after a sufficiently long time, say 8 or 9 h, with depletion of glycogen reserves, and the decrease in heat generation will lead to a precipitous drop in core temperature.

No correlation has been observed between rate of cooling and shivering activity. Golden et al. (56) showed that there is a wide individual variation in shivering intensity that is not correlated with skinfold thickness or rate of cooling but that there was a correlation instead with $\dot{V}O_{2max}$ during exercise. During maximum shivering, the $\dot{V}O_2$ uptake of Golden's subjects was approximately 46% of their $\dot{V}O_{2max}$.

The question of efficacy of shivering of nearly nude subjects in cold water immersion has not yet been settled: Is the heat that is generated quickly dissipated by convective cooling because it is produced superficially? Earlier experimental data indicated that this might indeed be what happens, particularly at very great depths. Modelling of this question for air or hyperbaric gas exposures indicates that it is of benefit, especially for thermally insulated subjects. When shivering stops or declines, the body core cooling rate should increase.

A decrease in rate of shivering had been postulated earlier (2) for core temperatures below 35°C; shivering ceased altogether to be replaced by muscle rigidity for core temperatures in the range of 30–32°C, beginning again during rewarming for core temperatures in excess of 30°C. This postulation of decline of shivering below 35°C can be questioned. It is possible that with prolonged

cooling fatigue sets in and shivering declines, or it may be possible that in acute immersion cooling shivering does not occur because of a spastic response. Violent shivering, however, has been observed in many hypothermic victims, some with core temperatures as low as 27°C. Because the literature contains so little definitive information or observations on the shivering and metabolic responses of accidental hypothermia victims, this decline of shivering remains unsubstantiated. It may be that shivering appears in a spurious form on initial cold water immersion in some subjects and reappears on continued cooling to lower core temperature.

A paper (57) presented in this *Proceedings* examines the threshold to shivering as a consequence of gradual cooling in a hyperbaric chamber at two different pressures, 26 and 51 ATA. Although thermal data showed that shivering occurred quicker in the environment that was more stressful, 51 ATA, it also showed that the divers' bodies were able to detect subtle heat loss and increase metabolic output before significant temperature changes had occurred.

In summary, shivering is considered to do two things: it increases the rate of metabolic heat generation and it increases peripheral perfusion. The former action helps to maintain core temperature and the latter phenomenon diminishes it. The net balance of thermal benefit to the individual depends on the overall convective and respiratory heat demand as well as the thermal insulation that is worn.

## Biochemical Changes Due to Cold Stress

Biochemical studies during the last 4 years are also worthy of mention. Galbo and colleagues (58) showed that, whereas increases in body temperature of exercising swimmers were associated with exercise-induced increases in plasma noradrenaline, cortisol, growth hormone and glucagon, decreased body temperature was associated with catecholamine secretion, and shivering was shown to be associated with decrease of insulin secretion. Work performed by Wiehl and his colleagues (59) showed that immersion without cold stress suppressed plasma epinephrine without affecting plasma norepinephrine and that cold with immersion stress caused significant increases in plasma norepinephrine. This research group progressed further to suggest (60) that plasma norepinephrine levels increased by cold stress (through immersion in water at 1 or 4 ATA) could be used to assess overall general cold stress and also the degree of cold stress inherent in hyperbaria. Another research group (61) also demonstrated that plasma levels of norepinephrine during cold water immersion and subsequent rewarming by heat cradle or hot water tub were significantly higher than control values. A linear relationship was also established between time lag and plasma norepinephrine during immersion. In other studies, metabolic adaption (62) was shown to have a role in the subjective assessment of coldness. The cold stress of hyperbaric chamber exposure was shown to be best detected in terms of induced thyroidal response. The pan-

creas was advocated as being the best organ for monitoring in the detection of very mild forms of hypothermia.

## Cold-Induced Psychological Distress

The limit of subjective cold tolerance is more related to peripheral cold stress than to any core temperature changes. Subjective cold sensation is based on afferent impulses from thermal receptors in the skin (53). Recent work (51,52) has shown that such sensation can be attenuated or ameliorated by mental concentration or by the attainment of experience (habituation). Paul Webb (63) presents work in this *Proceedings* that shows impaired mental performance from mild body cooling, namely, a slowing of serial choice reaction time, an increased number of decision errors, and overloading in a dual task. This work is important in that it demonstrated that mild hypothermia, gradually incurred without discomfort, can impair cognitive performance.

## Effects of Cold Water on Diver Decompression

The thermal state of the diver can have an important bearing on the success of various decompression schedules and tables. Work conducted recently (64,65) documented the effect of peripheral circulation on postdive Doppler scores of divers undergoing decompression. Divers who are cold during their bottom exposures take up less gas in solution in body tissues than those who are warm. Consequently, on decompression, the diver who was warm at the bottom phase of the dive and cold during decompression may have more decompression problems. Anecdotal accounts exist describing divers working without hot water suits as being able to eliminate some decompression stops that were needed when hot water suits were used. This effect of cold exposure, however, is not recommended for reduction of decompression stress, because cold water divers can experience loss of capability and judgment, which is more important to their survival than the greater risk of decompression sickness due to being kept warm.

## Diver Hyperthermia

Although the accent on use of limits for respiratory gas heating is presently centered on the minimal limits that will prevent hypothermia, problems of hyperthermia are becoming more and more evident (66,67). Respiratory burns have already been identified as a hazard, but use of gas heaters with hot water suit technology can result in such heat stress in water exposures that divers will encounter heat syncope on re-entering a bell. The fainting usually occurs in the act of leaving the water and losing support from the hydrostatic column. Blood pooling then occurs in the lower regions of the body and the brain loses the circulatory support sufficient for consciousness. Other hyperthermic risks exist in the deck decompression of divers in surface-mounted chambers exposed to hot ambient thermal conditions in tropical regions (68).

Several deaths have been reported due to such hyperthermia and are considered to have been preventable through the use of adequate cooling measures involving shade or spray cooling.

## DIVER MONITORING

The principal reason for diver monitoring is the enhancement of diver safety: to determine if the diver is becoming hypothermic or if his performance is being impaired or jeopardized. Such monitoring is also important in obtaining knowledge of diver physiology and protection. The efficacy of such monitoring in operational diving is questionable, because most divers will voluntarily end their exposure when experiencing the initial effects of hypothermia, although search divers or military divers may not have that option. Most surface-supplied divers experience minor peripheral cold stress in shallow water diving and are accustomed to it. The value of monitoring is more important in bounce diving, either from the surface or from a bell, in which such monitoring would be of use in determining the thermal state of a diver before each successive exposure. There have been numerous calls for such technology in the literature (17,69), particularly because of the concern for symptomless hypothermia that is conjectured to be the cause of many diving accidents.

The state of monitoring technology is considered poor despite elegant transmission means that are available (optical fibers, hard wiring, ultrasonics) (25,26). Hot water temperatures for suits or gas heaters may be known at the point of source but are rarely known at the bell or at the point of entry to the diver's suit. A risk of hyperthermia or burning exists and several cases of fainting by heat syncope have been reported.

The parameters for monitoring are several: subjective verbal comments, deep body (core) temperature, selective or mean skin temperatures, heart rate, and direct heat flow measurements. No *one* physiological variable alone is an accurate and completely reliable indicator of a decrement in diver performance or development of a thermal problem. Furthermore, monitoring of most of the aforementioned parameters involves some invasion of personal privacy of the diver, either by having transducer leads affixed to him or inserted in various body orifices. Aside from the extra time and involvement that use of such transducers requires in the dressing of divers, these transducers may also adversely affect diver performance or act as irritants in the conduct of diver operations. For this reason, the single most useful and easily implemented diver monitoring parameter is direct verbal contact (70,71).

Because of a perceived lack of cooperation from divers, environmental monitoring should be used, wherever possible, to gather information on diver health and safety. For example, the one vital measurement for use in a hot-water-suited diver would be the temperature of the hot water on entry into the suit. If hot water is also used for heating of the breathing gas on entry into the diver's helmet, then the hot-water temperature measurement also suffices for

determination of respiratory thermal stress. The measurement of hot water temperature for the breathing gas heater should be compared to a lower limit, to avoid hypothermia induced by respiratory heat loss, and an upper limit to avoid respiratory burns. To avoid complexity, it is possible to use the hard-wired communications umbilical for transmission of the thermal information to the dive supervisor.

Instrumentation used in a helmet or on the diver umbilical is considered to be more acceptable to the diver than any transducers placed in or on his body. Several suggestions have been made as to new possibilities for this purpose. The scalp is one site of the body that is always vasodilated and which could serve as a site for surficial detection of either heart rate (by optical plethysmography or temperature (by contact thermistor). The apparatus for such measurement could be enclosed in the diver's protective bunny cap and would require relatively little attention or special involvement during the diver's dressing procedure.

Classical diver physiological monitoring has usually involved measurement of rectal temperature, not because it is the best deep body core site but because it has historically been a widely reported parameter and is relatively easily determined for comparison to results gathered in earlier studies. It is not the preferred site, because it is a slowly responding thermal area and one that is much affected by the *afterdrop* phenomenon. Still, its relative ease of access and the amount of experience involved in its use have shown it to be a relatively good indicator of general thoracic and head temperature during cooling but not during rewarming. Deep esophageal temperature is considered to be the best analogue of intrathoracic temperature during rewarming, because even tympanic temperature does not correlate well with the latter during this experience. To be successful, rewarming strategies must heat the heart; therefore, the most suitable temperature analogues to arterial temperature during this exposure have been shown to be esophageal, gastric, tympanic, and rectal, in descending order of applicability.

Whatever the method of measurement for deep body core temperature, it has been shown that it is not well correlated with the quantity of heat lost by the body. The heat loss-core temperature relationship is strongly dependent on rate of cooling; furthermore there are important individual differences in cooling rate, based on amount of body fatness and habituation.

Although monitoring of core temperature is useful in determining how cold a diver is, it doesn't relate necessarily to what his performance is, or is likely to be, and its use as a "gauge" of hypothermic stress is questionable. The level of unacceptable core temperature is still set at 35°C, but it is recognized that this measurement value is not as important as would be a definitive measurement of decrement of diver performance. No significant decrement in mental performance has been demonstrated for core temperatures as low as 34°C, although signs of decrement of peripheral physical performance are apparent at that temperature.

In rewarming, core temperature should only be used in the sense of differential diagnosis. Is there a problem of hypothermia or not? If not, then

attention should be directed to other potential diver biomedical concerns. The core temperature measurement value of 35°C is a useful indicator in that regard. If the core temperature is less than 35°C, then potential hypothermia should be considered to be a problem and the diver should be removed from the exposure. Temperature measurement in rewarming, then, may not be as important as it was once thought to be. It is the consequence of temperature that deserves consideration, i.e., respiration, cardiovascular support, fluid balance.

Measurement of heart rate was considered to be a good indicator for monitoring of diver cold stress, particularly during rewarming in a stable controlled environment. It decreases under the influence of diver cooling but is also influenced by diver work and pressure-related bradycardia. It drops precipitously on exposure of the diver to a controlled hot water bath along with a decrease of oxygen consumption. It is the first body parameter to show response to rewarming: it starts to increase before any body temperature site responds. It is therefore useful in monitoring of immersion or hypothermic victims during rewarming to prevent rewarming collapse or heat syncope. In such cases, if it starts to increase markedly, the bath temperature should be quickly lowered until it returns to stable lower values, at which point bath temperature can be slowly increased.

The mean skin temperature is considered to be an important parameter for diver monitoring. Indeed, some workers have proposed the measurement of the temperature of the big toe alone as a good parameter for correlation with diver performance and willingness to become cold! The diver sensation of thermal comfort is based upon afferent impulses from thermal receptors in the skin and such impulses are elemental in the shivering response, even for divers with elevated core temperatures.

Little agreement exists among thermal researchers as to the method of choice for measuring mean skin temperatures. Whereas various methods based on 4-site, 7-site, or 12-site indices agree fairly well in steady-state conditions, no agreement exists for the case of transient exposures. All such indices were derived for use in laboratory experiments on nude subjects in cool air environments. Such indices assume that every body site considered in the weighting has the same thermal sensitivity; however, a difference in density of cold thermal receptors exists over the body, which could explain certain of the anomalies of the shivering response. Indices of mean skin temperature as presently calculated may not be good representations of peripheral input. A more soundly based scheme of physiological weighting for determination of mean skin temperature is warranted.

## DIVING TECHNOLOGY

The goal of all diving technological development is equipment reliability and performance so that diving equipment no longer remains as the limiting factor in diver safety and productivity.

## Diving Suit Technology

Until recently, diver thermal protection was based on either passive neoprene foam-based insulation or expensive commercial hot-water heating systems (72,73), but various studies (74,75) have confirmed that such garments do not provide thermal comfort or safety for more than a few minutes at deep cold-water depths in the former equipment or until the hot water supply fails in the latter. Significant advances have since been made in passive and active diver thermal protection, particularly by the U.S. Navy, during the last 8 years. The active diver heating program is centered around a portable individual diver heater such as that based on magnesium/oxygen capable of providing power of 500 W of heat over a 6-h mission.

The passive thermal protection program is centered around the Diver Thermal Protection Passive System Prototype (76,77) which consists of a dry suit outergarment, to act as a flexible water barrier with minimum insulation, worn over a thermal undergarment that is made of a recently developed hydrophobic material called Thinsolite, a 3M-produced material that retains the majority of its insulation qualities even when wet. Having a higher insulation value than foam neoprene, it can serve as an insulation undergarment in both wet- and dry-suit ensembles even though mathematical modelling work (78) has shown that, with such a passive garment, it is difficult to satisfy the requirements of a diver during resting and working conditions with a single suit.

Copper-man studies (79,80) along with range-of-motion studies (77) have demonstrated that suits using this material possess thermal insulation values approaching that of stagnant air, which is the maximum value attainable by passive insulation technology. Typical suit insulation values are approximately 1 to 1.5 clo in normobaric conditions, depending on suit inflation; such insulation is intended to provide passive thermal comfort for a diver operating in 2°C water over a 6-h mission. These insulation values have been verified at depths of 10–70 fsw in long-duration air dives in water of 2–6°C, conducted by the U.S. Navy (81). Such studies confirmed the thermal benefit of such suits over 6 h for working divers and 3 h for resting divers, although it was noted that the divers' heads needed more protection and that the sealing of the dry suit undergarment was inadequate.

A resulting problem now considered with such suit technology is alleviation of hyperthermia, which is a problem common to all constant insulation suits, especially when worn at the surface before or after a dive. One solution will be to design a family of such garments with varying insulations for different thermal requirements. New sensing technology involving direct-reading heat flow discs (82,83), used with computer-based data acquisition systems (84–86) in corporation with mathematical models or predictive equations (87,88), will expedite such solutions.

The future of diving suit design will include the incorporation of regional differences in insulation to improve the physiological effectiveness of the suit and to bring about a reduction of suit squeeze. As to suit composition,

development of materials that are partially evacuated (89), or capable of holding a partial vacuum, may lead to improved thermal insulation values that are 1 order of magnitude better than those of presently used materials. Use will be made of localized chemical heating pads (90) for supplementary heating of the hands.

Control of effective diver insulation to avoid user hyperthermia will become an ever-increasing problem with use and further development of future suit technology. Even presently used hot water suits suffer from inadequate control of the hot water supply.

## Respiratory Gas Heating Technology

The technology for respiratory gas heating has been markedly improved over the last 5 years in terms of practicality and breathing resistance; however, the new minimal respiratory gas heating limits set for this technology are such that they cannot presently be met unless use is made of "active hot water insulation." Aside from the difficulty of meeting the new limits, another problem besets this technology, namely, convincing operational divers that this technology should be used and that the control of divers' inspired temperature should be improved.

Open-circuit demand technology is one of the easiest ways to effect respiratory heating. Closed-circuit systems use gas heaters as well, but such heating technology is fairly primitive in terms of thermal engineering. The most critical area for technological improvement is the interface at the helmet. Depth or pressure exposure is not a problem as the heaters are placed on the diver, close to the helmet. Other pertinent problems of this technology are the questions of what design limits there should be for heating for thermal comfort and for the avoidance of hypothermia.

Various experiments (43,91,92) have demonstrated that substantial core cooling transpires in deep water (300–500 msw) divers following loss of their hot water supply. The tolerable loss of respiratory gas heat is approximately 200 W and several authors (43,91,93) have stressed the need for ensuring that the inspired gas must be kept warm between the heat exchanger and the helmet, as this is one area where temperature drops noticeably (94).

Although active heat exchangers have been used universally for several years for heating of respiratory gas at deep depths, there is some potential for development of passive respiratory heat exchangers, particularly for shallower depths, in the air diving range. Riegel (45) has reviewed this rapidly developing technology and several navies have supported new developments of this sort (95–99). Some passive devices are marketed for regenerative recovery of exhaled respiratory heat (100,101), but there has been no wide acceptance of such breathing apparatus in spite of its obvious advantages.

## Thermal Power Sources for Divers

With over two decades of development, hot-water heating systems have been successfully applied to the problems of the shallow-depth surface-sup-

ported diver (102). Such commercial systems that exist easily support two divers in less than 100 fsw and are reliable and easily portable so that they can be used with rudimentary support in hitherto-inaccessible areas. There is a limit, however, to such surface-supported systems. As the depth of diving increases, the hot water pump pressure must also increase and the hot water umbilical becomes unwieldly and must be made stronger to withstand the extra pressure. Successful commercial deployments have been made (103) for hot-water heating systems to be mounted on the diving bell, circulating hot water to a closed-circuit liquid loop diving suit. These systems now permit diver cold-water lockouts of 6 hours or more at considerable depths (104,105). Suit hot-water flow rates are as high as 14 L/min but, even in cases where the hot water flow is constant, the correlation between divers' mean skin temperatures and the temperature of the hot water supply is such that considerable variation in physiological benefit is observed, presumably due to differences in mixing of the hot water within the suits. These observations (105) demonstrate that monitoring of the temperature of the hot water supply at entry into the diver's suit may not be sufficient as an index of diver thermal safety.

Various novel technologies have been proposed to improve the generation of heat at the bell. Heat, power, and oxygen can be obtained from the catalyzed exothermic decomposition of hydrogen peroxide (106), for example. Battelle Laboratories (107) have developed a hydrogen-fuelled backpack heater as part of the U.S. Navy Diver Thermal Protection Program which supplies up to 2 kw of heat to a diver for as long as 6 h at depths of 450 msw. New local heating concepts have been developed elsewhere (108), which involve salt-water activation of magnesium/iron mixtures in small sachets situated at various body sites. The French Climataseur Diver Heating System (109,110) has seen continued development to the point of operational application. This system involves thermal energy storage in molten salts in an insulated back-pack device, which transfers heat to closed-circuit liquid loop heating coils inside the diver's dry suit. Claims of thermal comfort for divers for over 6 h in 2°C water at 300 msw have been made.

## THE PROBLEM OF THE "LOST BELL" DIVER

Unfortunate hypothermic deaths of divers inside lost or trapped diving bells (111,112) have caused attention in this field to be focused on problems of diver survival in such a situation, not the least of which is the problem of relocating the bell to effect the rescue (113).

The lost bell problem is one that is analogous to the hydration problem in survival of individuals in a life raft with a limited supply of water (104). Various recommendatons for extending survival time exist and are being implemented (114,115). These include better insulation of the diver, removal of the diver from contact with the sides of the bell, adoption of a foetal position for reduction of heat loss, avoidance of any physical activity, use of all available heat retained in the bell after its "loss" and use of emergency

respiratory heat reclaimers/$CO_2$ scrubbers. In uninsulated bells, a major factor in the cooling of the bell environment is the thermal capacity of the bell structure itself. The effect of shivering on the boundary layer coefficient for convective heat transfer is an unknown but presumably large and important factor.

Various theoretical papers have been presented in which the survival of divers in a lost bell was modelled. One model (116) involved the diver with, and without, 75% body heat reclamation but without respiratory heat conservation in the survival experience. The end point of this model was a body heat loss of 400 kcal, calculated to lower the body core temperature to 33°C. Depending on the depth, predictions of survival for isolation divers were of the order of one to several hours. In consideration of the possible benefit of shivering, involving only the metabolic heat generated in comparison with the associated increase in respiratory heat loss, shivering was found to be beneficial except for very deep exposures of the order of 1000 fsw and temperatures of about 5°C. This pessimistic appraisal would not pertain if the respiratory heat loss was reclaimed. A different mathematical approach (117,118) also predicted short survival times for the relatively unprotected diver, but this analysis was extended to show that, with proper thermal insulation (1 clo in the environment of concern) and reclamation of 75% of respiratory heat loss, survival of the diver should be possible. A 500-W heat source and 1 clo of diver insulation (in the environment of concern) was suggested as being sufficient to provide extended survival at a depth of 150 msw and environmental temperature less than 5°C. Concern was demonstrated for the beneficial effect of shivering. Shivering fatigue is important in that this analysis has shown that the thermal protection is marginal once shivering ceases. It was also shown that re-entry into the cold water by the hypothermic bell diver should not be attempted. This work has been extended and is reported in a paper in this *Proceedings* (119).

Several sets of experimental data were presented that had a bearing on this problem. The first such study was conducted at the Norwegian Underwater Institute (NUI) in 1980 (120,121) involving 4 divers in 2–6°C oxyhelium at 16 bars (150 msw). Wearing survival equipment (two different sets of commercial lost bell insulation ensembles and two different respiratory gas thermal regeneration systems), 2 divers were able to endure approximately 10 h of exposure but it was considered that the feasibility for 24 h of protection had been demonstrated. A similar study was conducted in England a year later (122) on 3 commercial bell survival systems in temperatures of 7–8°C and a bell internal pressure of approximately 250 msw. Although there was no carbon dioxide problem, the test had to be terminated at the 6-h mark because diver core temperatures decreased to values as low as 35.5°C.

A repeat of the NUI test ended with more positive results (123,124). Two different commercially available bell survival systems were tested in 8–10°C oxyhelium at 31 bars for approximately 10 h. No noticeable diver hypothermia occurred in these tests, primarily because the efficacy of the thermal rebreather/scrubbers kept inspired gas temperatures above 37°C. An American

test conducted at approximately the same time (125) involved one test subject in a bell survival system at 0–3°C oxyhelium temperatures at a pressure of 3 ATA for 24 h. No undue cold stress was evident physiologically and the diver was "too warm" except when sleeping.

A definitive collaborative test was conducted by NUI and AMTE (PL) recently, the results of which are reported in this *Proceedings* (126). This test of 2 commercial survival systems at 16 bars over 24 h with 2 subjects, precooled as they would be in actual bell diving, showed that 24 h of thermal safety was assured with only minor rectal temperature change. A Swedish study (127) is also reported that demonstrated similar beneficial results using a commercial survival system tested at 150 msw by 2 divers for over 16 h in 2°C conditions. Again, respiratory heat conservation was shown to be essential to such success.

From these experiments, there has developed a survival technology for trapped bell divers based on a passive insulation garment ensemble to be used with a reclamative thermal regenerator/$CO_2$ scrubber (128). The emphasis on such development was cost-effectiveness in terms of design simplicity, space required, and maintainability (129). A key requirement is high thermal efficiency of the respiratory gas thermal exchanger/scrubber and significant technical work has brought this about (130–132). It was considered that if a bell rescue was not accomplished in 6 or 8 h, then protection of more than 24 h may be warranted, but if survival protection can last 24 h, then several days survival is likely as long as other life support elements do not become threatening, e.g., food, carbon dioxide, and the like. As much as possible, the survival equipment of a diver should be the equipment that he normally uses and wears. One example is the use of the diver umbilical for thermal insulation if it is wrapped in the bell trunk. Various new technologies have been proposed for emergency generation of heat in the lost bell, such as the catalytic decomposition of hydrogen peroxide to generate heat, power, and oxygen (106).

## MODELLING OF DIVING THERMAL STRESS

The importance of mathematical modelling of diver thermal exposure is that it permits elucidation of likely thermal and physiological phenomena associated with severe body core cooling, often involving rapid calculations from large on-line computers (133). Experimental investigations of such phenomena are limited either to animal data or to data collected during treatment of accidental hypothermia victims. Ethical considerations and funding problems limit human diving thermal experiments to mild hypothermia only. Mathematical modelling can be used to extend this experimental data into cooling scenarios that are ethically or experimentally unobtainable.

Biomathematical modelling has a brief history and came into prominence through application to astronaut exposures in space-related activities. The essence of a good thermal mathematical model is that it can be based on sound

physical principles of heat flow. The scale of complexity of such models applied to diving can range from simple spheres (134,135) to complex composites of many cylinders, each with radial layers simulating bone, muscle, viscera, fat, and skin of a particular body component (136). In such models, arterial blood temperature is usually taken as body core temperature and provision is made for thermal effects of blood circulation and varying metabolic rate. Modelling of heat loss from the body surface by convection is very important as is that of the internal physiological counter-current heat exchange system. One elegant model in particular, that of Wissler (136), incorporates a rate of shivering driven not only by core and skin temperatures but by other thermal sensors as well, analogous to those considered intrinsic to the respiratory tract. It incorporates a fatigue factor for shivering, with elimination of shivering occurring within 8 or 9 h of exposure onset.

The predictions of the various models have been found to be in good agreement with the limited amount of human experimental data available on severe hypothermia (133,137,138). However, the effects of shivering are dependent on distribution of thermogenesis over the body and this information is not yet well known. Only a continued collection of pertinent thermal data will lead to refinement and improvement of such models in the future.

## REWARMING OF THE HYPERBARIC HYPOTHERMIA VICTIM

### Progress on Normobaric Rewarming of Hypothermic Victims

Considerable interest has developed currently as to the most efficacious method for rewarming of victims of accidental hypothermia (139), whether such hypothermia is due to cold water immersion or to cold air exposure. There is a range of advocacy for such treatment (140), from simple passive techniques such as blankets and body warming to more active clinical techniques such as peritoneal dialysis, extracorporeal circulation, and inhalation of warm humidified air or oxygen. The latter technique in particular has attracted considerable attention and is the subject of some debate. Despite early claims (54,141) for its thermal efficacy and suitability, such as the fact that heat is transmitted into the hypothermic core of a victim where it is needed most, most recent investigations (139,142,143) show that it is not an efficacious technique and that it is fraught with a hazard of inducing respiratory burns. Indeed, the less complicated and sophisticated the treatment, particularly in the field, the greater the chance of assuring victim survival, but even such customarily accepted techniques as showers and hot water tubs can be associated with physiological problems and dangers (144).

Much of the recent debate on the subject of rewarming from accidental hypothermia centers around the concept of *afterdrop*. The consideration of afterdrop of core temperature, usually denoted in terms of rectal temperature, has been defined as the *continued cooling of the core during the rewarming process,* and has been considered (presumably) to result from the return of

cold blood from the periphery to the core under the influence of vasodilation induced by the rewarming. Such continued cooling has been considered to be dangerous because the extent of afterdrop may be as great as 1 or 2°C, and if the heart, which may have been cooled to temperatures at which it becomes hyperexcitable (i.e., less than 34°C), is exposed to colder temperatures, ventricular fibrillation has been suspected to be a dangerous possibility.

This conventional explanation can be questioned. The evidence for afterdrop is based almost entirely on observations of rectal temperature, which is determined by cold venous blood return from the legs of a cold-exposed body, arterial blood input from the abdomen, some venous blood return from the superficial layers on the trunk, as well as the extent of body insulation provided by gluteal muscles and tissue. It is now considered that the rectal afterdrop can be explained sufficiently as a conductive phenomenon, that is, as being due to a time-lag in conductive heat transfer between the core and the shell. A hemodynamic explanation may be involved but it is not necessary for explanation. Afterdrop is now considered to be an important factor in the extended cooling of a diver or immersion victim due to exercise-induced venous return on coming out of the hydrostatic column. It is not considered to warrant significant concern in treatment regimens for accidental hypothermia, and such adages as ensuring that one's arms and legs are not immersed in cold water during hot tub rewarming are no longer taught. Nevertheless, strict concern for the sensitivity of cardiac tissue during rewarming must still be exercised because of the dangers of fibrillation.

As to explicit concern for normobaric rewarming of hypothermic divers, relatively little work has been done. That which has taken place (145–147) indicates no marked difference as compared with rewarming of accidental immersion victims or victims of cold air exposure. One concern that has been noted is that divers, accustomed as they are to the discomfort associated with cold water exposure, may be more ready to re-enter the hypothermic environment before rewarming to thermoneutrality and also may be less willing to admit to a hypothermic distress because of their habituation.

## Rewarming Treatment of the Rescued Lost Bell Diver

In the lost bell incidents that have occurred, the unconscious divers have fallen down into the bell trunk and the weight of their bodies has prevented easy or quick opening of the bell door on rescue. It has been proposed in the diving industry that the divers lash themselves in a seated position on the side of the bell to prevent this from happening, but this technique could lead to suffocation of the divers because the weight of their heads would occlude their airways when unconscious. It is recommended that they not be tied up in a seated position and that consideration be given to special yokes or harnesses for prevention of the probable suffocation.

Decompression of a rescued lost bell diver should only take place after rewarming has been completed. Death should not be pronounced in the case of a body that is recovered in a presumably hypothermic condition. If the diver is

conscious on rescue, then rewarming may take place in the deck decompression saturation chamber. It should consist of passive rewarming to avoid the dangers of hypovolemic collapse (rewarming shock). Any maneuver that would lead to a demand on cardiac output is to be avoided and the diver should be laid prone or horizontal with his head slightly down. If the diver is found unconscious in the recovered lost bell that is brought to the surface, it may be preferable to leave him in it and stabilize him there until he recovers consciousness by passive rewarming. He should be moved as little as possible and an air mattress and inflatable splints may be considered for use in preventing rewarming shock. If he is alive and kept insulated, his residual metabolism will bring him back to consciousness. Once conscious and near normothermia, consideration may be given to use of warm water flushing of his hot water suit. During the rewarming, his pulse should be noted and recorded. If the pulse starts increasing rapidly, the temperature of the oxy-helium environment should be lowered to reduce it. Once the pulse has recovered, the temperature of the bell can be raised again. This technique will prevent rewarming shock from developing.

## References


1. Webb P. Current concepts of metabolism and thermophysiology. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:493–502.
2. Webb P. Thermal problems in diving. The sixth Undersea Medical Society workshop. Bethesda: Undersea Medical Society, Inc., 1974:1–29.
3. Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society workshop. Bethesda: Undersea Medical Society, Inc., 1981.
4. Webb P. Body heat loss in diving. Report on U.S. Navy Contract N00014–72–C–0057, 1980.
5. Annis J, Troutman S, Webb P, Kuehn L. The effect of cooling rate upon the tolerance and thermal responses of mildly hypothermic man. Proceedings of the annual scientific meeting of the Aerospace Medical Association, 1979:161–162.
6. Hellon R. Neurophysiology of temperature regulation: problems and perspective. Fed Proc 1981;40:2804–2807.
7. Veicsteinas A, Ferratti G, Rennie DW, Superficial shell insulation in cold water in resting and exercising man. Fed Proc 1981;40:580.
8. Golden F. Diver monitoring. Thermal constraints in diving In: Kuehn LA, ed. The twenty-fourth Undersea Medical Society workshop. Bethesda: Undersea Medical Society, Inc., 1980:117–132.
9. Påsche A, Tønjum S, Wissler EH. Body cooling rate in hyperbaric heliox. In: Elliott DH, ed. Proceedings of European Undersea Biomedical Society seventh annual congress, 1981;2:379–389.
10. Påsche A, Tønjum S, Onarheim J. Body cooling rate in heliox at 21, 31, 41, and 51 ATA. Undersea Biomed Res 1982;9(Suppl):13–14.
11. Wade C, Decaney S, Smith R. Regional heat loss in resting man during immersion in 25.2°C water. Aviat Space Environ Med 1979;49:590–593.
12. Golden F, Hampton I, Smith D. Lean long distance swimmers. J R Nav Med Serv 1980;66:26–30.
13. Veicsteinas A, Rennie DW, Thermal insulation and shivering threshold in Greek sponge divers. J Appl Physiol: Respir Environ Exercise Physiol 1982;52:845–850.
14. Park YS, Lee IS, Paik KS, Kang, DH, Suh DJ, Lee SH, Hong, SY, Rennie DW, Hong SK. Korean women divers revisited: Current status of cold adaption. Undersea Biomed Res 1981;8(Suppl):25.
15. Park YS, Rennie DW, Hong SK. Status of cold acclimatization in contemporary Korean women divers wearing wet suits. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of

the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:373–384.
16. Childs C, Norman J. Unexplained loss of consciousness in divers. Med Aero Spat Med Sub Hyp 1978;17:127–128.
17. Hayward M, Keatinge W. Progressive symptomless hypothermia in water-possible cause of diving accidents. Br Med J 1979;1:1182.
18. White M, Allan D, Light I, Norman J. Thermal balance in divers. Lancet 1980;2:1362.
19. Keatinge W, Hayward M, McIver N. Hypothermia during saturation diving in the North Sea. Br Med J 1980;1:291.
20. Hamilton SJC. Hypothermia and unawareness of mental impairment. Br Med J 1980;1:565.
21. Baker S, Atha J. Canoeist's disorientation following cold immersion. Br J Sports Med 1981;15:111–115.
22. Kurss DI, Lundgren CEG, Påsche AJ. Effect of water temperature and vital capacity in head-out immersion. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:297–301.
23. Malkinson T, Martin S, Simper P, Cooper K. Expired air volumes of males and females during cold water immersion. Can J Physiol 1981;59:843–846.
24. Dahms T, Lind A, Williams C, Cooper K. Ventilatory response to immersion and isometric exercise in cold water. Fed Proc 1979;38:1227.
25. Oruc T, Terzioglu M. The sensitivity of hypoxia of peripheral and central respiratory control mechanisms under normo- and hypothermic conditions. Bull Eur Physiopathol Respir 1979;15:1117–1128.
26. Sterba J, Lundgren CEG. Influence of water temperature on breath-holding time in submerged man. Undersea Biomed Res 1979;6(Suppl):29–30.
27. Keatinge W, Hayward M. Sudden death in cold water and ventricular arrhythmia. J Forensic Sci 1981;26:459–461.
28. Shiraki K, Konda N, Sagawa S, Nakayama H, Matsuda M. Body heat balance and urine excretion during a 4-day saturation dive at 4 ATA. Undersea Biomed Res 1982;9:321–323.
29. Tomiyasu K, Nakayama H. Study on body heat loss and heat insulation or heating on divers. Report 3: Body heat loss under hyperbaric helium-rich environment. Tech report JAMSTEC 1981;7:121–140.
30. Tomiyasu K, Nakayama H. Study on body heat loss and heat insulation or heating on a diver. Report 2: The change in rectal, skin, and exhaled gas temperatures of saturation divers. Tech report JAMSTEC 1981;6:115–135.
31. Nakayama H, Hong SK, Claybaugh JR, Matsui N, Park YS, Ohta Y, Shiraki K, Matsuda M. Energy and body fluid balance during a 14-day dry saturation dive at 31 ATA (*SEADRAGON* IV). In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:541–554.
32. Påsche A, Tønjum S, Holand B, Dryseth A, Olsen C. Deep Ex 80, Project II, Thermal studies in diving: thermal model. Bergen: Norwegian Underwater Institute 1980. (NUI report 42–80)
33. Brubakk AO, Tønjum S, Holand B, Peterson RE, Hamilton RW, Morild E, Onarheim J. Heat loss and tolerance time during cold exposure in heliox atmosphere at 16 ATA. Undersea Biomed Res 1982;9:81–90.
34. Wilcock SE, Flook V. Environmental and body temperatures of 52 divers in hyperbaric heliox. Undersea Biomed Res 1980;7:225–239.
35. Garrard MP, Hayes PA, Carlyle RF, Stock MJ. Metabolic and thermal status of divers during simulated dives to 55 bars. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:517–538.
36. Sheehan M, Brauer RW. Implications of changes in temperature perception and temperature regulation in high pressure heliox oxygen environments. In: Proceedings of fourth international symposium on pharmacology of thermoregulation. 1980:232–237.
37. Imbert G, Naraki N, Le Doucen J. The relationship between heat production, non-evaporative heat loss and core temperature in mammals breathing helium-oxygen under hyperbaric conditions. Undersea Biomed Res 1982;9(Suppl):13.

38. Naraki N, Imbert G. Discharge of energy, pulmonary ventilation, and thermal balance in mammals in a hyperbaric heliox environment. Med Aero Spat Med Sub Hyp 1981;20:338–343.
39. Nuckols M. Heat and water vapour transfer in the human respiratory system at hyperbaric conditions. Dissertation. Durham, NC: Duke University, 1981. (U.S. Naval Coastal Systems Center Tech report 364–81)
40. Piantadosi C. Respiratory heat loss limits in helium-oxygen saturation diving. Panama City, FL: U.S. Navy Experimental Diving Unit, 1980. (NEDU report no. 10–80)
41. Piantadosi C, Thalmann ED, Spaur WH. Metabolic response to respiratory heat loss-induced core cooling. J Appl Physiol: Respir Environ Exercise Physiol 1981;50:829–834.
42. Zumrick JL, Jaeger MJ, Piantadosi CA. Effects of cold gas breathing on forced expiratory flow to 1800 fsw. Undersea Biomed Res 1981;8(Suppl):63.
43. Hayes P. Cooling in divers following loss of hot water heating: Limits of endurance and the subsequent rewarming procedures. In: Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society workshop. Bethesda: Undersea Medical Society, Inc., 1981;83–100.
44. Hayes P, Padbury E. Respiratory heat transfer and oxygen consumption when breathing warm humidified gas at 250 m (76 bar) following cold water diving. J Physiol (Lond) 1980;308:110P.
45. Riegel P. Investigation of methods of inspired gas heating. Summary Report on U.S. Navy Contract No. N61331–81–M–2369, 1981.
46. Schmidt TC. The advantages of helium-oxygen breathing gas for cold water diving in the air depth range. Undersea Biomed Res 1982;9(Suppl):15.
47. Burse R, Stroschein L. Metabolic and temperature responses of men working partially submerged in very cold water. Fed Proc 38:1227.
48. Iaizzo PA, Petry RW, Pozos RS. An electromyographic study of shiver in immersed human subjects. In: Program, abstracts, and mini papers. The Undersea Medical Society annual scientific meeting, 7th symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1980:19.
49. Pozos R. Frequency analysis of shiver in humans and its alteration by the temperature of inspired air. In: Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society workshop. Bethesda: Undersea Medical Society, Inc., 1981:55–80.
50. Schneider M, Brooke J. Bimodal relationship of human tremor and shivering on introduction to cold exposure. Aviat Space Environ Med 1979;50:1016–1019.
51. Martin S, Cooper K. Factors which affect shivering in man during cold water immersion. Pfluegers Arch Eur J Physiol 1981;391:81–83.
52. Jorna P. Stress, information processing, and diving. Netherlands Institute for Perception, 1981. (IZF Report 1981–4)
53. Stevens J, Marks L. Spatial summation of cold. Physiol Behav 1979;22:541–547.
54. Collis M, Steinman A, Chaney R. Accidental hypothermia: An experimental study of practical rewarming methods. Aviat Space Environ Med 1977;48:625–632.
55. Kuehn L, Zumrick J. Assessment of thermal protection afforded by hot water diving suits. Engineering aspects of diver thermal protection. American Society of Mechanical Engineers, 1980. (ASME 80–WA/OCE–4)
56. Golden FStC, Hampton IFG, Hervey GR, Knibbs PAV. Shivering intensity in humans during immersion in cold water. Physiol (Lond)1979;290:48P.
57. Bolstad G, Brubakk AO, Påsche A, Holand B. Shivering threshold in man. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:343–348.
58. Galbo H, Houston M, Christensen N. The effect of water temperature on the hormonal response to prolonged swimming. Acta Physiol Scand 1979;105:326–337.
59. Wiehl A, Langworthy HC, Manalaysay AR, Layton RP. Metabolic response of resting man immersed in 25.5°C and 33°C water. Aviat Space Environ Med 1981;52:88–91.
60. Manalaysay AR, Langworthy HC, Layton RP. Catecholamine levels in resting divers exposed to the stress of cold water immersion and hyperbaria. Undersea Biomed Res 1981;8(Suppl):62.
61. Martin S, Bauce L, Cooper K. Continuous sampling of plasma catecholamines in man during cold water immersion and rewarming. Fed Proc 1981;40:580.

62. Cross M, Booth L. The biochemical assessment of hypothermia. In: International conference Divetech '81. The way ahead in diving technology, Workshop A. London: Society for Underwater Technology, 1981.
63. Webb P. Impaired performance from prolonged mild cooling. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:391–400.
64. Dunford R, Hayward J. Venous gas bubble production following cold stress during a no-decompression dive. Undersea Biomed Res 1981;8:41–49.
65. Dunford R, Hayward J. The effects of cold stress on venous gas emboli. In: Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society workshop. Bethesda: Undersea Medical Society, Inc., 1981;81–83.
66. Cox R, McIver N, King J, Calder I. Hyperthermia in hyperbaric activities. Lancet 1980;2:1303–1304.
67. Turner B, Pennefather J, Edmunds C. Cardiovascular effects of hot water immersion. Med J Aust 1980;67:39.
68. Bondi K. An analysis of heat stress under hyperbaric conditions. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:503–507.
69. Davis F. Diving and hypothermia. Br Med J 1979;2:494.
70. James PB. Human limitations and physiological monitoring in commercial diving. In: International conference Divetech '81. The way ahead in diving technology, Workshop B5. London: Society for Underwater Technology, 1981.
71. Pearson RR. Why do we need diver monitoring? In: International conference Divetech '81. The way ahead in diving technology, Workshop B5. London: Society for Underwater Technology, 1981.
72. Tomiyasu K, Nakamura M, Yamada M, Murai T. Study of body heat loss and heat insulation or heating of a diver: Effects on heat insulation of underwear for dry diving suit. Tech report JAMSTEC 1980;5:187–189.
73. Yamada M, Kadomoto Y, Inagaki N, Kanda S, Murai T. Operational study of the diver heating system. Tech report JAMSTEC 1980;4:215–238.
74. Allin L, Bowen C. Open water comparison of two surface-supplied hot water diving suits. Downsview, Ontario: Defence and Civil Institute of Technical Environmental Medicine, 1979. (DCIEM commun no. 79–C–27)
75. Middleton J. Evaluation of Poseidon Unisuit and O'Neill Supersuit systems. Panama City, FL: U.S. Navy Experimental Diving Unit, 1979. (NEDU report no. 4–79)
76. Nuckols M. The development of thermal undergarments for diver's dry suits. U.S. Naval Coastal Systems Center Report, 1979.
77. Nuckols M. The development of thermal protection equipment for divers. In: Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society workshop. Bethesda: Undersea, Medical Society, Inc., 1981:133–166.
78. Wissler E, Nuckols M. Integration of physiological and physical factors in the design of passive thermal garments for divers. Undersea Biomed Res 1979; 6(Suppl):28.
79. Audet N, Orner G, Kupferman Z. Thermal insulation materials for diver's underwear garment, 1980. U.S. Navy Clothing Textile Res Facility, Tech report 139.
80. Richardson D, Audet N. A 10-zone thermal mannikin for evaluating personnel protective garments in cold air and water immersion environments, 1981. U.S. Navy Clothing Textile Res Facility, Tech report 142.
81. Piantadosi C, Ball D, Nuckols M, Thalmann E. Manned evaluation of the NCSC diver thermal protection (DTP) passive system prototype. Panama City, FL: U.S. Navy Experimental Diving Unit, 1979. (NEDU report 13–79).
82. Wissler EH, Ketch RB. Errors involved in using thermal flux transducers under various conditions. Undersea Biomed Res 1982;9:213–231.
83. Nuckols ML, Piantadosi CA. Calibration and characterization of heat flow transducers for use in hyperbaric helium. Undersea Biomedical Research 1980;7:249–256.
84. Layton RP, Mintz WH. An amplifier and multiplexer system for heat flux and temperature measurements. Undersea Biomed Res 1979;6(Suppl):29.

85. Layton RP. Data acquisition and analysis software for thermal stress studies. Bethesda: Naval Medical Research Institute, 1982. (NMRI report 82–3).
86. Zumrick JL, Kuehn LA. A computerized system to evaluate diver convective heat loss. Undersea Biomed Res 1979;6(Suppl):29.
87. Johnson CE, Collins JD. The development of predictive engineering formulations for diver heating, vols 1 and 2. Durham, NC: Duke University, 1982:437p. (Duke Univ report on contract N000014–79–C–0379).
88. Johnson CE, Collins JD, Piantadosi CA. Equations for predicting diver regional skin temperatures as a function of mean skin temperature. Undersea Biomed Res 1982;9:59–74.
89. Pogorski L, Kuehn L. Evacuated diving suit insulation. U.S. Patent No. 4,274,158, 1981.
90. Burton DR. Underwater engineering research at CSIRO. SPUMS (Suppl):1981;22–24.
91. Hayes PA, Padbury EH. Rewarming after diving to the limit of cold (4°C) endurance down to 300 m. Undersea Biomed Res 1981;(Suppl):26.
92. Kuehn LA, Zumrick J. Thermal protection afforded by hot water diving suits. Undersea Biomed Res 1979;6(Suppl):28–29.
93. Tønjum S, Påsche A, Holand B, Svendson E, Olsen C. Thermal condition of divers using hot water suits at 300 msw In: Elliott DH, ed. European Undersea Biomedical Society 7th annual congress, 1981: Abstr no. 26.
94. Middleton J. Evaluation of kinergetics breathing gas heaters for use in open-circuit demand underwater breathing apparatus. In: Kuehn, LA ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society workshop. Bethesda: Undersea Medical Society, Inc., 1981:167–184.
95. McKay W. Passive respiratory heat exchange in diving. Dissertation. Salford, UK: University of Salford, 1979.
96. Thornton AG. An investigation of some simple regenerative heat exchangers for use with divers' breathing apparatus. R N Nav Serv 1980;7:247–251.
97. Thornton AG. Potential of passive methods for protection of divers. In: International conference Divetech '81. The way ahead in diving technology, Session 2A. London: Society for Underwater Technology, 1981.
98. Kuehn L, Pogorski L. Heat receiver for divers. U.S. Patent 4,201,206, 1980.
99. Dingwall R, Dundas C, Norman J. A technique for evaluation of respiratory exchangers at simulated depth. In: Seventh international congress on hyperbaric medicine, 1981:431–432.
100. Cardone B. Warm up with WABA. Skin Diver 1979;28:104–105.
101. Williscroft R. Heater and humidifier for breathing apparatus, NOAA Diver 1979;3:5–6.
102. Harrison J. Test and evaluation of DUI ECONO II Hot Water Heater. Panama City, FL: U.S. Navy Experimental Diving Unit, 1979. (NEDU report no. 16–78).
103. Bramham E. Diver heating by a liquid loop hot water system. International Underwater Systems Design 1979;1:8–14.
104. Anonymous. Heat pump diver heating system. International Underwater Systems Design 1980;2:31.
104. Kuehn LA. Proposals for survival in isolated diving bells. In: Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society workshop. Bethesda: Undersea Medical Society, Inc. 1981:253–275.
105. Påsche A, Tønjum S, Holand B. Diver heating during cold water dives at 51 ATA. Undersea Biomed Res 1982; 9(Suppl):14.
106. MacNair E. Hydrogen peroxide for an underwater heat source. In: International conference Divetech '81. The way ahead in diving technology, Session B4. London: Society for Underwater Technology, 1981.
107. Anonymous. Hydrogen-fueled back-pack heater. Faceplate 1982;13:5.
108. Chan CYL, Burton DR. A portable thermochemical heat source for divers. In: International conference Divetech '81. The way ahead in diving technology, Session 2A. London: Society for Underwater Technology, 1981.
109. Rouhet JC, Crocq L, Lemasson Y, Petretto G. System for divers in cold water: A molten salt heat storage system associated with a dry type suit with hot water circulation. In: Proceedings of ADC international diving symposium 1979:171–177.
110. Anonymous. French heater for divers. Offshore Services; 1979;12:50.

111. Arnoux G. Rescue of the standard divers of the STENA Seaspread by divers of the *Uncle John*, 21 January 1981. In: International conference Divetech '81. The way ahead in diving technology, Session B4. London: Society for Underwater Technology, 1981.
112. Anonymous. "Lost bell" incident: Surviving hypothermia. SPUMS 1981:3–5.
113. Kelland N, Partridge C. Equipment to relocate a lost bell. In: International conference Divetech '81. The way ahead in diving technology, Session B4. London: Society for Underwater Technology, 1981.
114. Diving Medical Advisory Committee (DMAC). Thermal stress in relation to diving, In: Elliott D, Golden F, Chairman. DMAC workshop proceedings session three, 1981:23–30.
115. Thornton A. The protection of man in lost diving bells. In: International conference Divetech '81. The way ahead in diving technology, Session B4. London: Society for Underwater Technology, 1981.
116. Schmidt T. Thermal considerations for survival in emergency hyperbaric situations. In: Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society workshop, Bethesda: Undersea Medical Society, Inc., 1981:217–234.
117. Wissler E. An analysis of the "lost bell" problem. In: Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society workshop. Bethesda: Undersea Medical Society, Inc., 1981:315–344.
118. Wissler E. An analysis of the "lost bell" problem. In: International conference Divetech '81. The way ahead in diving technology, Session B4. London: Society for Underwater Technology, 1981.
119. Wissler EH. Predicted survivability of "lost bell" accidents under various conditions. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:401–412.
120. Tønjum S, Hamilton RW, Brubakk AO, Peterson RE, Youngblood DA. Project *Polar Bear*: Testing of diver thermal protection in a simulated "lost bell." Bergen: Norwegian Underwater Institute, 1980. (NUI report 2–80).
121. Tønjum S, Project *Polar Bear I*: Testing of diver thermal protection in a simulated "lost bell." Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society workshop. Bethesda: Undersea Medical Society, Inc., 1981:345–358.
122. Hayes P, Padbury E, Atherton P. Validation of emergency procedures—the lost bell situation. In: International conference Divetech '81. The way ahead in diving technology, Session B4. London: Society for Underwater Technology, 1981.
123. Tønjum S, Påsche, Furevik D, Holand B, Brubakk A, Olsen C. Test of survival systems for stranded bell divers. In: International conference Divetech '81. The way ahead in diving technology, Session B4. London: Society for Underwater Technology, 1981.
124. Tønjum S, Påsche A, Furevik D, Holand B, Brubakk A, Olsen C. Test of survival systems for stranded bell divers at 31 bars. In: Elliott DH, ed. European Undersea Biomedical Society 7th annual congress, 1981: Abstr no. 24.
125. Egstrom GH, DiCharo A. Emergency thermal protection for saturation diving. In: Program, abstracts, and mini papers. Undersea Medical Society Annual Scientific Meeting, 7th symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1980:19.
126. Tønjum S, Påsche A, Onarheim J, Hayes P, Padbury H. Cold exposure in heliox environment at 16 bars for 24 hours. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:385–390.
127. Magnusson J, Holmer I, Elnäs S, Hamilton RW. Metabolic rate of divers in simulated "lost bell" survival test. In: Bachrach AJ, Matzen MM, eds. underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:349–360.
128. Murray B, Clarke D. Development of hyperbaric survival equipment. In: International conference Divetech '81. The way ahead in diving technology, Session B4. London: Society for Underwater Technology, 1981.
129. Long R. Development and performance standards of DUI's Polar Bear System. In Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society workshop. Bethesda: Undersea Medical Society, Inc., 1981:373–390.

130. Hamilton RW. Design of a combination thermal regenerator $CO_2$ cannister for hyperbaric survival. In: Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society workshop. Bethesda: Undersea Medical Society, Inc., 1981:359–372.
131. Lloyd E. Diving and hypothermia. Br Med. J 1979;2:668.
132. Furevik D, Påsche A, Tønjum S. Endurance test of lung-powered scrubbers designed for hyperbaric rescue chambers and standard diving bells. In: International conference Divetech '81. The way ahead in diving technology, Session B4. London: Society for Underwater Technology, 1981.
133. Wilcock S, Flook V. A computer model designed to make rapid predictions of diver temperature changes. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:555–563.
134. Troutman SJ Jr, Webb P, Annis JF. Estimating body heat loss from temperature changes during cooling. Undersea Biomed Res 1979;6(Suppl):27–28.
135. Webb P. Predicting $T_{re}$ from heat loss. In: Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society workshop, Bethesda: Undersea Medical Society, Inc., 1981:35–44.
136. Wissler EH. A mathematical model of the human thermal system with reference to diving. In: Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society workshop. Bethesda: Undersea Medical Society, Inc., 1981:181–212.
137. Baker E, Harnett R, and Renguest J. An evaluation of protection equipment. U.S. Coast Guard Contract Report CG–D–80–79.
138. Nunnely S, Wissler EH. Immersion hypothermia: Validation of model and prediction of cooling rates. In: Kuehn LA, ed. Thermal constraints in diving. The twenty-fourth Undersea Medical Society workshop, Bethesda: Undersea Medical Society,Inc., 1981:213–252.
139. Kuehn L. Rewarming strategies: Evaluation of techniques. Human performance in the cold. Proceedings of symposium held at 33rd Alaska science conference. U.S. Office of Naval Research, 1982. N00014–76–C–0990. In press.
140. Anonymous. Symposium of first aid, treatment, and transport of diving casualties. In: Proceedings of the South Pacific Underwater Medicine Society annual meeting, Melbourne, 1980.
141. Morrison J, Conn M, Hayward J. Thermal increment provided by inhalation rewarming from hypothermia. J Appl Physiol: Respir Environ Exercise Physiol 1979;46:1061–1065.
142. Hoskin R, Goode R, Layton R, Kuehn L. An evaluation of three rewarming methods for mildly hypothermic subjects. Undersea Biomed Res 1982;9(Suppl):15–16.
143. Conn M, Hayes P, Morrison J. Contribution of metabolic and respiratory heat to core temperature gain after cold water immersion. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:509–516.
144. Buckingham I, Kuehn L. Diagnosing and treating hypothermia. Can Med. Assoc. J 1982; 126:1276–1277.
145. Strauss MB, Vaughan WS Jr. Rewarming experiences with hypothermic scuba divers. Undersea Biomed Res 1981;8(Suppl):25–26.
146. Hayes P, Bramham E. Physiological and engineering aspects of diver thermal protection and rewarming. In: Proceedings of international diving symposium '81. New Orleans: Association of Diving Contractors, 1981:73–87.
147. Mekjavic IB, Morrison JB. The importance of static and dynamic characteristics of thermoreception to thermogenic response in the human. In: Bachrach AJ, Matzen MM, eds. Proceedings of the eighth symposium on underwater physiology. Underwater physiology VIII. Bethesda: Undersea Medical Society, Inc., 1984:361–371.

# Part VI

## INTERACTIONS OF VENTILATORY CONTROL, HYPEROXIA, AND INCREASED GAS DENSITY AT HIGH AMBIENT PRESSURES

# RELATIONSHIP BETWEEN VENTILATION PATTERN AND VENTILATORY REQUIREMENT IN CATS AT DEPTHS DEEPER THAN 750 MSW

*H. Burnet, N. Naraki, and G. Imbert*

Experiments performed on animals at very high pressure can be used for prediction of efficiency of the ventilatory system at depths greater than those reached by human divers up to this time.

According to Dejours et al. (1), when one has to compare under different conditions the ventilatory flow rate of a medium, absolute values of medium-convection flow rates ($\dot{V}E$) become more meaningful when they are related to the flow rates per unit of oxygen consumed ($\dot{M}O_2$). The ratio $\dot{V}E/\dot{M}O_2$, which represents *respiratory equivalent* or *ventilatory requirement,* signifies that for a unit of oxygen taken up in the course of the external respiration, the body has to move a certain volume of medium over the gas exchange surfaces.

Imbert (2) reported an increase in ventilatory requirement associated with high respiratory frequency during a 2-h stay at 1000 msw. To verify if this increase in $\dot{V}E/\dot{M}O_2$ ratio is related to changes in the breathing pattern, we studied the relationship between $\dot{V}E/\dot{M}O_2$ ratio, tidal volume (VT), and respiratory frequency (f) during long stays (12–14 h) under high pressure (750, 900, and 1000 msw).

## MATERIAL AND METHODS

### Subjects

Eleven cats weighing between 2.6 and 3.6 kg were used as subjects in separate simulated dives.

## Measurement of Respiratory Data

Using volumetric plethysmography, we measured continuously VT and f. The $\dot{M}O_2$ was measured by the decrease of oxygen partial pressure in the hyperbaric chamber during stays at high pressure. These methods have been described previously (2,3).

## Diving Protocols

Nine dives were performed with He-$O_2$ normoxic mixtures. The compression rate decreased with depth: 180 msw·$h^{-1}$ from surface to 180 msw; 120 msw·$h^{-1}$ from 180 to 600 msw; 60 msw·$h^{-1}$ from 600 to 1000 msw (Fig. 1). Depending upon the dive, the maximal pressure was 750, 900, or 1000 msw. During compression, 2-h stops were allowed at 300 and 600 msw, when maximal pressure was 750 or 900 msw. A 2-h stop at 900 msw was added to the compression schedule for deeper dives. Two additional dives at 1000 msw were performed with He-$N_2$-$O_2$ (trimix) mixtures ($FI_{N2}$ = 0.05) with the same compression schedule.

Oxygen partial pressure and temperature were monitored continuously. Oxygen partial pressure was measured by a galvanometric cell (SEDAM) placed inside the chamber and was regularly checked with a paramagnetic

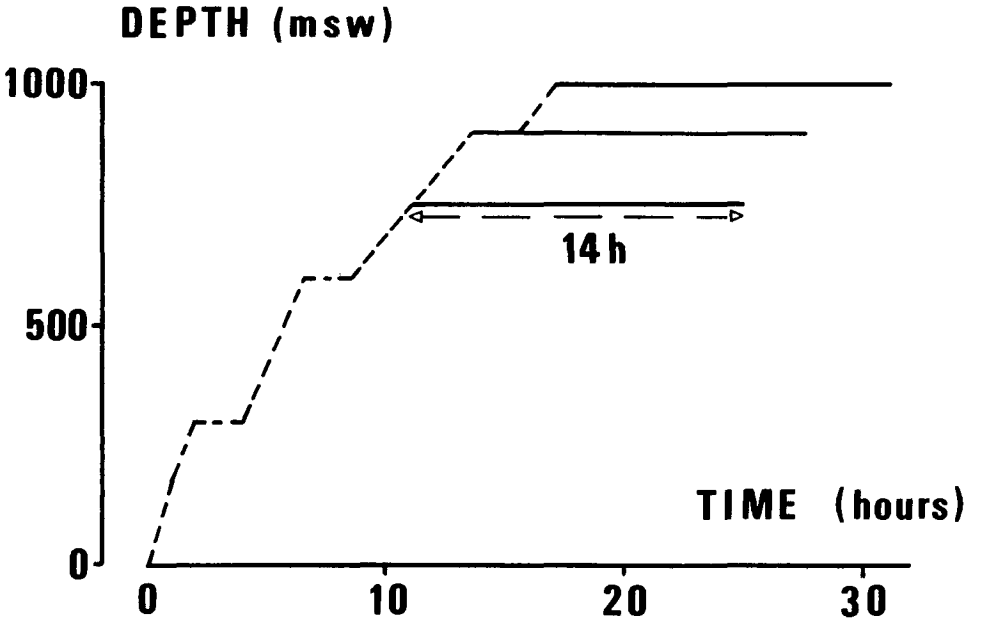



Fig. 1: Compression schedule used in all simulated dives.

analyzer (SERVOMEX OA 184). Ambient temperature in the hyperbaric chamber was 30.5°C at 300 msw, 32°C at 600 msw, 33°C at 900 msw, and 33.3°C at 1000 msw. These values were selected for thermal comfort in cats (Naraki, unpublished observations).

Carbon dioxide levels were monitored in expanded gases by infrared analysis and maintained below 5 mb by use of soda lime as the $CO_2$ scrubber in the life-support system; relative hygrometry was maintained below 50% with silica gel.

## RESULTS

Relationship between $\dot{V}E/\dot{M}O_2$, VT, and f during the stay at high pressure is presented in Table I. Spearman's rank-correlation test showed: a positive correlation between $\dot{V}E/\dot{M}O_2$ ratio and f; a negative correlation between $\dot{V}E/\dot{M}O_2$ ratio and VT or between VT and f. When f decreased, conversely VT increased (Fig. 2).

For 1000-msw He-$O_2$ dives, ventilatory requirements decreased with time spent at depth (Fig. 3). We assume that a steady state is reached after about 7.5 h at depth. Trimix 1000-msw dives didn't show such a pattern: ventilatory requirements remained constant throughout the 14-h stay at maximal depth. Therefore, we compared the different values of $\dot{V}E/\dot{M}O_2$ ratio, $\dot{V}E$, $\dot{M}O_2$, VT, and f during the steady state in He-$O_2$ and trimix dives (Table II).

## DISCUSSION

Trimix is heavier than He-$O_2$ mixtures. For the same depth, the respiratory work would be increased in trimix dives because of density. Moreover, $O_2$ diffusivity is lower in trimix than in He-$O_2$ mixtures (4). Therefore, from the

TABLE I

Relationship between Ventilatory Requirement ($\dot{V}E/\dot{M}O_2$), Tidal Volume (VT), and Respiratory Frequency (f) during Long Stays at High Pressure in He-$O_2$ Simulated Dives

| | $\dot{V}E/\dot{M}O_2$ vs. f | $\dot{V}E/\dot{M}O_2$ vs. VT/B | f vs. VT/B |
|---|---|---|---|
| 750 msw ($n$ = 20) | $r$ = + 0.904<br>$P <$ 0.001 | $r$ = − 0.677<br>$P <$ 0.01 | $r$ = − 0.788<br>$P <$ 0.001 |
| 900 msw ($n$ = 19) | $r$ = + 0.779<br>$P <$ 0.001 | $r$ = − 0.514<br>$P <$ 0.05 | $r$ = − 0.545<br>$P <$ 0.05 |
| 1000 msw ($n$ = 19) | $r$ = + 0.717<br>$P <$ 0.001 | $r$ = − 0.805<br>$P <$ 0.001 | $r$ = − 0.717<br>$P <$ 0.001 |

r: Spearman's rank-correlation coefficient. B: body weight.

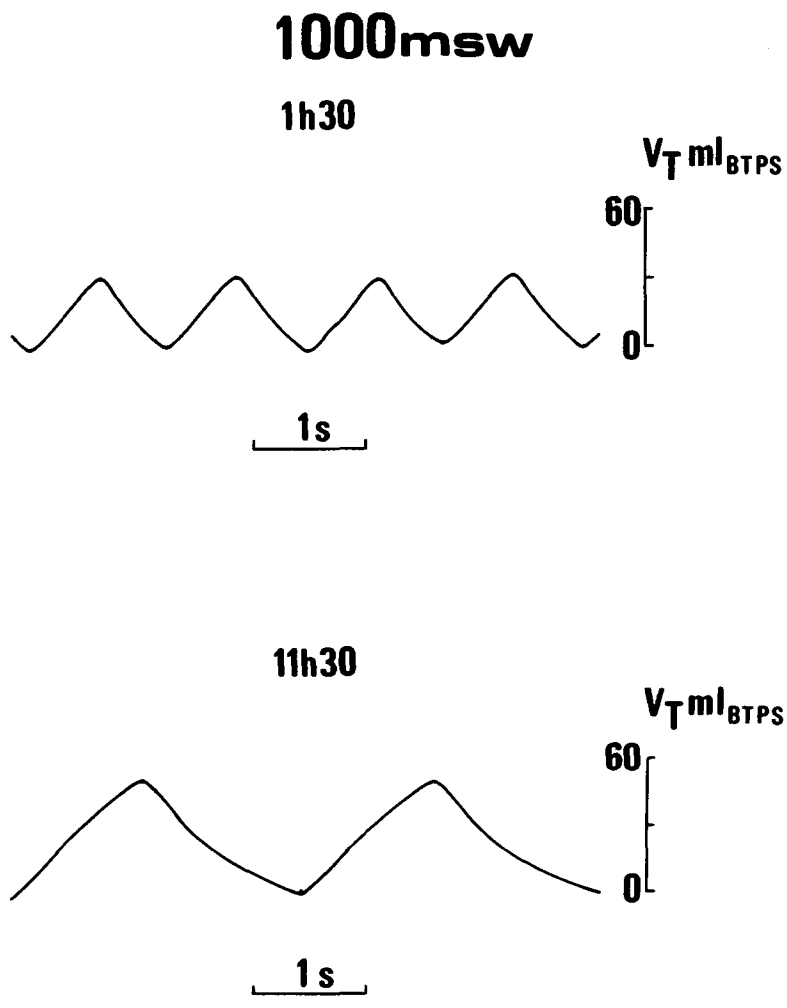



Fig. 2: Spirograms recorded in the same cat 1 h, 30 min and 11 h, 30 min after arrival at 1000 msw in an helium-oxygen simulated dive.

point of view of efficient breathing, for the same depth and the same breathing pattern He-$O_2$ mixtures theoretically would be preferable to trimix ones.

Soon after reaching maximal pressure, ventilatory requirements are lower in trimix environments than in He-$O_2$ ones. From the viewpoint of efficient breathing, the breathing pattern developed in He-$O_2$ mixtures at arrival at maximal pressure (increased f, low VT) is not the best—which would be increased VT and low frequency. The breathing pattern observed soon after reaching maximal pressure is expected to enhance alveolar stratification of gases and exert limiting effects on alveolar $O_2$ and $CO_2$ exchanges (5–7). The

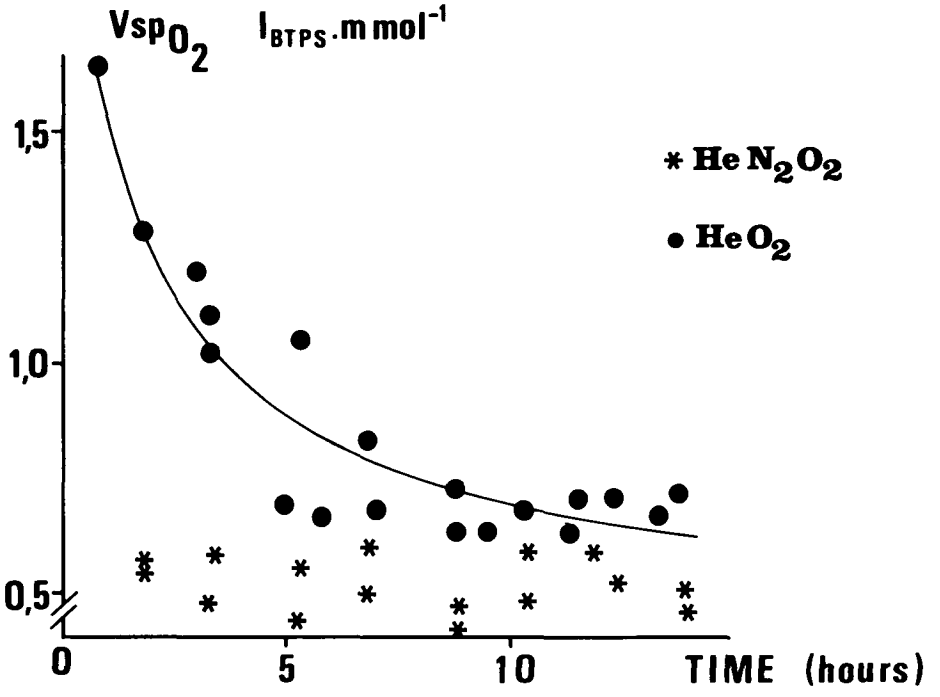



Fig. 3: Evolution as a function of time of ventilatory requirement for helium-oxygen and trimix dives.

use of trimix by decreasing the ventilatory rate through the decreased f diminishes the work of breathing and the alveolar dead-space. This effect benefits the animal subjects as is shown by the lower ventilatory requirement observed in our trimix series as compared to He-$O_2$ ones.

If we compare the respiratory data at steady-state (i.e., for longer than 7.5 h), the ventilation rate is lower in the trimix series than in the He-$O_2$ ones. These low ventilatory rates result from decreased f and VT. On the other hand, $O_2$ consumption is increased in He-$O_2$ environments as compared to trimix ones.

We suggest that high pressure helium may induce central nervous system hyperexcitability. Such a general hyperexcitability would induce increased metabolism, which is evidenced by the increased $O_2$ consumption in He-$O_2$ mixtures as compared to trimix.

High pressure nervous syndrome (HPNS) is believed to be divided in two components: the first is compression-rate dependent, which would disappear during the stay at maximal pressure; the second is pressure-dependent, which remains constant throughout the stay under high pressure. Therefore, at arrival at depth the two components are synergistic and hyperexcitability is maximal. As the compression-dependent component decreases with the stay at depth,

TABLE II

Comparison between the Respiratory Data Recorded in He-$O_2$ and Trimix Dives at 1000 msw

| Mixture | f $c \cdot min^{-1}$ | $V_T$ $mL_{BTPS}$ | $\dot{V}_E$ $L_{BTPS} \cdot min^{-1}$ | $\dot{M}O_2$ $mmol \cdot min^{-1}$ | $\dot{V}_E/\dot{M}O_2$ $L_{BTPS} \cdot mmol^{-1}$ |
|---|---|---|---|---|---|
| He-$O_2$ | $n = 9$ | $n = 9$ | $n = 9$ | $n = 9$ | $n = 9$ |
| | 31.4 | 49.1 | 1.538 | 2.259 | 0.681 |
| | SD 3.9 | 3.3 | 0.171 | 0.236 | 0.039 |
| He-$O_2$-$N_2$ | $n = 16$ | $n = 16$ | $n = 16$ | $n = 16$ | $n = 16$ |
| | 21.6*† | 35.7*† | 0.763*† | 1.461*† | 0.513*† |
| | SD 3.5 | 4.9 | 0.775 | 0.026 | 0.058 |

Respiratory data recorded during the steady-state period. *: significantly different from He-$O_2$ value, $P \leq 0.01$ (using the Mann-Whitney U test). †: significantly different from He-$O_2$ value, $P < 0.001$ (using $t$ test, assuming data are drawn from normally distributed populations).

hyperexcitability decreases. We observed in the 1000-msw He-$O_2$ series that ventilatory rate increased soon after reaching maximal pressure and tended to decrease to a lower steady state as time elapsed. This occurrence could be explained in terms of HPNS-dependent hyperexcitability of the respiratory system. Nitrogen is believed to decrease, or even to abolish, clinical symptoms of HPNS (8–10). In our trimix series we did not observe time-dependent disturbances of the breathing pattern; actually, breathing frequency was lower in trimix environments than in He-$O_2$ ones. All of these arguments favor the hypothesis that HPNS includes a ventilatory component that can be diminished by addition of nitrogen to the breathing mixture.

## CONCLUSION

We suggest that HPNS includes a ventilatory component that induces hyperexcitability of the respiratory system, and leads to increased ventilatory rate in He-$O_2$ dives at high pressure; the result is increased work of breathing and increased alveolar stratification of gases. The use of trimix, by decreasing the ventilatory rate through the decreased frequency, diminishes the work of breathing and the alveolar dead-space. This diminution benefits the animal subjects as is evidenced by the lower ventilatory requirement observed in trimix at the beginning of a stay at maximal pressure.

## Acknowledgments

This work was sponsored by C.N.E.X.O. (Grant 81–2557). We thank P. Giry for helpful discussions and criticism of the manuscript.

## References


1. Dejours P, Garey WF, Rahn H. Comparison of ventilatory and circulatory flow rates between animals in various physiological conditions. Respir Physiol 1970;9:108–117.
2. Imbert G. Des effets sur les animaux de la respiration d'oxygènehélium à des pressions élevées. Thèse de Doctorat d'Etat. Marseille, 1981;161.
3. Imbert G, Jammes Y, Naraki N, Duflot JC, Grimaud C. Ventilation, pattern of breathing, and activity of respiratory muscles in awake cats during oxygen-helium simulated dives (1000 msw). In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:273–282.
4. Van Liew HD, Paganelli CV, Sponholtz DK. Estimation of gas-phase diffusivities in hyperbaric environments. Undersea Biomed Res 1982;9:175–181.
5. Scheid P, Piiper J. Intrapulmonary gas mixing and stratification. In: West JB, ed. Pulmonary gas exchange, vol. I: Ventilation, blood flow, and diffusion. New York: Academic Press, 1980:88–130.
6. Okubo T, Piiper J. Intrapulmonary gas mixing in excised dog lobes studied by simultaneous wash-out of two inert gases. Respir Physiol 1974;21:223–239.
7. Adaro F, Piiper J. Limiting role of stratification in alveolar exchange of oxygen. Respir Physiol 1976;26:195–206.
8. Bennett PB, Blenkarn GD, Roby J, Youngblood D. Suppression of the high pressure nervous syndrome in human deep dives by He-$N_2$-$O_2$. Undersea Biomed Res 1974;1:221–237.
9. Rostain JC, Gardette-Chauffour MC, Naquet R. HPNS during rapid compressions of men breathing He-$O_2$ and He-$N_2$-$O_2$ at 300 m and 180 m. Undersea Biomed Res 1980;7:77–94.
10. Rostain JC, Imbert JP, Gardette B, Lemaire C, Dumas JC, Naquet R. Compression methods: II. Study of the effects of profiles and $N_2$ injections on HPNS of the baboon Papio papio. Undersea Biomed Res 1978;5(Suppl):46.

# THE CORRECTION OF HYPOVENTILATION BY HIGH-FREQUENCY OSCILLATION (HFO)

*J. R. Clarke, D. Kerem, E. T. Flynn, and M. E. Bradley*

During the inhalation of a dense gas, pulmonary flow resistance increases and diffusivity decreases. Increased flow resistance at depth accounts for reductions in maximal minute ventilation and exercise ventilation. Low diffusivity hampers mixing of inspired gas with alveolar gas: the result is an apparent increase of dead space. The end result of dense gas breathing is often $CO_2$ retention and hypercapnea. Severe bouts of dyspnea seemingly unrelated to chemical changes (1,2) may result from respiratory muscle fatigue secondary to the increased work of breathing. Under such conditions some form of ventilatory assistance may be beneficial.

Classical methods of ventilatory assistance result in cardiovascular perturbations, encroach on the spontaneous breathing pattern, and, on the whole, are subject to limitations resulting from increased airway resistance. They are thus unsuitable for application to diving. However, a new method of ventilation, called *high-frequency oscillation* (HFO) (3–5), lacks the disadvantages of the older methods. High-frequency oscillation involves application of small pressure and flow oscillations to the trachea or chest wall (6) at frequencies above 5 Hz and at stroke volumes far below anatomical dead space. High-frequency oscillation is distinguished from the older technology of high-frequency positive pressure ventilation (HFPPV) (7) by its higher frequencies, smaller stroke volumes, and still somewhat mysterious mode of action.

As used experimentally and clinically, HFO is administered to anesthetized, usually paralyzed and intubated subjects as a total mode of ventilation, coupled with a substantial flow of fresh gas (bias flow) to the upper airways. It is believed that the oscillations serve to enhance diffusion and extend the alveolar space toward the mouth, where $O_2$ is supplied and $CO_2$ is removed by

the continuous flow of gas. In effect, the mixing action of oscillations may reduce dead space.

In one study on conscious, nonintubated humans, a substantial amount of $CO_2$ was removed by applying oscillations to the mouth of breath-holding subjects (8). It may thus be feasible to use HFO as a means of enhancing gas exchange or of reducing ventilatory requirements in conscious, spontaneously breathing, but hypoventilating subjects.

The applicability to divers seems particularly attractive, since unlike conventional ventilation methods that rely on convective transport of gas and are compromised during dense gas breathing (e.g., HFPPV), HFO is theoretically free of convective limitations. Furthermore, any reduction of dead space by an enhancement of gas mixing would at worst partly compensate for and at best reverse the opposing effect of the lower diffusivity. This study is the first examination of the effect of prolonged ventilation by HFO without a bias flow, but rather with the oscillations superimposed on negative-pressure-induced hypoventilation. The effects of gas density are also examined. The study presents the first requirements in adapting HFO to reduce hypoventilation during diving.

## METHODS

Mongrel male dogs weighing 6–10 kg were given the preanesthetic xylazine (2.2 mg/kg i.v.), followed by sodium pentobarbital (11 mg/kg i.v.). Following intubation and surgical placement of catheters in the femoral vein and pulmonary and femoral arteries, dogs were placed in a temperature-regulated box that served as a negative-pressure ventilator (hereafter referred to as the *Drinker* ventilator). The animals' endotracheal tubes were connected to a port in the box wall. While anesthetized, the animals were paralyzed with pancuronium bromide (0.2 mg/kg) and ventilated by the Drinker at a fixed rate with a tidal volume suitable for the desired degree of hypoventilation. Anesthesia was maintained by periodic bolus injections of sodium pentobarbital. Tidal volume was controlled in the following manner: a saw-toothed voltage signal of fixed periodicity was applied to a device controlling two solenoid-operated valves. One valve opened the box to a vacuum source, which resulted in inflation of the animal. The inspired gas was pulled from a Douglas bag containing either air, or a mix of 80% $SF_6$-20% $O_2$. At a predetermined point in the inflation, the vacuum valve was shut and the second valve opened, venting the Drinker to atmosphere and allowing passive deflation of the animal. The rate at which the box was evacuated (and thus the resulting tidal volume) could be varied by a valve in the vacuum line. Tidal volume (VT) and minute ventilation (MV) were obtained by integration of the flow signal from a Fleisch pneumotachograph, which was attached to the endotracheal tube and located outside the box.

The high-frequency ventilator (Bunnel Life Systems Puffer) is a computerized, pressure-regulated jet ventilator with a frequency range of 0.2–32

Hz. Gas for the Puffer was delivered by a 50-psi line containing either air or 80% $SF_6$-20% $O_2$. Oscillation frequency, airway pressure, and inspiratory and expiratory times could all be monitored independently and controlled by the ventilator. The Puffer was attached to the endotracheal port and was parallel to the Douglas bag (Fig. 1). The connection between the Drinker and the bag was sized to allow unimpeded ventilation with the Drinker, while serving as a high impedance to the high-frequency flows emanating from the Puffer. As a result, most, but not all, of the gas leaving the Puffer was delivered to the animal rather than to the atmosphere.

The following variables were monitored continuously: rectal temperature, arterial blood pressure (BP), pulmonary artery pressure (PAP), transcutaneous oxygen tension ($TCO_2$), low-pass filtered, transrespiratory pressure (TRP = box pressure − tracheal pressure) and end-tidal $CO_2$ concentration ($ETCO_2$). Arterial blood gases and pH, as well as cardiac output (by thermodilution), were measured at specific points in the course of the experiment; PAP and cardiac output were not measured in all animals. The animals were "sighed" to a TRP of 20 cm $H_2O$ every 5 min by manual override of the Drinker controls.

During an initial period of spontaneous breathing, baseline values for all variables were obtained. The animal's resting ventilation was then mimicked with the Drinker. Following a second sampling sequence, inspiratory flow was

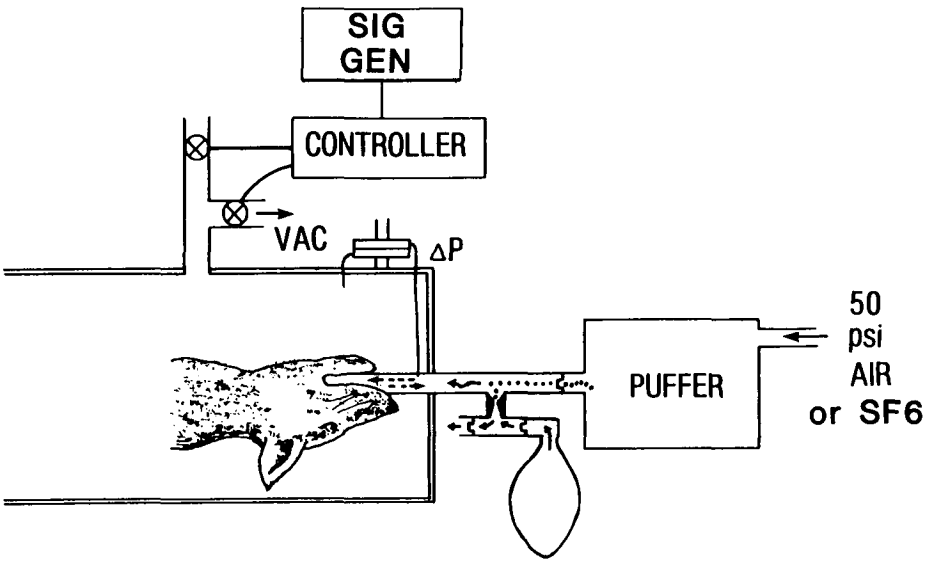



Fig. 1. Experimental setup. A controller driven by a signal generator was used to vary the pressure in a Drinker respirator. ΔP: transrespiratory pressure (tracheal pressure − box pressure). Inspired gas (either air or 80% $SF_6$-20%$O_2$) was drawn from a Douglas bag during ventilation by the Drinker alone, or from the Puffer and bag during combined operation.

decreased by varying amounts, while frequency remained constant; thus hypoventilation was induced. After stabilization of cardiovascular and respiratory parameters, a blood sample was taken and the Puffer was turned on at frequencies of 15–20 Hz. The positive end-expiratory pressure (PEEP) imposed by the Puffer (1–4 cm $H_2O$) caused a slight increase in functional residual capacity (FRC), as well as a reduction in the VT obtained with a given Drinker setting. Inspiratory flow was adjusted to keep pre- and post-Puffer tidal volumes constant by reference to the low-pass filtered TRP because the Fleisch was rendered useless during oscillation. After a new steady state was obtained, blood samples were drawn.

## RESULTS

Figure 2 shows the typical cardiopulmonary response of a hypoventilated dog to the Puffer. Transcutaneous $P_{O_2}$ rose and end-tidal $CO_2$ dropped. Pulmonary artery pulse pressure and mean PAP decreased (probably signifying a reversal of a hypoxic pulmonary vasoconstrictions), along with mean systemic arterial pressure, whereas cardiac output remained constant.

In each of 30 tests on 7 dogs, the combination of the Drinker and the Puffer resulted in better gas exchange than the use of the Drinker alone during hypoventilation (Fig. 3; Table I). $Pa_{O_2}$ rose an average of 21 Torr and $Pa_{CO_2}$ dropped an average of 8 Torr. The Puffer by itself, however, was grossly inadequate as a ventilator at the frequencies and pressures used here. For example, during 3 min of apnea in one animal, $Pa_{O_2}$ dropped from 87 to 14 Torr, and $Pa_{CO_2}$ rose from 37 to 58 Torr. Three minutes on the Puffer alone resulted in a change of $Pa_{O_2}$ from 85 to 31 Torr and a change in $Pa_{CO_2}$ from 39 to 59 Torr. The Puffer was slightly better than no ventilator at all.

As minute ventilation increased there was a reduction in the degree to which $O_2$ and $CO_2$ tensions could be altered by HFO. When relating an initial blood-gas value to the change in value produced by the combined use of the Puffer and the Drinker, the slopes of the regression were significantly different from zero ($P<0.05$; Fig. 4). The alveolar air equation predicts that when respiratory frequency is held constant while tidal volume varies, and $V_A = V_E - V_D$, the effect of reducing dead space becomes less as total ventilation increases.

There were multiple sites where gas was exhausted from the system; the result was high-impedance pathways that constituted leaks in low-frequency gas flows. Therefore, a portion of the gas from the Puffer was shunted to the atmosphere without reaching the dog's lungs. It was thus impossible in this system to collect the expired gas samples required for calculating dead space from the Bohr equation. The apparent reduction in dead space resulting from high-frequency oscillation, however, could be estimated, as described in the *Appendix*. Using this procedure, we found that the observed reduction in $Pa_{CO_2}$ (Table I) during use of the Puffer-Drinker combination on air could be explained by a reduction in dead space of 20% or more ($V_{D_2}/V_{D_1} = 0.80 \pm 0.02$ (mean ± SE); *see Appendix*).

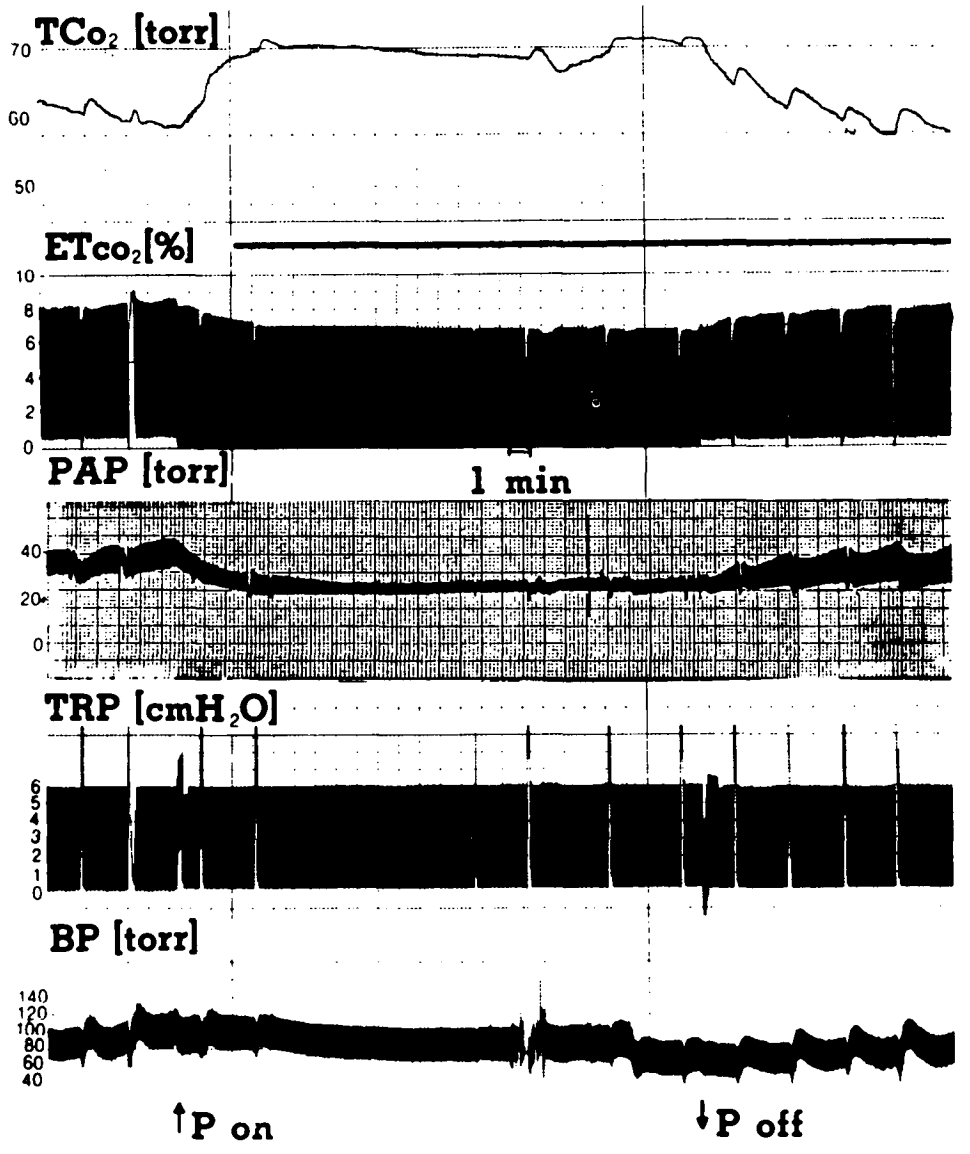



Fig. 2. A typical cardiopulmonary response of one dog to HFO following hypoventilation. $T_{CO_2}$: transcutaneous $P_{O_2}$; $ET_{CO_2}$%: end-tidal $CO_2$ concentration; PAP: pulmonary artery pressure; TRP: transrespiratory pressure; BP: systemic arterial pressure. The Puffer was turned on and off at the *arrows*. *Spikes* in the *TRP tracing* indicate the points at which the animal was sighed.

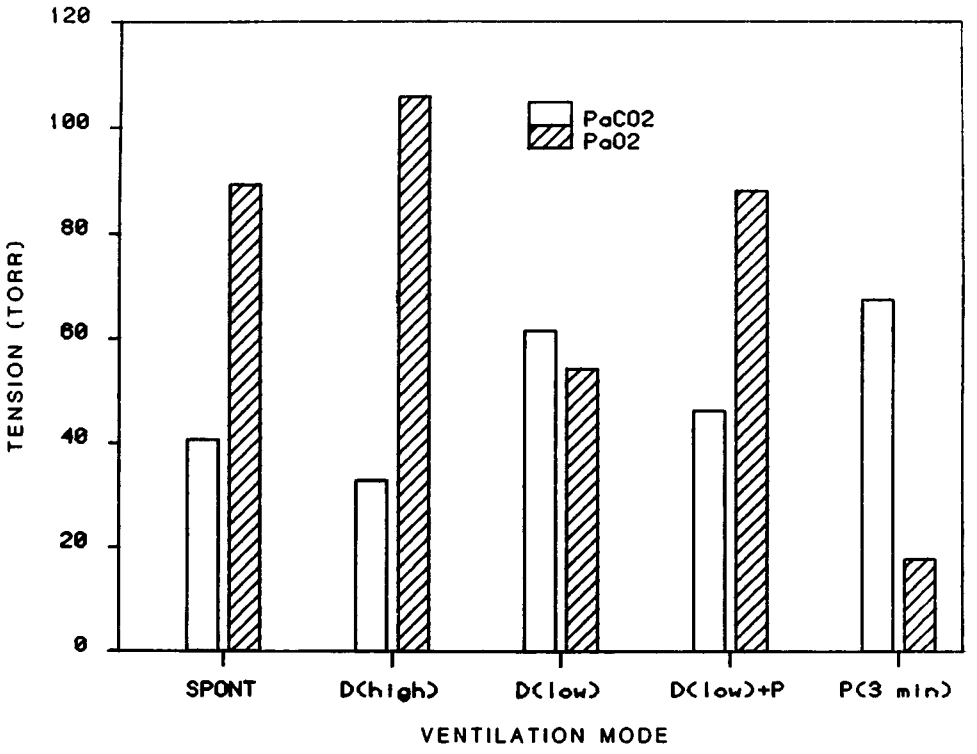



Fig. 3. Mean arterial gas tensions in one animal during various types of ventilation on air. SPONT: the animal's spontaneous breathing; D: Drinker respirator alone, at either *high* or *low* tidal volumes; P: Puffer. For both the low Drinker setting and after 3 min on the Puffer alone, $Pa_{CO_2}$ tensions were higher than $Pa_{O_2}$. Because of the poor performance of the Puffer as used here, the animal was allowed on the Puffer alone for only 3 min.

TABLE I

Blood Gas Tensions before and after Use of the Puffer during Hypoventilation

| | Tension$_1$* | Tension$_2$' | ΔTension | *T* | *P* |
|---|---|---|---|---|---|
| $Pa_{CO_2}$ | 57.19 ± 1.76 | 48.75 ± 1.57 | 8.43 ± 0.97 | 8.734 | <0.005 |
| $Pa_{O_2}$ | 61.94 ± 3.06 | 82.99 ± 2.74 | 21.05 ± 2.28 | 9.248 | <0.005 |

*Tension$_1$: blood gas tension during hypoventilation with the Drinker respirator. 'Tension$_2$: blood gas value following combined ventilation with the Drinker and Puffer. Mean gas tension ± 1 SE. *T* value is given for the paired *t*-test.

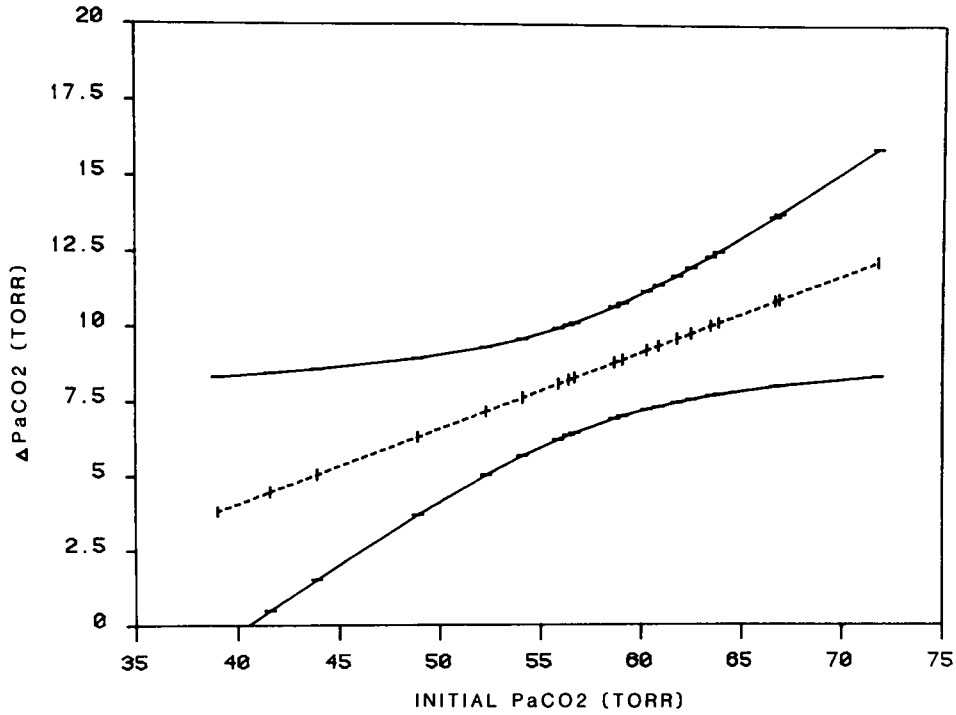



Fig. 4. The relationship between the initial level of $Pa_{CO_2}$ preceding application of the Puffer, and the resulting change in $Pa_{CO_2}$ with the Puffer and Drinker operating on air. The best linear fit to the data and 95% confidence limits for the regression are shown.

The ability of the high-frequency oscillator to improve gas exchange was unaffected by a mixture of $O_2$ and the dense gas $SF_6$ (80% $SF_6$-20% $O_2$) (Fig. 5). In one animal, $Pa_{CO_2}$ was reduced an average of 10.1 ± 1.4 Torr (mean ± SE) by using the Puffer, while the dead space ratio $V_{D_2}/V_{D_1}$ was only 0.73 ± 0.04 (mean ± SE).

## DISCUSSION

Gas exchange, impaired by hypoventilation, can be improved by the superposition of high-frequency oscillations on tidal breathing, at least in the experimental system described here. Furthermore, this improvement is achieved without reducing cardiac output.

Assumed values for the initial dead space ratio, $V_{D_1}/V_{E_1}$, had a large effect on the estimated $V_{D_2}/V_{D_1}$. For example, for one set of measurements with assumed dead space ratios of 0.5, 0.4, and 0.3, estimated $V_{D_2}/V_{D_1}$ was 0.65, 0.48, and 0.19, respectively. Simply stated, the smaller the initial dead

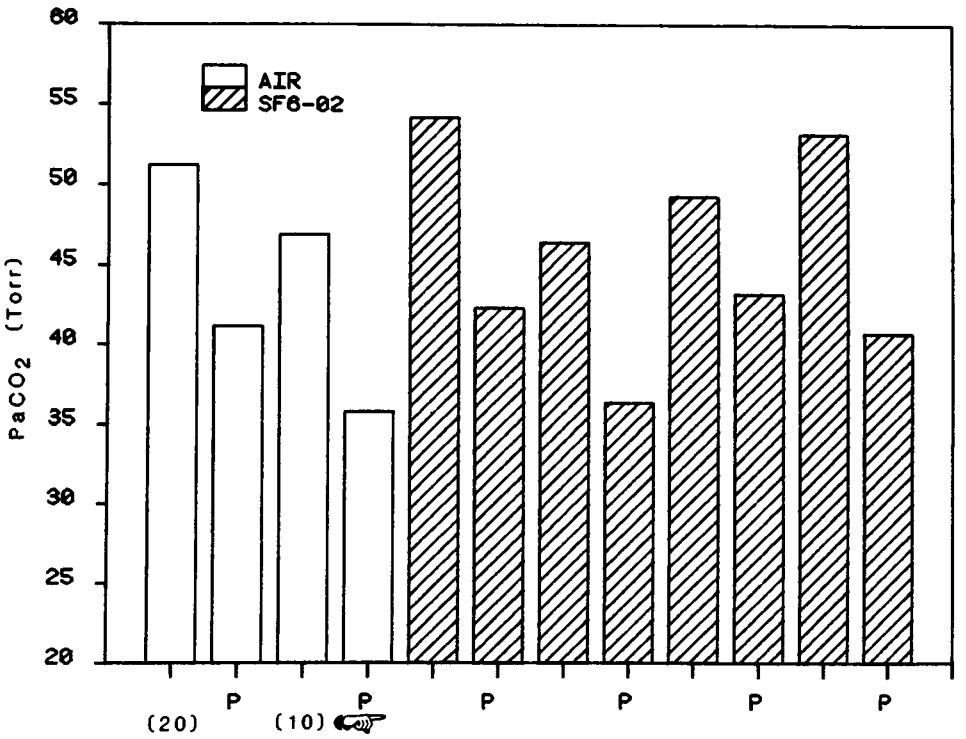



Fig. 5. Levels of arterial $P_{CO_2}$ during a sequence of ventilations with and without the Puffer in air and 80% $SF_6$-20% $O_2$. The *unlabeled bars* show the $Pa_{CO_2}$ levels resulting from hypoventilation. Where the Puffer was turned on (*P*), blood gases were drawn again. *Numbers in parentheses* represent the respiratory frequency in $min^{-1}$ of the Drinker respirator. After an initial frequency of 20 $min^{-1}$, the frequency was reduced to 10 $min^{-1}$ and remained there for the duration of the experiment.

space, the greater must be the reduction in dead space to account for a given fall in $Pa_{CO_2}$ during HFO. Values for $V_{D_2}/V_{D_1}$ given above are for an assumed $V_{D_1}/V_{E_1}$ of 0.5, a conservative estimate including both physiological and an estimated 25–30 mL equipment dead space.

This and other studies to date suggest that dense gases have little effect on the efficiency of HFO. Solway et al. (9) recently reported that in anesthetized, paralyzed dogs, the removal of $CO_2$ from lungs during HFO is independent of the density of gas in the lungs, be it air, $SF_6$-$O_2$, or He-$O_2$ mixtures. Since HFO does not augment axial convection (conduction down the length of the airways) (4), the high resistance to flow of a dense gas may be inconsequential. While the exact mechanism of HFO is still unknown, an emerging concept is that HFO simply helps mix alveolar and dead-space gas (9). In mechanical, two-compartment models (10), large vortices were formed during HFO, causing rapid mixing of gases between two compartments. We might speculate that once an angular velocity has been imparted to a small volume of

gas, the dissipation of that angular momentum by friction with adjacent gases of differing velocity or by collisions with neighboring heavy molecules may be offset by the inertia of the rotating gas. We might predict that the only effect of increasing gas density may be to require larger energy expenditures to initiate mixing.

It should be noted that the $SF_6$-$O_2$ mixture at 1 ATA has a binary diffusivity for $O_2$ and $CO_2$ that is 0.55 that of air, far removed from the 0.048 value experienced in the deepest chamber dives (11). Thus, the degree to which the adverse effects of the lowered diffusivity may be alleviated by HFO remains unsettled.

It is encouraging that a substantial improvement in gas exchange can be demonstrated without a steady flow of fresh gas down the trachea because a modification of the technique employed here presumedly could be used to aid a free-breathing subject without impeding his normal ventilation.

Although this is only a first step toward solving the problem of ventilatory limitation in exercising divers, we believe that the above findings constitute sufficient grounds for relevant human experimentation and for hyperbaric studies.

## Appendix

We wish to express the effect of HFO as a reduction of physiological dead space.

The Bohr equation, which is usually used for this purpose, however, requires the collection of expired gas. Because that was not possible in this instance, we derived a means of estimating changes in ventilation of dead space by measuring $Pa_{CO_2}$ before and after application of HFO. The derivation is based on the alveolar gas equation with zero $CO_2$ in the inspired gas.

$$P_{A_{CO_{2(n)}}} = (P_b - 47) \cdot \frac{\dot{V}CO_{2(n)}}{\dot{V}A_{(n)}} \qquad (1)$$

Substituting $\dot{V}E - \dot{V}D$ for $\dot{V}A$:

$$P_{A_{CO_{2(1)}}} = \frac{K \cdot \dot{V}CO_{2(1)}}{\dot{V}E_{(1)} - \dot{V}D_{(1)}} \quad ; \quad P_{A_{CO_{2(2)}}} = \frac{K \cdot \dot{V}CO_{2(2)}}{\dot{V}E_{(2)} - \dot{V}D_{(2)}}$$

After rearranging:

$$\dot{V}CO_{2(1)} = \frac{P_{A_{CO_{2(1)}}}}{K} \cdot \left(\dot{V}E_{(1)} - \dot{V}D_{(1)}\right)$$

If we assume $\dot{V}CO_{2(1)} = \dot{V}CO_{2(2)}$ and $P_{A_{CO_2}} = Pa_{CO_2}$:

$$Pa_{CO_{2(1)}} \cdot \left(\dot{V}E_{(1)} - \dot{V}D_{(1)}\right) = Pa_{CO_{2(2)}} \cdot \left(\dot{V}E_{(2)} - \dot{V}D_{(2)}\right) \qquad (2)$$

We further assume that $\dot{V}E$ is controlled:

$\dot{V}E_{(1)} = \dot{V}E_{(2)} = \dot{V}E$ , which yields after rearrangement:

$$\dot{V}D_{(2)} = -\left(\frac{\dot{V}E \cdot Pa_{CO_{2(1)}}}{Pa_{CO_{2(2)}}}\right) + \left(\frac{\dot{V}D_{(1)} \cdot Pa_{CO_{2(1)}}}{Pa_{CO_{2(2)}}}\right) + \dot{V}E$$

$$\text{therefore } \frac{\dot{V}D_{(2)}}{\dot{V}D_{(1)}} = -\left(\frac{\dot{V}E \cdot P_1}{\dot{V}D_{(1)} \cdot P_2}\right) + \left(\frac{P_1}{P_2}\right) + \left(\frac{\dot{V}E}{\dot{V}D_{(1)}}\right) \quad \text{where } P_1 = Pa_{CO_{2(1)}} \text{ and } P_2 = Pa_{CO_{2(2)}}$$

$$\text{or} \quad \frac{\dot{V}D_{(2)}}{\dot{V}D_{(1)}} = \left[\left(\frac{\dot{V}E}{\dot{V}D_{(1)}}\right) \cdot \left(1 - \frac{P_1}{P_2}\right)\right] + \frac{P_1}{P_2}$$

## Acknowledgments

D. Kerem was affiliated with the Naval Medical Research Institute, Bethesda, MD during the time this research was accomplished.

Our thanks go to Kevin Conrad and Kathy Byrne for their excellent technical assistance. Mrs. Maureen Darmody, Mrs. Ellen Hughes, and Ms. Janet Gaines graciously provided editorial services.

This work was supported by NMRDC Task No. M0099PN.01B.0009. The opinions and assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting views of the Navy Department or the Naval Service at large.

The experiments supported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animals Resources, National Research Council, DHEW, Pub. No. (NIH) 78–23.

## References

1. Dwyer J, Saltzman HA, O'Bryan R. Maximal physical-work capacity of man at 43.4 ATA. Undersea Biomed Res 1977;4:359–372.
2. Spaur WH, Raymond LW, Knott MM, Crothers JC, Braithwaite WR, Thalmann ED, Uddin DF. Dyspnea in divers at 49.5 ATA: mechanical, not chemical in origin. Undersea Biomed Res 1977;4:183–198.
3. Slutsky AS, Drazen JM, Ingram RH Jr, Kamm RD, Shapiro AH, Fredberg JJ, Loring S, Lehr J. Effective pulmonary ventilation with small-volume oscillations at high frequency. Science 1980;209:609–611.
4. Bohn DJ, Miyasaka K, Marchak BE, Thompson WK, Froese AB, Bryan AC. Ventilation by high-frequency oscillation. J Appl Physiol:Respir Environ Exercise Physiol 1980;48:710–716.
5. Clarke JR. Gas exchange during high frequency ventilation. Ann Biomed Eng (in press).
6. Bitterman H, Kerem DH, Shabtai Y, Gavriely N, Palti Y. Respiration maintained by externally applied vibration and tracheal insufflation in the cat. Anesth Analg 1983;62:33–38.
7. Oberg PA, Sjostrand U. Studies of blood pressure regulation III. Dynamics of arterial blood pressure on carotid-sinus nerve stimulation. Acta Physiol Scand 1971;81:96–109.
8. Goldstein D, Slutsky AS, Ingram RH Jr, Westerman P, Venegas J, Drazen J. $CO_2$ elimination by high frequency ventilation (4 to 10 Hz) in normal subjects. Am Rev Respir Dis 1981;123:251–255.

9. Solway J, Gavriely N, Slutsky AS, Drazen JM. Effect of resident gas composition on $CO_2$ output during high frequency ventilation. Fed Proc 1983;42:1350.
10. Clarke JR, Homer LD, Flynn ET. Efficiency of high frequency ventilation as determined by nitrogen washouts: a model study. Bethesda: Naval Medical Research Institute, 1983:1–19. (NMRI Tech report 83–90)
11. Van Liew HD, Paganelli CV, Sponholtz DK. Estimation of gas-phase diffusivities in hyperbaric environments. Undersea Biomed Res 1982;9:175–181.

# TRAINING TO BREATHE AGAINST ADDED EXTERNAL RESISTANCES AT ATMOSPHERIC PRESSURE

*P. B. L. Giry, R. Hyacinthe, A. Battesti, and B. Broussolle*

To insure their ventilation divers must breathe gas in pressure in equilibrium with the surrounding medium. Breathing gas density will therefore linearly increase with pressure (expressed as absolute atmospheres: ATA). To move this gas, the breathing effort of the diver should overcome two types of resistance: resistance from gas flow through intrapulmonary airways (internal resistance); and resistance from underwater breathing apparatus (external resistance).

Because of increased gas density and for the same pressure gradient, gas flow will decrease approximately as the square root of gas density (1–4). Consequently, breathing resistances will vary as the square root of absolute pressure.

The driving force for gas flow is the alveolo-buccal pressure gradient developed by the respiratory muscles and the elastic recoil of the lungs. The limit of the first has been estimated at 200 cm $H_2O$ (5). It is therefore logical to consider the possibility of a limit to ventilation at pressure due to increased gas density (4).

To improve a diver's capability against increased resistances, one must try to improve the performance of respiratory muscles by increasing either their force (the maximal pressure gradient they can develop) or their endurance (the duration of maximal breathing effort), or both. Demedts and Anthonisen (6) and Leith and Bradley (7) have already done this, using specific ventilatory training.

To train any musculature, one should impose a sustained but tolerable effort. In our situation, this can be realized through increased breathing resistance or breathing demand for respiration, or both.

It has been demonstrated (8) that adding diaphragms (less than 10 mm in diameter) will lead to limitation to ventilation by external breathing resistance. This method is simple enough and can simulate increased external resistance from an Underwater Breathing Apparatus.

However, external resistance alone is unlikely to increase ventilatory performance for a resting subject. Ventilatory demand to the respiratory musculature should also be elevated to increase work of breathing. In their experiment, Leith and Bradley (7) asked their subjects to perform long-duration maximum voluntary ventilation (MVV). Such a situation seems remote from that which the divers will encounter. Furthermore, their research effort was to show the possibility of specific ventilatory training. Ours was to improve ventilatory efficiency.

Another possibility is for subjects to perform physical exercise, which will increase the ventilatory demand by both neurologic and metabolic processes. This is the method we chose because of its simplicity, its realism, its possibility for increasing general fitness of subjects, and its relative independence of subject willingness.

During preliminary testing of the method, it was observed that external limitation to ventilation may lead to hypoxia. To limit such hypoxia, our subjects performed exercise under slightly hyperoxic conditions, which also simulate the slightly hyperoxic environment usually realized in pressure chambers.

## MATERIAL AND METHODS

### Subjects

Seven divers volunteered for the study. Their characteristics are given in Table I. They were young (age range 29–35 years), with fair physical fitness. Six were professional divers, four French Navy and two civilians.

For the Navy divers, physical fitness is regularly checked, and general physical training was already performed before starting ventilatory training (1 hour/day cross-country running).

Clinical examinations and lung function tests performed pretraining were within the normal limits.

Mechanical workloads leading to cardiac frequency to 75% of maximal cardiac frequency were determined to be 150 watts (W) for all Navy subjects, 120 W for one subject, and 100 W for the two remaining subjects.

### Tests Performed

Three series of tests were performed throughout the study: Lung function tests; breathing capability tests (endurance and performance); and tolerance to exercise without (supramaximal exercise) or with (submaximal exercise) added external breathing resistance.

TABLE I

Characteristics of Subjects Submitted to Ventilatory Training

| Subject | | *S1* | *S2* | *S3* | *S4* | *S5* | *S6* | *S7* | *mean* | *SD* |
|---|---|---|---|---|---|---|---|---|---|---|
| Height | (cm) | 178 | 182 | 183 | 171 | 174 | 159 | 176 | 175 | 8 |
| Weight | (kg) | 78 | 84 | 87 | 68 | 74 | 50 | 64 | 72 | 13 |
| Age | (years) | 38 | 35 | 31 | 31 | 39 | 29 | 30 | 33 | |
| Body area | (m) | 1.95 | 2.06 | 2.08 | 1.80 | 1.88 | 1.49 | 1.79 | 1.86 | .20 |
| Workload* (pre-training) | (W) | 150 | 150 | 150 | 150 | 120 | 100 | 100 | 131 | 24 |
| Workload* (post-training) | (W) | 150 | 150 | 150 | 150 | 140 | 100 | 100 | 134 | 24 |
| Pretraining† Fitness Level | | ‡ | § | § | ‖ | ? | § | ? | | |

*: workload (in watts, W) allowing the cardiac frequency to reach 75% of $Fc_{max}$ during exercise with external limitation to ventilation; †: known training level before starting of the experiment; ‡: good; §: fair (average military diver training); ‖: excellent (amateur competition); ?: unknown.

*Respiratory function tests.* We measured: Vital capacity: CV; forced expiratory volume in 1 s: $FEV_1$; lung diffusion capacity for carbon monoxide steady-state method): DLCO; maximum voluntary ventilation without added external resistances, on 15 s extrapolated to 1 min MVV 15-s flow/volume curves.

*Breathing capability.* These tests were specially designed for the experiment, but derived from classical ones:

Maximum voluntary ventilation was measured on 2 min without added breathing resistance: MVV 2 min expressed in $L \cdot min^{-1}$. Measuring was done while subjects were rebreathing in an air-filled dry spirometer with accumulation of metabolic $Co_2$. None of the subjects complained of problems that could be related to hypoxia or hypercarbia. On untrained subjects, MVV 2 min represented 75% of MVV 15 s, a finding that shows it explores breathing muscle sensitivity to fatigue (respiratory muscle endurance).

Maximum voluntary ventilation (MVV 15 s) was performed with added external resistance (diaphragm of 5-mm diameter): MVV 5 mm, to explore respiratory muscle sensitivity to increased external resistance.

*Exercise tolerance tests.* Two type of tests were performed. The first was without added breathing resistances, ventilation during supramaximal exercise: $VE_{max}$. The second was with added external breathing resistances.

*$VE_{max}$ (9).* This test was performed on an electrically braked bicycle ergometer. After 2-min rest and 5-min warm-up (100 W) periods, workload was increased by 12 $W \cdot min^{-1}$ until exhaustion. We recorded maximal ventilation measured during the test (measured with a pneumotachograph): $VE_{max}$; and

maximal cardiac frequency achieved during the test measured on control (ECG): $Fc_{max}$.

*Tolerance to exercise with added respiratory resistances.* This test always was performed 1 day after $VE_{max}$. The subject was connected to the same testing rig used for training (described in Fig. 1). On both inspiratory and expiratory sides of the breathing apparatus were placed the external resistances (diaphragms of 6.5-mm diameter corresponding to 21 cm $H_2O \cdot L^{-1} \cdot s$ measured on a sinusoidal breathing pump with $VT = 2$ L and $f = 20 \cdot min^{-1}$. On the inspiratory side was added extra oxygen flow (3 $L \cdot min^{-1}$) diluted to VE with room air. On the expiratory side (downflow of the diaphragm) was inserted a pneumotachograph for ventilation measurements. On the mouthpiece were two ports: one for mouth pressure measurements (dP); the other for gas analysis (continuous sample of 0.5 $L \cdot min^{-1}$; ventilation measurements were corrected for this leak). Oxygen was analyzed breath-by-breath with a Beckman OM11 polarographic analyzer, $CO_2$ (end tidal), with a Beckman LB3 infrared analyzer. The ECG was continuously recorded through standard derivations.

After a 2-min warm-up period (50 W), the workload was increased in square step change to the one determined for the subject's cardiac frequency to reach 75% of $Fc_{max}$ (determined during the $VE_{max}$ test). The test was continued for 15 min or until exhaustion, whichever occurred first.

All parameters were continuously recorded. Statistical analyses were made of these measures taken during the last effective minute of exercise: duration of the test (when less than 15 min); mean VE; mean $PET_{CO_2}$; mean

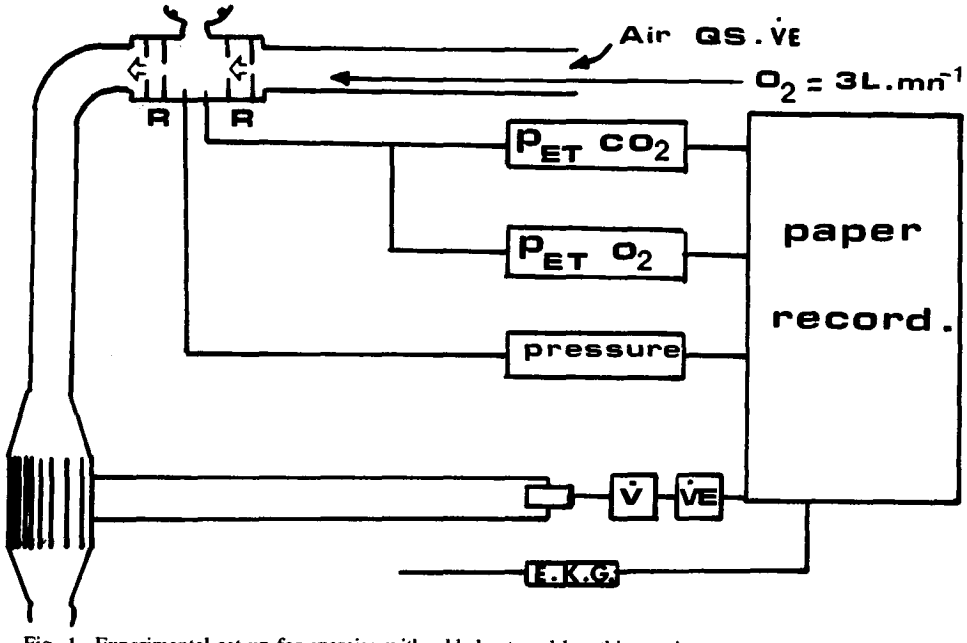



Fig. 1. Experimental set-up for exercise with added external breathing resistances.

peak inspiratory and expiratory pressures: $dP_{Insp}$ and $dP_{exp}$, the sum of which represents the maximum pressure swing, $dP_{max}$.

During these tests, subjects were not informed of their performance ($PET_{CO_2}$, VE, $dP_{max}$).

## Training Protocol

Training was composed of exercise bouts, identical to the ones described under *Tolerance to exercise...*; only the diameters of the diaphragms varied:

*Week 1:* 7.5 mm (resistance: 14 cm $H_2O \cdot L^{-1} \cdot s$);
*Week 2:* 7.0 mm (resistance: 18 cm $H_2O \cdot L^{-1} \cdot s$);
*Week 3:* 6.5 mm (resistance: 21 cm $H_2O \cdot L^{-1} \cdot s$);
*Week 4:* 6.0 mm (resistance: 28 cm $H_2O \cdot L^{-1} \cdot s$).

Resistances were measured as the peak resistance with sinusoidal 1-ATA air flow of 40 $L \cdot min^{-1}$ at a breathing frequency of $20 \cdot min^{-1}$.

Specific training was done 4 days per week, with 2 exercise bouts per day (within 2 h, separated by 30 min rest). The fifth day was devoted to general exercise (swimming). During the training period the subjects were continuously informed of their performance (real time information: $PET_{CO_2}$, VE, $dP_{insp}$, $dP_{exp}$), and requested to maintain $PET_{CO_2}$ below 45 Torr.

# RESULTS

Results are described in Tables II and III. They are expressed as mean ± standard deviation. Statistical analyses were performed using Student's paired *t* test and were considered significant if $P < 0.05$.

## Standard Pulmonary Function Tests

No significant variation was observed in VC, $FEV_1$, $DL_{CO}$, flow-volume curve, MVV 15 s (Table II). For this last, frequency was increased by 17%, but this increase was compensated for by a decrease in tidal volume, VT; the product VT · frequency remained nonsignificantly changed.

## Breathing Capability (Table III)

*MVV 5 mm.* This test went from 51.8 ± 7.0 $L \cdot min^{-1}$ pretraining to 57.2 ± 4.8 $L \cdot min^{-1}$ post-training. This represents an increase of 13% ($P < 0.05$). There was no systematic trend in the breathing pattern: some subjects increased frequency but not tidal volume (*S2, S3*); others increased tidal volume, the respiratory frequency remained unchanged.

*MVV 2 min.* This test explored the sensitivity to fatigue of the subject's respiratory muscles. MVV 2 min went from 159 ± 24 $L \cdot min^{-1}$ pretraining to

TABLE II

Standard Pulmonary Function Tests

| Subject | | *S1* | *S2* | *S3* | *S4* | *S5* | *S6* | *S7* | *P* |
|---|---|---|---|---|---|---|---|---|---|
| VC (L) | pre | 6.3 | 6.3 | 6.2 | 5.8 | 5.7 | 4.1 | 4.0 | NS |
| | post | 6.5 | 6.2 | 6.1 | 5.0 | 5.6 | 4.2 | 4.4 | |
| $FEV_1$ (L) | pre | 4.6 | 4.7 | 5.0 | 3.7 | 5.0 | 3.4 | 3.7 | NS |
| | post | 5.0 | 4.5 | 5.1 | 3.1 | 5.0 | 3.6 | 3.4 | |
| $DL_{CO}$ | pre | 33.4 | 28.9 | 29.1 | 29.9 | 25.8 | 19.4 | 18.6 | NS |
| | post | 37.4 | 30.8 | 29.0 | 25.0 | 29.0 | 18.6 | 17.4 | |
| Peak flow | pre | 11.8 | 12.3 | 11.8 | 10.7 | 12.5 | 8.8 | 12.3 | NS |
| | post | 12.7 | 12.6 | 11.3 | 6.8 | 12.4 | | 12.7 | |
| V 50% of CV | pre | 5.5 | 5.7 | 5.6 | 3.8 | 7.4 | 4.6 | 6.1 | NS |
| | post | 6.5 | 5.0 | 6.0 | 3.4 | 7.0 | | 5.1 | |
| V 25% of CV | pre | 3.9 | 2.1 | 2.6 | 1.6 | 3.6 | 1.9 | 2.5 | NS |
| | post | 3.0 | 1.8 | 2.9 | 1.8 | 4.7 | | 1.4 | |
| MVV 15 s | pre | 213 | 209 | 186 | 175 | 217 | 156 | 171 | NS |
| | post | 226 | 210 | 242 | 161 | 219 | 162 | 194 | |

pre: pretraining measurement; post: post-training measurement; volumes were measured in litres (BTPS); flows were measured in $L \cdot s^{-1}$ (BTPS).

$175 \pm 33$ $L \cdot min^{-1}$ post-training. This represents an increase of 10% ($P < 0.05$). Most of the gain was obtained at *Week 3*.

*Tolerance to exercise: $VE_{max}$.* For MVV 2 min, this test was performed pretraining (at *Week 3*) and post-training (at end of *Week 4*). Results are summarized in Table III and Fig. 2. No significant difference in $Fc_{max}$ was observed, a finding that shows subjects indeed went on maximal capability.

Increase in $VE_{max}$ was progressive; values at *Week 3* were intermediary between pre- and post-training ones. The total increase in $VE_{max}$ 20% ($P < 0.001$), starting from $114 \pm 13$ $L \cdot min^{-1}$ pretraining (range: 103–133) to reach $136 \pm 13$ $L \cdot min^{-1}$ post-training (range: 110–152).

*Tolerance to exercise: Exercise with external limitation to ventilation.* Results are given in Table III and in Fig. 3. During pretraining, out of 7 subjects, 3 were unable to run 15 min of exercise with external limitation to ventilation. Post-training, all of the subjects did complete the full extent test (15 min). The measured parameters show an increase in performance between pretraining and post-training tests.

Between pretraining and *Week 3*, most ventilatory parameters reached maximal values:

VE increased by 15% ($P < 0.025$) from $43 \pm 7$ $L \cdot min^{-1}$ to $49 \pm 5$ $L \cdot min^{-1}$; mean peak inspiratory pressure ($dP_{insp}$) increased by 87% ($P < 0.001$); mean peak expiratory pressure ($dP_{exp}$) increased by 161% ($P < 0.01$). No modification in respiratory frequency was observed. Therefore, VT was in-

TABLE III

Results of Long-Duration Tests: $W_{max}$, MVV 2 min, and Exercise 6 mm

| Subject | *S1* | | | *S2* | | | *S3* | | | *S4* | | | *S5* | | | *S6* | | | *S7* | | | % | % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Week | 0 | 3 | 4 | 0 | 3 | 4 | 0 | 3 | 4 | 0 | 3 | 4 | 0 | 3 | 4 | 0 | 3 | 4 | 0 | 3 | 4 | 0–3 | 0–4 |
| $W_{max}$ (W) | 295 | 310 | 364 | 300 | 350 | 371 | 291 | 308 | 330 | 335 | 350 | 350 | 262 | 290 | 315 | 182 | 202 | 205 | 168 | 220 | 235 | 12 ± 4* | 20 ± 5* |
| $Fc_{max}$ ($min^{-1}$) | 182 | 178 | 176 | 204 | 200 | 195 | 195 | 191 | 190 | 192 | 185 | 200 | 190 | 185 | 188 | 200 | 190 | 196 | 190 | 190 | 192 | −2 ± 1 | −1 ± 1 |
| $\dot{V}E_{max}$ (L · $min^{-1}$) | 119 | 121 | 141 | 113 | 141 | 144 | 123 | 135 | 152 | 133 | 145 | 134 | 118 | 108 | 135 | 93 | 109 | 110 | 103 | 125 | 139 | +11 ± 5* | +20 ± 4* |
| MVV 2 min (L · $min^{-1}$) | 188 | 203 | 173 | 191 | 213 | 232 | 159 | 171 | 190 | 144 | 155 | 139 | 164 | 167 | 190 | 136 | 126 | 134 | 131 | 153 | 169 | + 7 ± 3* | +10 ± 6* |
| Exercise with Limitation (Results are mean of the last minute values) | | | | | | | | | | | | | | | | | | | | | | | |
| Duration (min) | 15 | | | 15 | | | 15 | | | 15 | | | 15 | 10 | | 13 | 15 | | 7 | 15 | | | |
| $\dot{V}E$ (L · $min^{-1}$) | 48 | 55 | 51 | 49 | 41 | 50 | 48 | 52 | 46 | 43 | 54 | 52 | 32 | 45 | 46 | 34 | 40 | 34 | 50 | 47 | 47 | +15 ± 6* | +9 ± 7* |
| $PET_{CO_2}$ (Torr) | 57 | 47 | 42 | 52 | 43 | 40 | 52 | 48 | 45 | 59 | 46 | 40 | 56 | 48 | 43 | 49 | 41 | 42 | 46 | 41 | 39 | −15 ± 2* | −21 ± 3* |
| $\Delta P_{insp}$ (cm $H_2O$) | 30 | 59 | 67 | 42 | 52 | 66 | 55 | 77 | 45 | 27 | 70 | 100 | 37 | 55 | 52 | 22 | 42 | 37 | 32 | 62 | 63 | +79 ± 19* | +91 ± 37 |
| $\Delta P_{exp}$ (cm $H_2O$) | 32 | 115 | 90 | 30 | 53 | 66 | 57 | 101 | 63 | 25 | 73 | 75 | 20 | 67 | 87 | 32 | 88 | 110 | 42 | 89 | 68 | +161 ± 30* | +165 ± 45* |
| $\Delta P_{insp} + \Delta_{exp}$ (cm $H_2O$) | 62 | 174 | 157 | 72 | 105 | 132 | 112 | 178 | 100 | 52 | 143 | 175 | 57 | 122 | 139 | 54 | 130 | 147 | 74 | 151 | 131 | +117 ± 21* | +119 ± 37* |
| $f$ ($min^{-1}$) | 17 | 15 | 24 | 22 | 36 | 23 | 19 | 26 | 23 | 23 | 19 | 26 | 14 | 11 | 16 | 21 | 18 | 16 | 27 | 24 | 25 | +3 ± 13 | +3 ± 18 |

*Statistically signifcant ($P < 0.05$) O: Pretraining; 3: On *Week 3;* 4: Post-training.

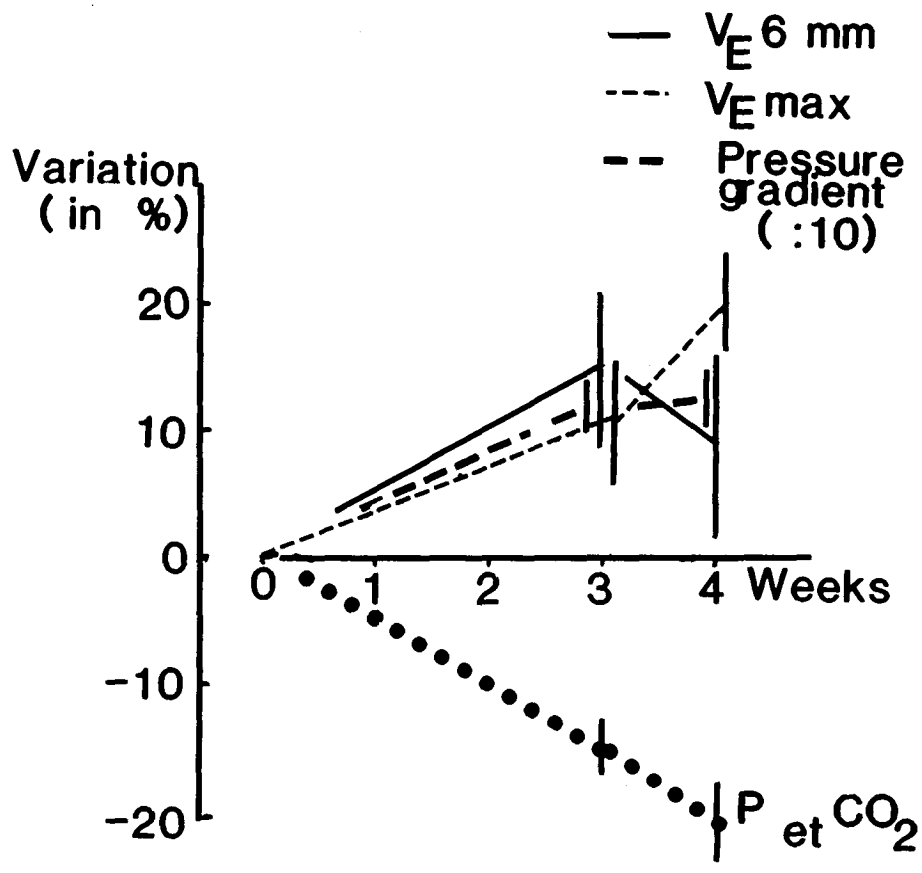



Fig. 2. Results of the exercise tests expressed in percentage of control values ($VE_{max}$ and exercise with 6-mm diaphragms). *Continuous line:* VE during the last minute; *dotted line:* $PET_{CO_2}$; *broken line:* pressure gradients; *dashed line:* $VE_{max}$.

creased because of increased pressure peaks. $PET_{CO_2}$ decreased by 15% ($P < 0.01$), from 53 ± 2 Torr to 45 ± 1 Torr.

Between *Weeks 3* and *4*, ventilatory parameters were on a plateau. However, $PET_{CO_2}$ continued decreasing, reaching 42 ± 1 Torr (−21 ± 3%), evidence of more efficient alveolar ventilation. These results lead one to the conclusion that 4 weeks of training allows better respiratory performance, shown mainly in the tests, which need high respiratory work or tolerance to fatigue.

## DISCUSSION

These results agree with those of Leith and Bradley (7). However, the increase in ventilation in our study is slightly lower than in theirs (10% in our

experiment on MVV 15 s instead of 20% in theirs). Two main differences must be noted:

1) Their training lasted 5 weeks—ours lasted 4 weeks (but it is unlikely that one extra week would give 10% extra performance).

2) They trained their subjects exclusively with MVV maneuvers, allowing for practice effects. In our study, the number of MVV's was too small (9 runs/subject over the whole training period) to have such an effect. Furthermore, if such a practice effect were to occur, it should have been seen at MVV 15 s in our experiment, but it was not.

It is known that general physical training increases ventilatory performance (10). Most of the experiments made on this point study the effects of training on sedentary (or untrained) subjects. In such conditions, training also induces increase in $VO_{2_{max}}$, $VE_{max}$, and modifications in $DL_{CO}$ (11). In our work, $DL_{CO}$ did not vary nor did the workload increase cardiac frequency to 75% of $Fc_{max}$ for 6 of our 7 subjects; it increased only 20 W in the 7th subject. General fitness was not drastically improved by our training program. Furthermore, four out of seven subjects had already achieved, before our ventilatory training period, a fair fitness level (1 hour/day general training). It is unlikely that, at least on these four Navy divers, general training had a noticeable effect.

We observed an increase in mouth peak-pressures (almost double) during the exercise with external limitation to ventilation. Such modifications need increased force for respiratory muscles, mainly due to intensive use of accessory ventilatory ones. There is therefore an increase in the strength of these muscles, because they are able to develop greater pressure gradients. Increased transpulmonary pressures will lead to increased gas flow, as shown by the augmentation in MVV 5 mm. How does it happen that such increase in muscle strength is not seen during other short-duration tests such as V′/V loops or MVV 15 s?

To explain such a phenomenon, we return to the regulation of muscular contraction. When a muscle contracts, stimulation of tendon and neuromuscular fiber receptors occurs, giving the central nervous system feedback information on the strength (pressure) and displacement generated. Articulated movement also informs about bone (i.e. in our case, costal) displacement, and therefore on the efficiency of the contraction. At the pulmonary level, intrapulmonary stretch receptors are also involved. The immediate information is thoracic cage position and rate of displacement (dV/dt; dP/dt).

During ventilatory tests with increased resistances (exercise with external limitation to ventilation or MVV 5-mm runs), information from receptors is abundant, and the reflexes fully play their role. Therefore, it is under such conditions that maximal dP's can be obtained. Without any resistance and mostly in subjects trained to fight against resistances, there is less feedback information from thoracic receptors, and ventilatory contraction may be less efficient. In such unlimited conditions, the limit to respiratory muscle performance is the speed of contraction (twitch contraction). It is possible that because of these phenomena tests performed on single breath (V′/V curve) or

short duration without any added external respiratory resistance (MVV 15 s) do not show any variation with training, because of lack of feedback information from lung and thoracic structures.

This experiment also reports increased performance (VE, dP, $PET_{CO_2}$) when long-duration high workloads are imposed on the ventilatory musculature, as during MVV 2 min and exercise with external limitation to ventilation. Figure 3 compares MVV 2 min and MVV 15 s pre- and post-training. Pretraining, the points are far from the identity line (the line for which MVV 2 min equals MVV 15 s). During MVV 2 min, respiratory muscle fatigue occurs before full completion of the test and ventilation decreases, leading to MVV 2 min being smaller than MVV 15 s. Post-training, MVV 2 min is closer to MVV 15 s (points are systematically closer to identity line). Such an effect is not because of increased strength, because MVV 15 s (corresponding to the ventilation during the first 15 s of the test) remains unchanged between pre- and post-training runs. Therefore, it must be concluded that the decrease normally occurring during MVV 2 min has been lessened by training. In other words, the maximum ventilatory level has been sustained longer, probably by an increase in the endurance of ventilatory muscles.

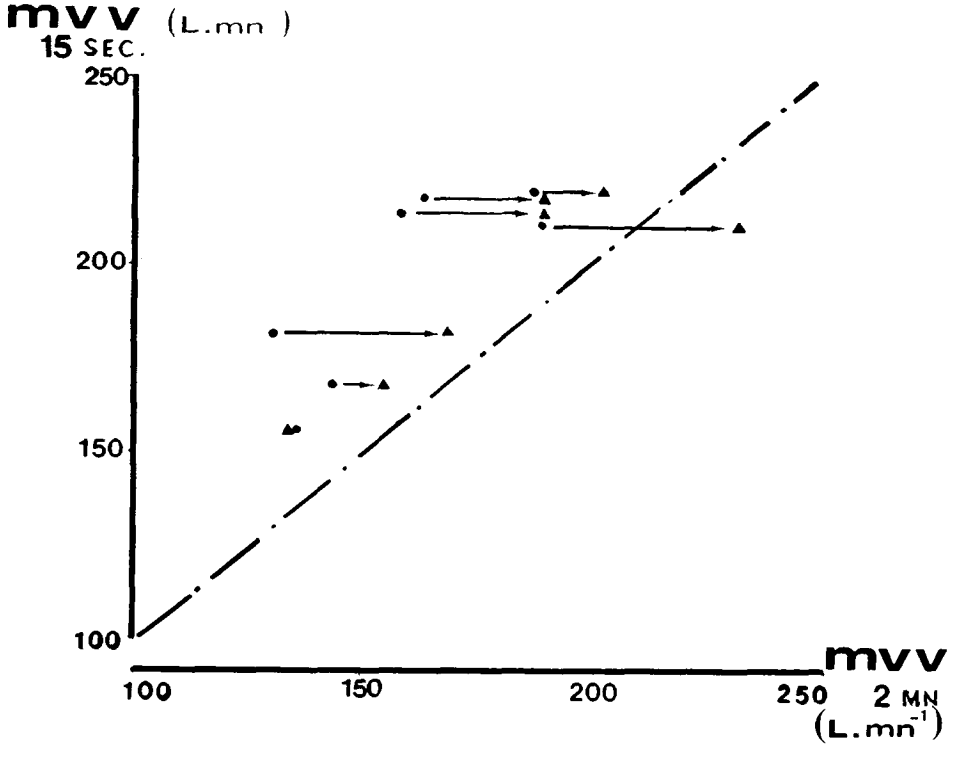



Fig. 3. MVV 2 min (*abcissas*) as a function of MVV 15 s (*ordinates*), pretraining (*circles*), and post-training (*triangles*).

Between *Week 3* and *Week 4*, $PET_{CO_2}$ continues to decrease while other ventilatory measurements ($V'E$, $dP_{max}$, etc.) do not change. This can be due to modifications in the breathing pattern leading to better efficiency of the alveolar ventilation. Our data do not allow any discussion on this point, because no systematic trend in the modification of ventilation has been recorded.

Retention of the benefits has not been verified because 1 week after completion of the training, subjects and experimenters entered the active phase of the deep-dive project for which the divers were being trained.

## CONCLUSION

Four weeks of 1-ATA ventilatory training performed by subjects exercising with external limitation to ventilation allowed an increased tolerance of respiratory musculature to: work of breathing and fatigue (duration of increased work of ventilatory efficiency at high workloads).

The increased ventilatory performance gained through this atmospheric pressure training should lead to better tolerance to increased breathing resistances at depth. However, in actual diving environment, divers must overcome both internal and external increased resistances. The gain reported is both on peak pressures and tolerance time to exercise. This increased performance would help to overcome any respiratory pressure gradient, caused by either internal or external resistances, and therefore should help the divers to postpone the limitation to ventilation caused by increased gas density under pressure.

## References

1. Maio DA, Farhi LE. Effects of gas density on mechanics of breathing. J Appl Physiol 1967;23:687–693.
2. Wood LDH, Bryan AC. Effect of increased ambient pressure on flow volume curve of the lung. J Appl Physiol 1969;27:4–8.
3. Vorosmarti J Jr, Lanphier EH. Effect of external resistances on maximum expiratory flow at increased gas density. In: Lambertsen CJ, ed. Underwater physiology V. Proceedings of the fifth symposium on underwater physiology. Federation of American Societies for Experimental Biology. Bethesda: 1976:101–107.
4. Broussolle B, Hyacinthe R, Giry P, Gaillard S. La ventilation en plongeé profonde. Med Aerospat Med Sub Hyp 1981;20:328–337.
5. Mead J. Resistance to breathing at increased ambient pressures. In: Goff Z, ed. Proceedings of the underwater physiology symposium. Washington DC: National Academy of Sciences—National Research Council Pub 377, 1955:112–120.
6. Demedts M, Anthonisen NR. Effects of increased external airways resistances during steady state exercise. J Appl Physiol 1973;35:361–366.
7. Leith DE, Bradley ME. Ventilatory muscle strength and endurance training. J Appl Physiol 1976;41:508–516.
8. Vorosmarti J. Jr. Influence of increased gas density and external resistance on maximum expiratory flow. Undersea Biomed Res 1979;6:339–346.

9. Brue F, Hyacinthe R. Détermination indirecte de la puissance maximale aérobie et de la consommation d'oxygène ($VO_{2max}$) sur ergocycle selon une méthode deriveé du test d'Astrand-Ryming. C.E.R.B. BP 610, 83 800. Toulon, France: Toulon-Naval (CERB report 80–22).
10. Morehouse LE, Miller AT. Training. In: Physiology of exercise. St. Louis: CV Mosby, 1967:253–259.
11. Vanroux R. Les mécanismes qui assignent les limites a l'exercice musculaire. In: Olivier RH, ed. Traite de biologie appliqueé. Paris: V.B. Maloine, 1970:1041–1047.

# ENHANCEMENT OF MAXIMAL VOLUNTARY VENTILATION AND EXPIRATORY FLOW AT DEPTH BY EXERCISE

*D. D. Hickey, C. E. G. Lundgren, and A. J. Påsche*

Earlier observations (1) in this laboratory of exercise ventilation levels approaching or occasionally even exceeding resting maximal voluntary ventilation (MVV) levels at high ambient pressures prompted this study. Maximal voluntary ventilation and forced expiratory flow at 40% of vital capacity ($\dot{V}E_{max}$, 0.4 VC) were recorded at pressures from 1.45 to 6.76 atm. The measurements were performed at rest and during leg exercise on a bicycle ergometer at 50, 125, and 200 W. To study the possible effect of $CO_2$ accumulation induced by exercise and diving, we performed some resting experiments during inhalation of $CO_2$-enriched air. Submersion was employed in most experiments to mimic in-sea conditions.

## MATERIALS AND METHODS

From three to six healthy, nonsmoking, male volunteers 21–30 years old were used as subjects. A bag-in-box system with a rolling seal spirometer was used for recording 15-s free-frequency MVV and $\dot{V}E_{max}$, 0.4 VC, the volume signal being differentiated for display of flow. For a full description of the apparatus, *see* Ref. 2. A full-face mask with an oronasal mask inserted connected the subject to the bag-in-box system. Breathing gas composition was analyzed by a mass spectrometer. The breathing gas was air while subjects performed leg exercise; in some resting experiments the subjects inhaled air-$CO_2$ mixtures humidified at ambient pressure and temperature.

Authors' names in alphabetical order.

All experiments were made on subjects sitting erect on a seat with a backrest. Immersion was performed by placing the subject behind the Lanphier-Morin barrier, which allows precise control of the hydrostatic pressure on the chest (2). When submersed, the subject's breathing gas pressure was set to equal the hydrostatic pressure at the equal pressure point on the subject's chest; thus a normal resting FRC (*cf.* Ref. 3) was essentially preserved. For the control measurements, a series of respiratory maneuvers consisting of VC (vital capacity), FEVC (forced expiratory VC), MVV, VC, and FEVC were performed at rest. The same series of maneuvers were also carried out while performing leg exercise for periods of 2–5 min at workloads of 50, 125, or 200 W. Recordings were obtained at 1.0, 1.45, 2.82, 4.65, and 6.76 atm. In three subjects the FEVC maneuver was performed three times at 2-min intervals after the 125-W load was terminated. This was done at 1.45 and 2.82 atm.

## RESULTS

Increasing pressure and gas density depressed MVV and $\dot{V}E_{max}$, 0.4VC both during rest and exercise (Figs. 1*a* and 1*b*). The influence of gas density on maximal expiratory flow at lung volumes larger than 25% of VC may be expressed as a power function of the gas density (5); in the present study the mean value of $a$ in $V \propto p^a$, where V = flow and p = gas density, was − 0.37 ± 0.06 (SD). The most notable finding was that both MVV and $\dot{V}E_{max}$, 0.4 VC were markedly higher during exercise (except at 50 W) at all pressure levels (Figs. 1*a* and 1*b*).

The results of all measurements were normalized for each subject relative to his resting results. A test based on the relationship between order statistics and empirical distribution was used to determine that the data were normally distributed and statistical evaluation was made by paired comparisons (*t*-test). The data in Table I show results obtained at exercise which differ significantly from resting values at $P < 0.05$. Analysis of all data indicated that the exercise effects on MVV and $\dot{V}E_{max}$, 0.4 VC were independent of pressure. Therefore the results of the $\dot{V}E_{max}$, 0.4 VC recordings at 125 W of exercise obtained at 1.45 and 2.82 atm were pooled for the two pressure levels. The results are shown in Fig. 2. Clearly, the enhancing effect of exercise on expiratory flow had disappeared within 2 min.

Adding $CO_2$ to the inhaled air to increase inspired $P_{CO_2}$ to between 35 and 55 mm Hg and end-tidal $P_{CO_2}$ by between about 5 and 20 mmHg had no marked effect on MVV or maximal expiratory flow with the exception of a 21% increase in $\dot{V}E_{max}$, 0.4 VC with 1.6% $CO_2$ mixture at 4.64 atm.

## DISCUSSION

The maximal expiratory flow and resting MVV were somewhat less influenced by high pressure in this study than in some other studies. Thus, the

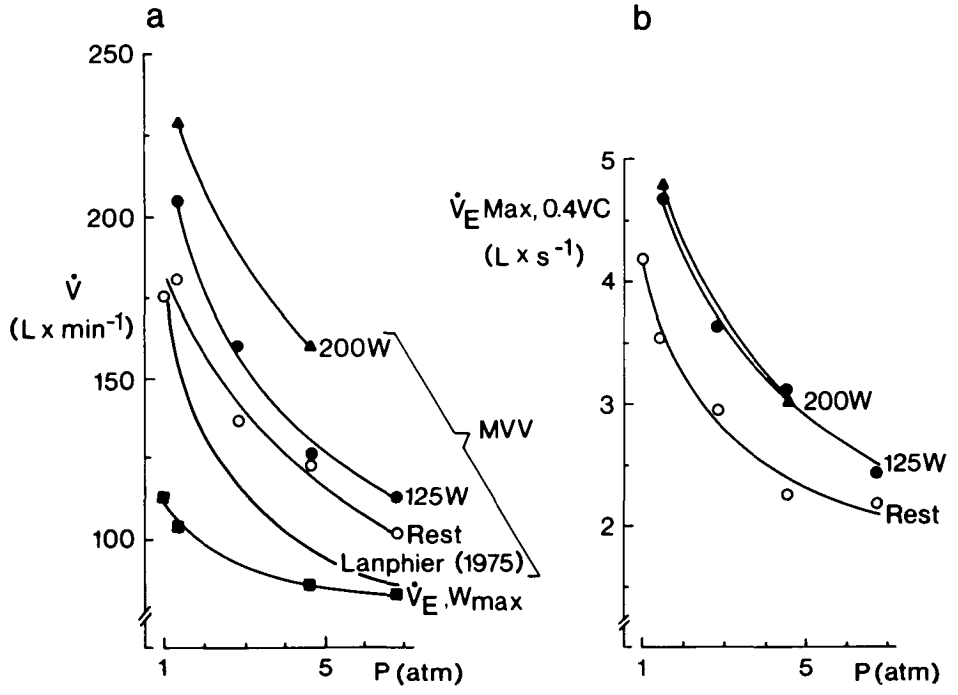



Fig. 1. *a*: Maximal voluntary ventilation (MVV) at increased ambient pressure (P) in submerged air-breathing subjects (means from 5 subjects) at rest and during leg exercise at 125 and 200 W. The MVV curve (modified from Ref. 4) is from four different studies in resting, nonimmersed subjects. The $\dot{V}E_{,W_{max}}$ curve represents mean spontaneous ventilation during maximal exertion in 5 subjects. *b*: Maximal expiratory flow at a lung volume of 40% of vital capacity ($\dot{V}E_{max}$, 0.4VC) under the same conditions as MVV measurements in *a*. (Figure from Ref. 5, Hickey *et al.* with permission.)

TABLE I

Exercise vs. Resting Data: MVV and $\dot{V}E_{max}$, 0.4 VC

| Pressure (atm) | 1.45 (%) | 2.82 (%) | 4.64 (%) | 6.76 (%) |
|---|---|---|---|---|
| MVV | | | | |
| 125 W | 114 ± 5 SE | 115 ± 2 | 111 ± 4 | 110 ± 4 |
| 200 W | 115 ± 2 | — | 117 ± 2 | — |
| $\dot{V}E_{max}$, 0.4 VC | | | | |
| 125 W | 136 ± 11 | 131 ± 7 | 140 ± 9 | 127 ± 8 |
| 200 W | 144 ± 8 | — | 148 ± 11 | — |

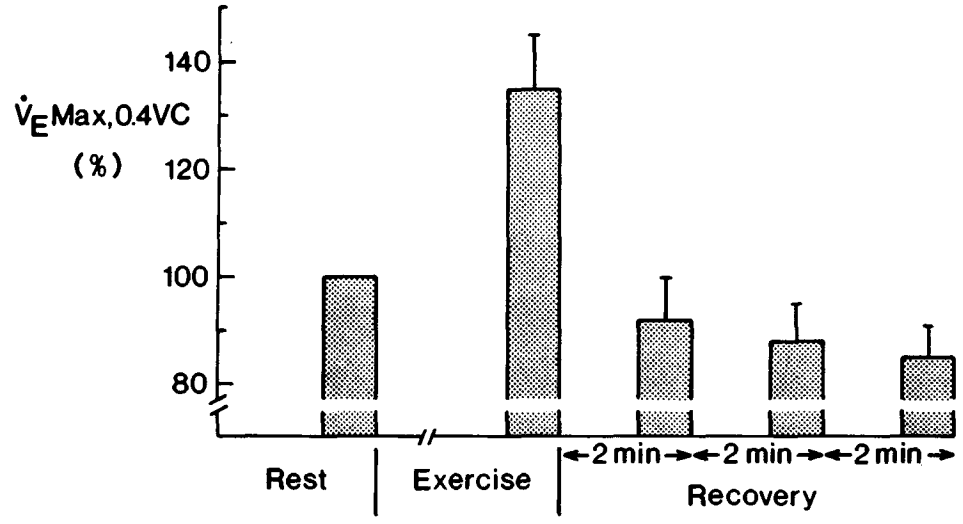



Fig. 2. Normalized $\dot{V}E_{max}$, 0.4VC values in 3 subjects before, during, and 2, 4, and 6 min after exercise. Means and standard errors of measurements at 1.45 and 2.8 atm are shown. (Figure from Ref. 5, Hickey, et al. with permission.)

expiratory flow was governed by the density raised to a power of −0.37 on the average compared to −0.45 in studies by Wood and Bryan (6) and Hesser et al. (7). The explanation for this difference is not clear. It may depend on methodological differences or it may reflect a more laminar gas flow with less energy expenditure on density-related flow phenomena in our subjects or recording apparatus. The MVV results at all pressures in the present study were consistently larger by 11–15 percentage points than what could be predicted based on the MVV values obtained at 1.0 atm and the density exponent (−0.37) derived from the maximal expiratory flow measurements. This finding is in fair agreement with the difference of 7–9 percentage points reported by Hesser et al. (7). One possible explanation favored by the latter authors is that the expiratory reserve and mid-expiratory volumes may have increased during the MVV maneuvers at depth, the larger lung volumes allowing higher expiratory flows. In addition, they have considered the possibility that hypocapnea during MVV at 1.0 atm would increase pulmonary resistance and reduce MVV while less hypocapnea would develop at depth, which would allow more favorable flow conditions and higher MVV. The present results neither contradict nor support these hypotheses.

The present effect of exercise at pressures between 1.45 and 6.76 atm to increase MVV by 10–17% and $\dot{V}E_{max}$, 0.4 VC by 27–48% in submersed subjects are in excellent agreement with observations of Hesser et al. (7; *cf.* Ref. 8), whose nonimmersed subjects showed MVV increases of 11–15% in response to exercise at between 1.0 and 6.0 bars.

The mechanism for the enhancing effect of exercise on ventilatory capacity at depth may be a reduction in flow resistance brought about by autonomic

nervous control. The rapid reduction of maximal expiratory flow back to the control level after cessation of exercise would speak in favor of this explanation, which was proposed by Kagawa and Kerr (9) for the increase in airway conductance that exercise has been shown to have at 1.0 atm. The latter authors found that the conductance increase could be prevented by atropine, but not by a beta-blocker, a finding suggesting that exercise may exert its influence via a reduced parasympathetic tone.

Alveolar $CO_2$ accumulation occurred to some extent during the exercise experiments at high pressures. However, in no case did the alveolar $CO_2$ levels exceed or even reach the highest values recorded during the inhalation of the $CO_2$-enriched air mixtures. Because the MVV and maximal expiratory flow values were largely uninfluenced by hypercapnea, it is reasonable to assume that $CO_2$ accumulation during exercise was not the cause of the exercise-linked enhancement of MVV and $\dot{V}E_{max}$, 0.4 VC. While flow mechanics may change because of changes in lung volumes (*cf.* Ref. 7), this was clearly not a mechanism behind the present flow enhancement by exercise as expiratory reserve volumes remained quite stable between resting and exercise measurements of MVV. Another factor that can also be ruled out as an explanation is the tendency for MVV to increase with breathing frequency (10) because there was a general trend for breathing frequency to decrease with the introduction of exercise at depth.

We conclude that the prediction of ventilatory capacity of divers at depth if based on MVV or expiratory flow measurements should take into account the enhancing effect of exercise on these ventilatory indices.

## Acknowledgment

This work was supported by ONR contract N00014-78-C-0205 with funds provided by the Naval Medical Research and Development Command.

## References

1. Thalmann ED, Sponholtz DK, Lundgren CEG. Effects of immersion and static lung loading on submerged exercise at depth. Undersea Biomed Res 1979;6:259–290.
2. Thalmann ED, Sponholtz DK, Lundgren CEG. Chamber-based system for physiological monitoring of submerged exercising subjects. Undersea Biomed Res 1978;5:293–300.
3. Paton WDM, Sand A. The optimum intrapulmonary pressure in underwater respiration. J Physiol 1947:106:119–138.
4. Lanphier EG. Pulmonary function. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving and compressed air work. 2nd ed. Baltimore: Williams & Wilkins, 1975:102–154.
5. Hickey DD, Lundgren CEG, Påsche AJ. Influence of exercise on maximal voluntary ventilation and forced expiratory flow at depth. Undersea Biomed Res 1983;10:241–254.
6. Wood LDH, Bryan AC. 1978. Exercise ventilatory mechanics at increased ambient pressure. J Appl Physiol: Respir Environ Exercise Physiol 1978;44:231–237.
7. Hesser CV, Lind F, Faijerson B. Effects of exercise and raised air pressures on maximal voluntary ventilation. In: Grimstad J, ed. Proceedings of the European Undersea Biomedical Society 5th Annual Scientific Meeting. Bergen, Norway: Christian Michelsens Institute, 1979:203–212.

8. Lundgren CEG, Påsche AJ. Maximal voluntary ventilation and forced expiratory flow at depth. In: Grimstad J, ed. Proceedings of the European Undersea Biomedical Society 5th Annual Scientific Meeting. Bergen, Norway; Christian Michelsens Institute, 1979:213–219.
9. Kagawa J, Kerr HD. Effects of brief graded exercise on specific airway conductance in modern subjects. J Appl Physiol 1970;28:138–144.
10. Shephard RJ. Some factors affecting the open circuit determination of maximum breathing capacity. J Physiol 1957;135:98–113.

# OXYGEN CONSUMPTION IN CATS DURING SIMULATED DIVES AND ITS RELATION TO MUSCULAR ACTIVITY

*G. Imbert, N. Naraki, L. Fagni, and H. Burnet*

An important goal of high-pressure animal experiments is to estimate the safe depth limit for human subjects and to delineate the potential causes of such limitation. In chamber dives with helium as the primary diluent gas, human subjects have reached depths close to 700 m, while laboratory animals of different mammalian species have currently withstood pressures corresponding to 1000 m or more. At these great depths, several effects are prominent and eventually lead to animal exhaustion and death if the pressure is continually raised. The most relevant of these effects are: labored breathing due to increased gas density (1,2); hyperexcitability with neuromotor disturbances and eventually convulsive seizures due to high pressure (3,4); and inability to achieve thermal homeostasis due to thermodynamic properties of hyperbaric gaseous environments (5,6). Although all of these effects should lead to an increase in energy expenditure, the extent to which such an effect occurs has received little attention. The goal of the present experiments was therefore to measure the oxygen consumption ($\dot{M}O_2$) in cats during hyperbaric exposures and to partition any increase over baseline among the factors described above by comparing different breathing mixtures at different simulated depths.

## METHODS

Seventeen male unanesthetized cats, age 9-27 months, and weighing 2.3–5.0 kg were used as subjects. Nine cats were involved in helium-oxygen (heliox) dives, five cats in nitrogen-oxygen (nitrox) dives, and three cats in helium-nitrogen-oxygen (trimix) dives. In six cats, electromyographical elec-

trodes were implanted in the trapezius and in the triceps brachii 5-12 days before the dive.

All dives were simulated in a small pressure chamber equipped with an atmosphere conditioning system (ACS). The total volume of the chamber plus life support loop was 0.31 $m^3$. One animal was exposed at a time. Food and water were supplied ad libitum. Excess of water vapor and ammonia were removed by a silica gel absorbent, and carbon dioxide was absorbed by soda lime (Fig. 1 shows the experimental set-up and the general configuration of the system).

The maximal depths were 1000 m in heliox and trimix dives, and 165 m in nitrox dives. Other dives were conducted at 750 m and 900 m with heliox, and at 125 m with nitrox.

At maximal depth the nitrogen percentage was less than 1% in heliox dives (corresponding to atmospheric nitrogen in chamber residual air), and was 5% in trimix dives (obtained by nitrogen addition in last stages of compression). All dives were preceded by a preliminary exposure at 0 or 10 m for 4 to 12 h, to obtain baseline $\dot{M}O_2$ and muscle activity measurements. Compression to 10 m and subsequent compression to the final pressure were achieved by adding pure inert gases (nitrogen for nitrox dives, helium for heliox dives, or helium followed by nitrogen for trimix dives), while automatically controlling the chamber oxygen pressure ($P_{O_2}$) between 20 and 25 kPa. The compression

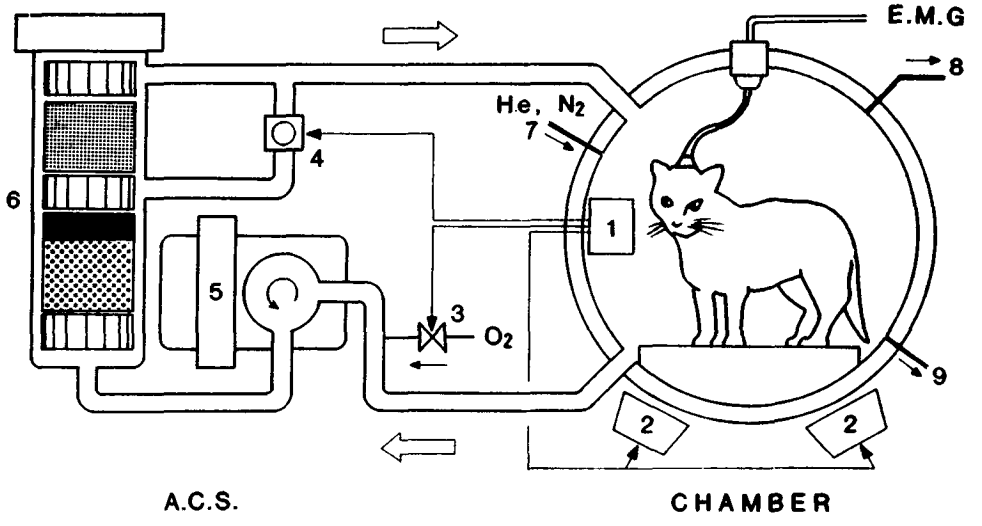



Fig. 1. Experimental set up: Hyperbaric chamber and atmosphere conditioning system. **1:** Sensor assembly ($O_2$, temperature and hygrometry controls); **2:** Heater; **3:** $O_2$ valve; **4:** Silica gel bypass (closed when too wet); **5:** Gas circulator; **6:** Filter assembly (from *bottom to top:* soda lime, charcoal, and silica gel); **7:** Inert gas input for compression; **8:** Gas sampling line for continuous $O_2$ and $CO_2$ analysis; **9:** Decompression line. Note that *lines* **3, 7, 8,** and **9** are closed during $\dot{M}O_2$ measurements; the change in $O_2$ content is read by the $O_2$ cell.

rates ranged from 3 to 1 m/min, decreasing with increasing depths. Compression stops of 2–14 h in duration to allow measurement of $\dot{M}O_2$ were performed at 300, 600, and 900 m in heliox and trimix dives, and at 70 and 125 m in nitrox dives. Excluding stops, compression times to maximal depths were approximately 3 h to 165 m (with nitrogen) and 11 h to 1000 m (with helium or helium-nitrogen). After the maximal exposure, $P_{O_2}$ was raised to 80 kPa and kept constant during decompression to 25 m. From 25 m to the surface, $P_{O_2}$ was progressively reduced to its normoxic level (21 kPa). Total decompression time was 46 h from 1000 m and 16 h from 165 m. No measurements of $\dot{M}O_2$ were obtained during decompression. An example of the dive profile to 1000 m (trimix) is shown in Fig. 2.

To insure adequate thermal conditions, we increased the chamber temperature during compression and decreased it during decompression (Fig. 2). The selection of chamber temperature was based on knowledge derived from previous experience. It ranged from 28 to 35°C from the surface to maximal depth in heliox or trimix dives and from 25 to 31°C from the surface to maximal depth in nitrox dives.

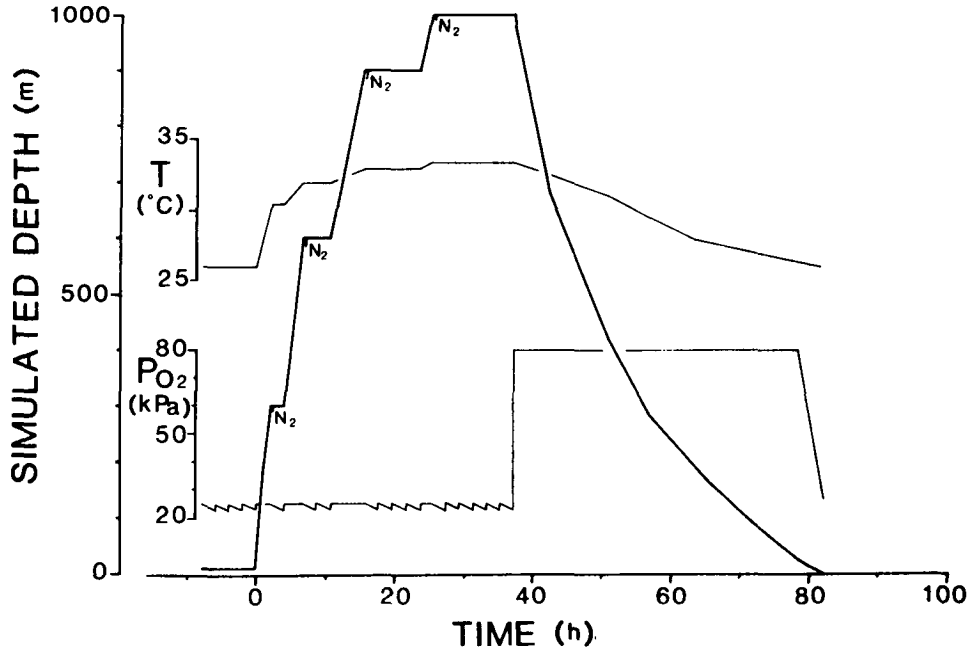



Fig. 2. Example of dive profile: Trimix Dive to 1000 m. Compression is achieved by injecting inert gases (helium followed by nitrogen), while controlling the oxygen pressure ($P_{O_2}$) at a normoxic level (20–25 kPa) and increasing chamber temperature ($T$). Throughout the predive exposure at 10 m and throughout exposures at 300, 600, 900, and 1000 m, $\dot{M}O_2$ is measured from the rate of change of chamber $O_2$ content during periods 90–120 min in duration (*saw-toothed $P_{O_2}$ curve*). During decompression from maximal depth to 25 m, $P_{O_2}$ is controlled at a hyperoxic level (75–80 kPa), and progressively reduced between 25 m and the surface.

Chamber temperature was measured with a Texas Instruments 145-02 pressure gauge, and chamber temperature was measured with a set of platinum resistances. During compression and decompression, the chamber atmosphere was continuously sampled and analyzed for its fractional oxygen concentration ($F_{O_2}$) with a paramagnetic oxygen analyzer, and for its fractional carbon dioxide concentration ($F_{CO_2}$) with an infrared spectrometer. The computed $CO_2$ partial pressure was in all circumstances kept at a value lower than 0.5 kPa, and the relative humidity was kept at a value lower than 60%. The relative variations in chamber $P_{O_2}$ were sensed by a galvanometric oxygen cell (SEDAM, 13190 Allauch, France) placed in the chamber.

At each depth, the measurement of $\dot{M}_{O_2}$ was made in isobaric and isothermal conditions over periods of 90–120 min in duration from the oxygen cell readings. No gas was added nor subtracted from the chamber during these periods, with the exception of the expired $CO_2$, which was continuously absorbed in the ACS. Thus, during the measurement, the change in oxygen content of the hyperbaric atmosphere resulted only from the animal's respiration. The value of $\dot{M}_{O_2}$ related to body mass (mmol/min·kg) was computed as:

$$\dot{M}_{O_2} = ß\ P\ V\ F_{O_2}\ \frac{1}{B}\ \frac{1}{\Delta t}\ \frac{\Delta E}{E_o} \qquad (1)$$

where β is the capacitance coefficient (7) of the gas mixture (mmol/Pa·m$^3$); β = 1 / ZRT (8); where Z is the compressibility factor (dimensionless); R the ideal gas constant (0.0083 J/mmol·K); and T the absolute temperature (K). P is the absolute pressure (Pa), one metre of sea water was considered equivalent to $10^4$ Pa. V is the total volume of the hyperbaric system (m$^3$). $F_{O_2}$ is the fractional concentration of oxygen in the gas mixture at the onset of the measurement (dimensionless). B is the body mass of the animal (kg). Δt is the duration of the measurement (min). $E_o$ is the voltage output of the galvanometric cell at the onset of the measurement (mV), corresponding to $F_{O_2}$. ΔE is the change in galvanometric cell output during the course of the oxygen consumption measurement (mV).

It was assumed that under isobaric and isothermal conditions and within the relatively short time of the measurement, the variation in the signal delivered by the galvanometric cell was linearly related to the variation in oxygen pressure and molar oxygen concentration in the hyperbaric atmosphere.

Electromyographic signals derived from the postural muscles were recorded on a tape recorder for subsequent analysis. The quantification of muscular activity was based on the summation of discrete values sampled by a digital voltmeter. Each sample corresponded to 400 ms of the integrated muscular potentials recorded and converted in arbitrary units. A series of 100 samples was registered during 200 s at 2-s intervals every 10 min during the measurement of $\dot{M}_{O_2}$. The corresponding averaged values were considered representative of the muscular activity during each run. They were compared and expressed relatively to the baseline value observed in each animal at rest and under normobaric thermoneutral conditions.

## RESULTS

All animals survived the dives with the exception of three that were decompressed from 1000 m and died from exhaustion and decompression sickness. Important behavioral changes were observed during the hyperbaric exposures, among them a marked reduction in spontaneous activity not related to the inert gas used for compression. Food intake was also reduced markedly, with a consequent loss in body mass (5–15%). There was little apparent change in consciousness: the animals remained conscious up to the highest pressure and reacted to various external stimuli.

In helium dives the classically described abnormal muscular activities (1–4) were apparent at simulated depths greater than 600 m. These abnormalities included limb and neck tremor, associated with whole body jerks. The amplitude of tremor and the frequency of jerks increased with increasing pressure. All these phenomena were also apparent on the electromyograms (Fig. 3). Tremor was signaled by rhythmic (8–13 Hz) bursts of activity; jerks were signaled by generalized and synchronized muscular discharges. An important feature was shown by electromyogram analysis: in all animals, the onset of tremor was preceded by a progressive hypertonia. An increase in postural tone at rest appeared at depths equal to or lower than 300 m (Fig. 3*b*). Increased tone evolved into tremor which appeared as bursts of muscular

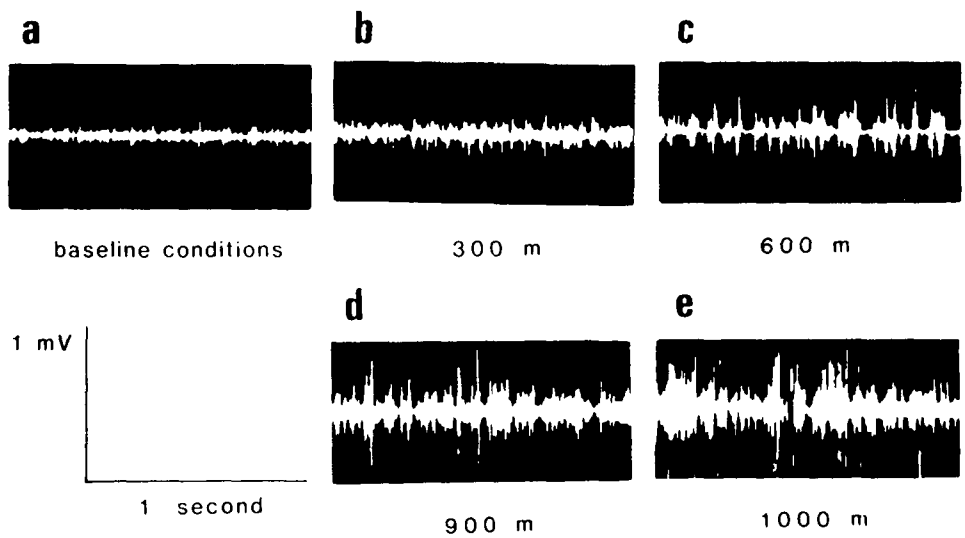



Fig. 3. Trapezius muscle EMG recorded at 10 m (**a**) and at different depths (**b, c, d, e**). Note the increase in postural tone at 300 m (**b**) and the 10-Hz tremor at 600 m (**c**) and beyond (**d, e**). *Baseline conditions* (**a**) refer to the predive 10-m period.

activity separated by brief silent periods (Fig. 3*c*). At later stages, with the progressive increase in postural muscle activity, tremor tended to be a rhythmic enhancement of the continuous discharge (Figs. 5*d* and 5*e*). The motor disorders disappeared during decompression between 600 and 300 m.

In trimix dives, the changes in muscular activity were also present but reduced markedly. They were absent in nitrox dives.

In all animals, $\dot{M}O_2$ clearly increased during heliox dives. Because measurements were impossible while compressing or decompressing, the data which are presented correspond only to mean values recorded under steady pressure and temperature conditions. In some animals, $\dot{M}O_2$ at a given depth was recorded for periods as long as 24 h and showed cyclic fluctuations over time. Figure 4 compares $\dot{M}O_2$ and its spontaneous variations during 24-h periods under baseline conditions (10 m), and under hyperbaric conditions at a simulated depth of 750 m (heliox dive) in the same animal. There was an average net increase of 32% in $\dot{M}O_2$ at depth; cyclic variations were still present and comparable to those observed at 10 m.

To compare $\dot{M}O_2$ at different depths, we considered only the value measured during the first 2 h upon arrival at a given depth. These values were compared to the last observed value under baseline conditions (last 2 h at 10 m or surface before compression). Results for all cats are presented individu-

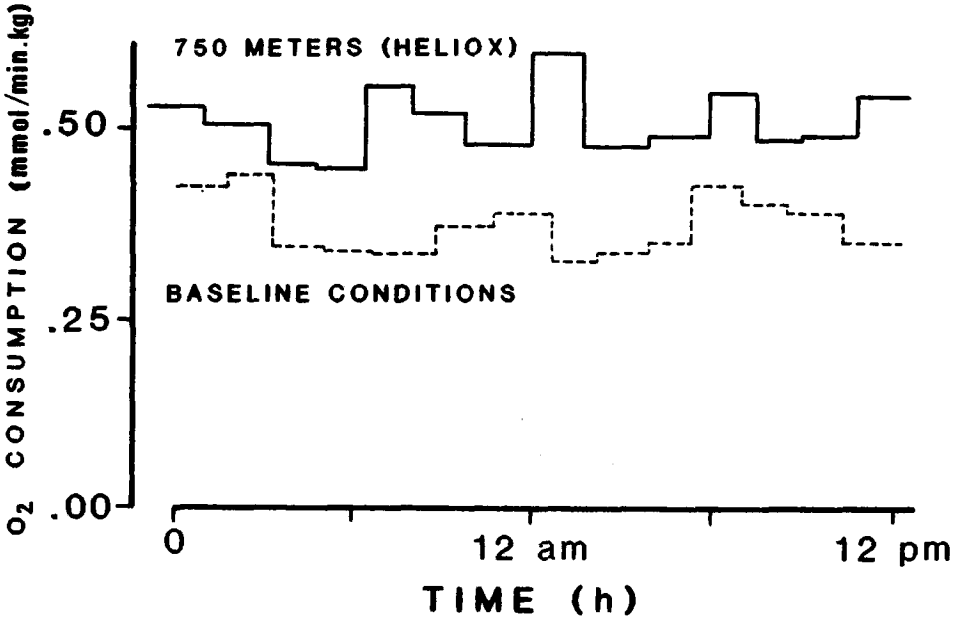



Fig. 4. Cyclic variations in $O_2$ consumption throughout 24-h periods in one cat. Body mass = 3.3 kg. *Baseline conditions* refer to a predive exposure at 10 m. Note the similarity of diurnal variations in $\dot{M}O_2$ under *baseline conditions* and at depth (*750 m, heliox*). The average value is markedly higher at depth.

TABLE I

Oxygen Consumption at Different Depths in Heliox Dives

| Simulated Depth | (metres of sea water) | 10 | 300 | 600 | 900 | 1000 |
|---|---|---|---|---|---|---|
| Chamber Gas Characteristics | | | | | | |
| Temperature | (°C) | 25.0 ± 1.0 | 30.5 ± 0.5 | 32.0 ± 0.4 | 33.0 ± 0.3 | 33.3 ± 0.2 |
| Oxygen Fraction | (%) | 10.5 | 0.70 | 0.34 | 0.22 | 0.21 |
| Nitrogen Fraction | (%) | 39.5 | 2.54 | 1.29 | 0.85 | 0.78 |
| Helium Fraction | (%) | 50.0 | 96.76 | 98.37 | 98.92 | 99.01 |
| Capacitance Coefficient | (mmol/Pa·m$^3$) | 0.41 | 0.40 | 0.39 | 0.38 | 0.38 |
| Absolute Density | (kg/m$^3$ BTPS ) | 1.2 | 5.8 | 10.8 | 14.7 | 16.2 |
| Cat Number | Body Mass (kg) | $\dot{M}O_2$ (mmolmin·kg) | | | | |
| H 1 | 4.7 | 0.43 | 0.53 | 0.62 | 0.73 | 0.67 |
| H 2 | 2.3 | | 0.52 | 0.64 | 0.80 | 1.07 |
| H 3 | 3.3 | | 0.53 | 0.45 | 0.73 | 0.81 |
| H 4 | 5.0 | 0.34 | 0.49 | 0.62 | 0.43 | 0.60 |
| H 5 | 5.2 | 0.47 | 0.61 | 0.66 | 0.45 | 0.57 |
| H 6 | 3.1 | 0.50 | 0.38 | 0.54 | 0.68 | 0.67 |
| H 7 | 3.7 | 0.46 | 0.48 | 0.50 | 0.51 | 0.66 |
| H 8 | 4.0 | | 0.43 | 0.40 | 0.58 | 0.73 |

Individual values measured during the first 2 h of sojourn at given depths.

TABLE II

Oxygen Consumption at Different Depths in Trimix Dives

| Simulated Depth | (metres of sea water) | 10 | 300 | 600 | 900 | 1000 |
|---|---|---|---|---|---|---|
| Chamber Gas Characteristics | | | | | | |
| Temperature | (°C) | 25.0 ± 1.0 | 30.5 ± 0.5 | 32.0 ± 0.3 | 33.0 ± 0.2 | 33.2 ± 0.2 |
| Oxygen Fraction | (%) | 10.5 | 0.70 | 0.34 | 0.22 | 0.21 |
| Nitrogen Fraction | (%) | 39.5 | 4.80 | 4.90 | 4.90 | 5.00 |
| Helium Fraction | (%) | 50.0 | 94.50 | 94.76 | 94.88 | 94.79 |
| Capacitance Coefficient | (mmol/Pa·m$^3$) | 0.41 | 0.40 | 0.39 | 0.38 | 0.38 |
| Absolute Density | (kg/m$^3$ BTPS ) | 1.2 | 6.5 | 12.6 | 18.7 | 20.9 |
| Cat Number | Body Mass (kg) | $\dot{M}O_2$ (mmolmin·kg) | | | | |
| T 1 | 2.6 | 0.60 | 0.37 | 0.46 | 0.33 | 0.53 |
| T 2 | 3.1 | 0.44 | 0.40 | 0.38 | 0.43 | 0.44 |
| T 3 | 4.0 | 0.48 | 0.47 | 0.39 | 0.51 | 0.52 |

Individual values measured during the first 2 h of sojourn at given depths.

ally in Table I for heliox dives, Table II for trimix dives, and Table III for nitrox dives.

Table I shows that $\dot{M}O_2$ increased in heliox dives by an average of 14% at 300 m, 25% at 600 m, 39% at 900 m, and 64% at 1000 m. The best-fit least-squares line relating $\dot{M}O_2$ to depth (expressed in m) was computed as the exponential function:

$$\dot{M}O_2 = 0.43\, e^{.00044P} \quad (2)$$

Only a slight increase in $\dot{M}O_2$ was found at maximal depth in trimix dives (Table II), and no systematic variation was found in nitrox dives (Table III). Data obtained at the same depths with heliox and with trimix were compared by a nonparametric Mann and Whitney U test. The addition of a slight amount of nitrogen (5%) to the breathing mixture reduced significantly $\dot{M}O_2$ at depth ($P < 0.05$).

Data obtained with different breathing mixtures may be compared with reference to their absolute gas densities. For example, the same density was achieved at 125 m in nitrox dives and at 900 m in heliox dives (14.6 and 14.7 g/L BTPS, respectively), or at 165 m in nitrox dives and at 900 m in trimix dives (18.9 and 18.7 g/L BTPS, respectively). Figure 5 shows that for a given absolute density, the mean $\dot{M}O_2$ values computed for all cats in trimix dives were intermediate between the mean $\dot{M}O_2$ values computed for all cats in heliox and in trimix dives.

TABLE III

Oxygen Consumption at Different Depths in Nitrox Dives

| Simulated Depth | (metre of sea water) | 10 | 70 | 125 | 165 |
|---|---|---|---|---|---|
| Chamber Gas Characteristics | | | | | |
| Temperature | (°C) | 24.6 ± 0.9 | 27.3 ± 0.8 | 28.0 ± 0.6 | 29.2 ± 0.4 |
| Oxygen Fraction | (%) | 21.0 | 2.62 | 1.55 | 1.20 |
| Nitrogen Fraction | (%) | 79.0 | 97.38 | 98.45 | 98.80 |
| Helium Fraction | (%) | 0.0 | 0.00 | 0.00 | 0.00 |
| Capacitance Coefficient | (mmol/Pa·m³) | 0.41 | 0.41 | 0.40 | 0.40 |
| Absolute Density | (kg/m³ BTPS ) | 1.1 | 8.7 | 14.6 | 18.9 |
| Cat Number | Body Mass (kg) | $\dot{M}O_2$ (mmolmin·kg) | | | |
| N 1 | 2.8 | | 0.50 | 0.26 | |
| N 2 | 3.2 | | 0.26 | 0.23 | |
| N 3 | 3.1 | 0.39 | 0.43 | 0.40 | 0.41 |
| N 4 | 4.3 | 0.28 | | 0.26 | 0.46 |
| N 5 | 2.5 | 0.33 | | | 0.34 |
| N 6 | 4.0 | 0.41 | 0.31 | 0.42 | |

Individual values measured during the first 2 h of sojourn at given depths.

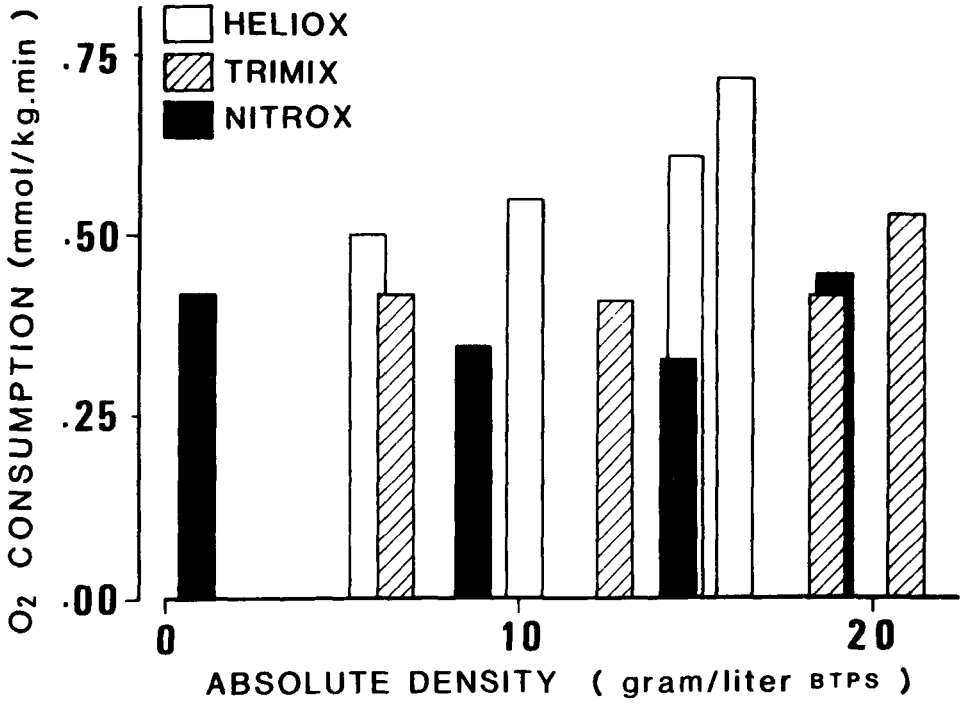



Fig. 5. Oxygen consumption plotted as a function of absolute gas density for different breathing mixtures (heliox, trimix, and nitrox) and different depths. Data pooled from all animals. Average $\dot{M}O_2$ values have been computed from individual values presented in Tables I, II, and III. A marked increase in $\dot{M}O_2$ is observed only in heliox dives.

Finally, $\dot{M}O_2$ was related to the electrical activity recorded in the postural muscles. In Fig. 6, $\dot{M}O_2$ is plotted as a function of the integrated electromyographic activity (EMGi) recorded in the trapezius muscle. The data obtained from six animals are pooled. Low $\dot{M}O_2$ values were associated with low levels of EMGi (nitrox dives or heliox dives at shallow depths), whereas high $\dot{M}O_2$ values were associated with high levels of EMGi (heliox dives at great depths). The net effect was a linear relationship between the relative increase in $\dot{M}O_2$ and the relative increase in EMGi.

## DISCUSSION

Neuromotor disturbances observed in cats during these experiments are similar to those commonly described in various mammalian species (3,4), as classical components of the high pressure neurological syndrome (HPNS). A full analysis and a general discussion of our electrophysiological data are

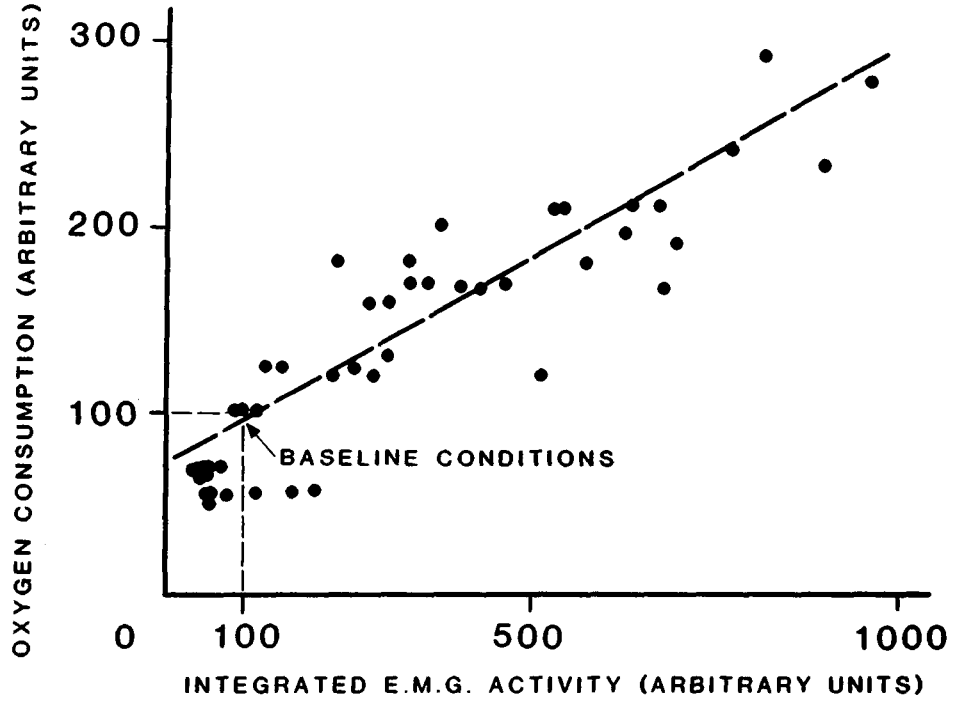



Fig. 6. The relation between $O_2$ consumption and the postural activity of trapezius muscle. The muscle activity is quantitated from the integrated electromyogram and expressed in arbitrary units, where the resting activity under baseline conditions (10 m) is considered equal to 100 arbitrary units. Similarly, the resting value to $\dot{M}O_2$ under baseline conditions is considered equal to 100 arbitrary units. This procedure allows the pooling of the data obtained from six animals during nitrox or heliox dives. The variation in $\dot{M}O_2$ is shown to be linearly related to the variation in postural muscle activity, for all depths and breathing mixtures (low values correspond to nitrox dives and to heliox dives at shallow depth; high values correspond to heliox dives at great depths).

presented elsewhere (9). The present discussion will focus on data related to $\dot{M}O_2$.

Because the temperature conditions were close to the apparent thermal comfort for all $\dot{M}O_2$ measurements, and because animals displayed a reduced level of activity, the recorded values are assumed to correspond to rough estimations of the standard metabolic rates (7), under the different diving conditions. The increase in standard $\dot{M}O_2$ can be explained in three ways:

1) *The increase in energy expenditure related to the mechanical work of breathing as a consequence of high gas density.* This increase cannot be considered as the primary factor since in nitrox dives the high gas density failed to cause any marked change in $\dot{M}O_2$. Moreover, comparison of heliox and trimix dives shows that at a given depth $\dot{M}O_2$ was lower in the second case, despite the higher gas density.

2) *The increase in heat losses under hyperbaric conditions.* Two factors may be considered: a) the thermal conductivity is higher for helium than for nitrogen (0.142 W/m·K and 0.024 W/m·K, respectively) and b) the gas molar concentration is roughly proportionate to depth and was higher in heliox and trimix dives than in nitrox dives. These factors could account for the difference between heliox or trimix dives and nitrox dives. But they could not account for the difference between heliox dives and trimix dives since the selected temperatures were identical, and since adding a small amount of nitrogen (5%) in helium could not change drastically the thermodynamic characteristics of the hyperbaric atmosphere.

3) *The motor excitation caused by increased pressure and related to the HPNS.* Several authors have shown that high hydrostatic pressure is able to increase $\dot{M}O_2$ in aquatic animals, fishes or invertebrates. This effect is probably mediated by an increase in motor activity (10,11). The present experiments demonstrated that the same effect can be observed in air-breathing animals, under the more complex conditions represented by simulated diving for a homeotherm. On the other hand, it is known that adding a small amount of nitrogen reduces the motor disturbances caused by high pressure (12,13). This could explain the difference observed at great depths between heliox and trimix.

## CONCLUSION

Measuring the oxygen consumption at rest under apparent thermoneutral conditions proved to be a simple and noninvasive method to quantitate the net effects of compression in the whole animal. Hence, in a helium-oxygen environment, the increase in standard metabolic rate was linearly related to the increase in postural muscle tone induced by pressure and evidenced by electromyogram analysis. On the other hand, the antagonistic effect of nitrogen was evidenced by a lower metabolic rate in trimix dives compared to heliox dives at the same depth.

Furthermore, the increase in energy expenditure at rest and, consequently, the increase in respiratory gas exchange and ventilatory activity, have to be considered with respect to breathing limitations in hyperbaric atmospheres (14,15). It may be that the raised energy expenditure under hyperbaric conditions represents a limiting factor in pressure acclimatization and a cause of progressive exhaustion.

## References

1. MacInnis JB, Dickson JG, Lambertsen CJ. Exposure of mice to a helium-oxygen atmosphere at pressures to 122 atmospheres (4000 feet of sea water). J Appl Physiol 1967;22:694–698.
2. Chouteau J, Imbert G, Le Pechon JC, Alinat J. Physiological aspects of prolonged exposures at extreme ambient pressures in an oxygen-helium atmosphere. In: Wada J, Iwa T, eds. Proceedings of the fourth international congress on hyperbaric medicine. Tokyo: Igaku Shoin, 1970:126–131.

3. Rostain JC, Nacquet R. Résultats préliminaires d'une étude comparative de l'effet des mélanges oxygène-hélium et oxygène-hydrogène et des hautes pressions sur le babouin *Papio papio*. In: Fructus X, ed. Proceedings des Troisièmes Journées Internationales d'Hyperbarie et de Physiologie Subaquatique. Paris: Doin, 1972:44–49.
4. Brauer RW. The high pressure nervous syndrome: animals. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving and compressed air work. London: Baillière Tindall, 1975:231–247.
5. Membery JH, Link EA. Hyperbaric exposure of mice to pressures of 60 to 90 atmospheres. Science 1964;144:1241–1242.
6. Stetzner LC, Deboer B. Thermal balance in the rat during exposure to helium-oxygen from 1 to 40 atmospheres. Aerosp Med 1972;43:306–309.
7. Dejours P. Principles of comparative respiratory physiology. Amsterdam: North Holland Publishing Company, 1975.
8. Imbert G, Dejours P, Hildwein G. The compressibility and the capacitance coefficient of helium-oxygen atmospheres. Undersea Biomed Res 1982;9:305–315.
9. Fagni L, Weiss M, Pellet J, Hugon M. The possible mechanisms of the high pressure-induced motor disturbances in the cat. Electroenceph Clin Neurophysiol 1982;53:590–601.
10. Fontaine M. De l'augmentation de la consommation d'oxygène des animaux marins sous l'influence des fortes pressions. Ses variations en fonction de l'intensité de la compression. C R Acad Sci (Paris) 1929:188:460–461.
11. Macdonald AG. Physiological aspects of deep sea biology. Cambridge: Cambridge University Press, 1975.
12. Rostain JC, Imbert JP, Gardette B, Lemaire C, Dumas JC, Naquet R. Compression methods: II. Study of the effects of profiles and $N_2$ injections on HPNS of the baboon *Papio papio*. Undersea Biomed Res 1978;5(suppl):46.
13. Miller KW. Inert gas narcosis and animals under high pressure. In: Sleigh MA, Macdonald AG, eds. The effects of pressure on organisms. Cambridge: Cambridge University Press, 1972:363–378.
14. Chouteau J. Respiratory gas exchange in animals during exposure to extreme ambient pressures. In: Lambertsen CJ, ed. Underwater physiology IV. Proceedings of the fourth symposium on underwater physiology. New York: Academic Press, 1971:385–397.
15. Imbert G, Jammes Y, Naraki N, Duflot JC, Grimaud C. Ventilation, pattern of breathing, and activity of respiratory muscle in awake cats during oxygen-helium simulated dives (1000 msw). In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:272–283.

# VENTILATORY RESPONSE TO $CO_2$ ELEVATION AND SUBMERGED EXERCISE AT 1 ATA IN NOVICE DIVERS

*D. Kerem, A. Ariel, E. Eilender, and Y. Melamed*

The topic of *$CO_2$ retention* during diving has recently earned an extensive review (1). The term refers to a mismatch between expected proportions of $\dot{V}CO_2$ and $\dot{V}A$ during exercise. In such a mismatch, the level of $\dot{V}A$ is abnormally low and results in an increase in $PA_{CO_2}$, possibly as high as 30 Torr. Although retention of $CO_2$ is accentuated by deep diving and the increased difficulty in breathing that is associated with higher gas density at deeper depths, this phenomenon has been noted in both shallow diving and dry exercise at normal pressure (2–5). Therefore, it commonly has been regarded as a subjective characteristic of many divers to fail to maintain normal $PA_{CO_2}$ values during exercise due to a low ventilatory response to exertion.

Since the degree of hypercapnia divers suffer from $CO_2$ retention is usually not harmful in itself, hypoventilation could be regarded as a successful and beneficial adaptation. On the other hand, hypercapnia enhances oxygen toxicity, nitrogen narcosis, and heat loss (1). Also, there are strong theoretical implications that it could increase the probability of decompression sickness (1). Because of these factors and implications, $CO_2$ retention should be considered maladaptive in military and commercial diving. It is thus unfortunate that this phenomenon seems to target the experienced diver over and above other athletes/workers (1,4,5) by putting him at risk of these hazards.

The basic question is whether the tendency to hypoventilate during exercise is a response acquired by anyone exposed to diving, or whether it is an inherent physiological characteristic prevalent among diving candidates. The answer has practical as well as theoretical implications. An acquired response would call for reconditioning the breathing pattern to combat $CO_2$ retention, while an inherent response would emphasize careful selection of diving candidates. Ideally, selection should rely on a simple test suited for screening a

large number of uninitiated individuals. This test could be either a direct measure of the ventilatory response to exercise or a measure of some other response closely correlated with hypercapnia resulting from exertion. One possibility for such a test might be the ventilatory response to $CO_2$ elevation, which has been shown to be relatively low in experienced divers (6,7). Even if the concerned response does not correlate with $CO_2$ retention, such a test has independent merit for candidates for closed or semi-closed scuba diving. In this form of diving, a weak response to elevation of $CO_2$ caused by faulty absorption of this gas could lead to $0_2$-induced convulsions or even to $CO_2$ intoxication and black-out (8), regardless of exertion. A test of ventilatory response to $CO_2$ could possibly screen for candidates at greater risk of encountering such hazards.

Comprehensive answers to the questions of the origin of hypoventilation during underwater exercise and the adequacy of proposed tests to screen for this problem can only be supplied by a longitudinal study. This investigation addressed those questions that would be included in the first stage of a longitudinal study on $CO_2$ retention. It gauged novice divers' ventilatory response to elevation of $CO_2$ and to underwater exertion. The following interrelated questions were addressed:

1) Is $CO_2$ retention present in novice divers? If so, to what degree?

2) What are the distribution pattern and degree of the ventilatory response to $CO_2$ in novice divers?

3) Is there a correlation between the above factors relative to the individual diver?

4) Could one or both of the above factors be used to select candidates for diving at a fairly early stage in their career?

The results of this phase of the longitudinal study indicate that novice divers as a group are similar to nondivers in regard to their ventilatory responses to $CO_2$ elevation and to exertion underwater. A weak correlation does exist between the two ventilatory responses, but is too small to form a basis for individual selection of divers.

## METHODS

Fifty-nine novice divers participated in the study. All had completed a basic air scuba course, which required 10 open-water dives for certification. Their physical characteristics are shown in Table I.

The experimental system was contained in a type of Lanphier-Morin wet compartment (9) of a 165-cm diameter pressure chamber. The wet portion seated a submerged diver hooked to a standard diving mouthpiece—double-hosed and undirectional. An air bubble with a pressure slightly lower than chamber pressure was maintained above the diver's head into which he could surface in case of difficulty (Fig. 1). Also in the wet portion was a chain-link extension of a bicycle ergometer (Ergometer 380, Siemens Elema), which was electromagnetically loaded and allowed the diver to exercise at rates controlled from outside.

TABLE I

Physical Characteristics of Subjects

| | |
|---|---|
| Age (yr) | 20.1 ± 0.5 |
| Weight (kg) | 70.8 ± 6.9 |
| Height (cm) | 176.9 ± 5.35 |
| VC (mL BTPS) | 4990.0 ± 420 |

The rest of the breathing system, situated in the dry portion of the chamber, consisted of a circuit that could be switched from an open-system mode (to and from chamber atmosphere) to a closed system. This included a 7-L bag-in-a-box in conjunction with a $CO_2$-absorber canister that could be bypassed in the rebreathing experiments. The bag composition could be changed by evacuating and filling from a built-in breathing system (BIBS) that supplied $O_2$ and a rebreathing mixture of 7.5% $CO_2$ in $O_2$. A demand regulator that could be attached to the inhalation port of the open system allowed the diver to inhale the above mixtures directly from the BIBS. A separate scuba regulator supplied with air extended into the diver's compartment to provide a supplemental breathing source.

Gas from the mouthpiece was led outside the chamber, and its $CO_2$ concentration was measured continuously by means of an infrared analyzer (Capnograph, Godart). Mouthpiece pressure, referenced to chamber pressure, and the pressure differential across a 7-cm diameter screen pneumotachograph connected to the bag-in-a-box were measured with differential pressure transducers (Model 270, Hewlett Packard). The mouthpiece pressure served to assess the hydrostatic load, to insure against either overinflation or underinfla-

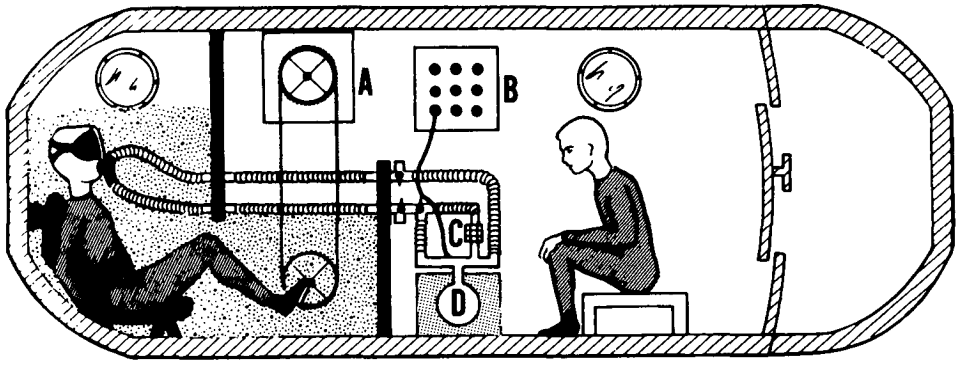



Fig. 1. General schematic of experimental system in chamber. **A**: ergometer; **B**: BIBS; **C**: $CO_2$-absorbent canister; **D**: bag-in-box.

tion of the bag, and to measure the work of breathing through the system. The pressure differential allowed on-line recording of tidal and minute volume by electronic integration of the flow signal (respiratory-integrator, Hewlett Packard). All variables were recorded and visualized on-screen on an eight-channel recorder (7788A Hewlett Packard).

The pneumotachograph and integrator were calibrated by means of a breathing simulator (Type I, Reimers Consultants) connected to the mouthpiece and set up to deliver known volumes (checked against a 120-L Tissot spirometer) of air. The work of breathing through the system (canister included) was determined with the simulator: pressure volume loops were recorded on an X-Y recorder (7045B Hewlett Packard) and their area was computed. Work of breathing was in the high range of commercial, closed system scuba diving (10) (Fig. 2): it was 0.06 kg m/L at a ventilation rate of 40 L/min.

## Protocol and Data Analysis

Subjects reported to the chamber in the morning after a light meal. They were briefed about the tests and the experimental system, including hand signs

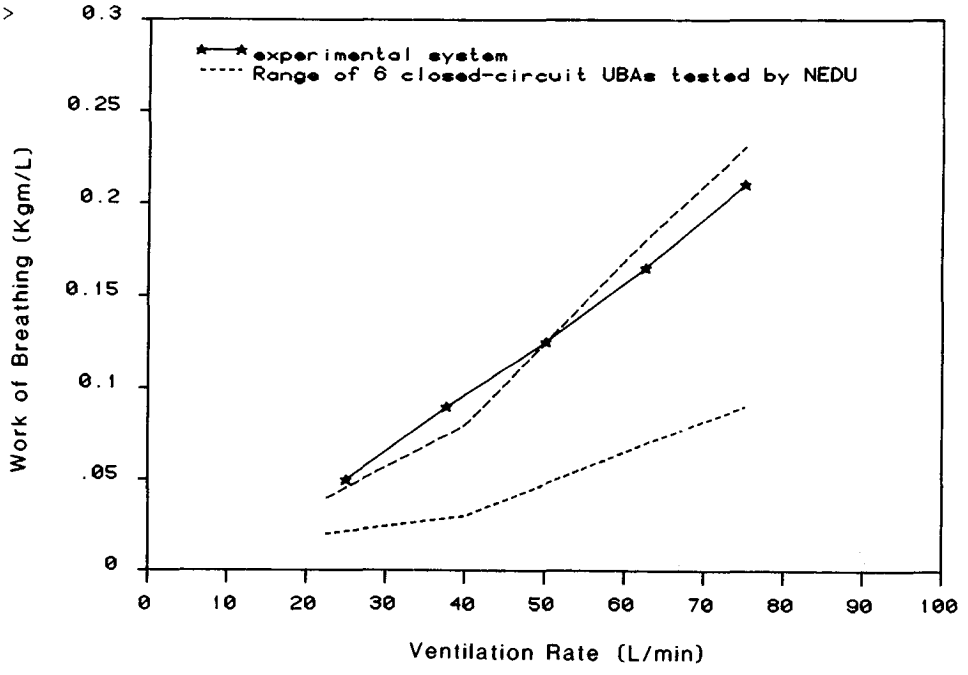



Fig. 2. Work of breathing through the experimental circuit. Work of breathing was determined in the closed-circuit mode, canister included. NEDU range from Ref. 10.

and safety procedures. Each subject performed a vital-capacity maneuver through the pneumotachograph, donned a full wet suit and weights, entered the wet compartment, and connected to the mouth-piece. Water temperature was kept at 25°C.

Subjects were given a 5-min adjustment period, resting on an open system until mouth pressures and end-tidal $P_{CO_2}$ stabilized. They were instructed to position their chest relative to the water level to maintain a zero or slightly positive alveolar pressure. Alveolar pressure was read with the mouth pressure transducer while subjects held their breath keeping the glottis open.

Following the adjustment period, a rebreathing test was begun by giving the subject three breaths of 7.5% $CO_2$ in $O_2$ and then switching him to the closed system, which was canister-bypassed and already primed with the same mixture. The subject rebreathed for about 4 min until $CO_2$ concentration in the mouthpiece reached 8.5–9%. He was then switched back to an open system to breathe oxygen through the BIBS and was allowed a 3- to 5-min recovery period.

A constant-rate exercise period followed, during which the subject pedaled at 600 kpm/min and breathed through the $CO_2$-absorbent canister in an $O_2$-flushed closed system. Oxygen was added periodically to the bag to compensate for the subject's level of $\dot{V}O_2$. Once stabilized (after a period of at least 4 min), end-tidal $P_{CO_2}$ was recorded as a measure of $CO_2$ retention.

Linear regression was used to compute the scaled $CO_2$ response slope ($\Delta\dot{V}E/VC/\Delta P_{CO_2}$) for each subject. The group mean slope as well as group mean values of ventilatory characteristics and end-tidal $P_{CO_2}$ during exercise were compared to corresponding group means of nondivers and experienced divers previously reported in this laboratory (5,7). Group means were compared by the Student's *t*-test, except for the $CO_2$ response slopes, which were distributed non-normally, and were compared by the nonparametric Wilcoxon-Mann-Whitney U test. Tests were made up to the 0.10 level of significance. Individual regression lines for the $CO_2$ response ($r \geq 0.8$) were correlated with the corresponding individual degree of $CO_2$ retention.

## RESULTS

### Degree of $CO_2$ Retention

The mean group value and ventilation rate and pattern are recorded in Table II, together with corresponding values for nondivers and experienced divers exercising on land (5). Mean end-tidal $P_{CO_2}$ and ventilation rates for subjects were found to be statistically different from corresponding values for experienced divers, but not from those for nondivers. Carbon dioxide retention was virtually nonexistent, and individual values never reached the extremes seen in experienced divers.

TABLE II

Ventilatory Response to Exercise and $CO_2$ Elevation in Novice Divers Compared to Nondivers and Experienced Divers

| Variable | Novice Divers | Nondivers | Experienced Divers |
|---|---|---|---|
| $V_T$ | 2.47 ± 0.61 (58)* | 2.08 ± 0.48 (33)† | 2.64 ± 0.56 (37)† |
| f | 19.0 ± 6.3 (58)* | 20.0 ± 6.8 (33)† | 13.0 ± 3.2 (37)† |
| $\dot{V}_E/VC$ | 9.0 ± 2.2 (58)* | 8.5 ± 1.4 (33)† | 6.83 ± 1.1 (37)† |
| End-tidal $P_{CO_2}$ | 39.4 ± 4.3 (59)* | 40.7 ± 3.8 (46)† | 48.5 ± 5.6 (21)† |
| $\Delta V_E/VC/\Delta P_{CO_2}$ | 0.57 ± 0.25 (59)* | 0.59 ± 0.36 (62)‡ | 0.38 ± 0.17 (22)‡ |

Means ±SD (*n*). *This series. †From Ref. 2. ‡From Ref. 4.

## Ventilatory Response to $CO_2$ Elevation

The group mean ventilatory response to $CO_2$ is also included in Table II and, again, is compared to the corresponding value for a group of experienced divers and a pooled mean from several nondiver groups (7). The distribution pattern of the individual responses in the same three subject groups is shown in Fig. 3. Means and distribution patterns of novice divers and nondivers were not found to be statistically different.

## Correlation Between $CO_2$ Retention and Ventilatory Response to $CO_2$

A significant but weak inverse linear relation between $V_E/VC/P_{CO_2}$ and level of end-tidal $P_{CO_2}$ during exercise was found:

$$\text{End-tidal } P_{CO_2} = -7.7\ \Delta V_E/VC/\Delta P_{CO_2} + 43.1 \quad (r = 0.30;\ P < 0.01)$$

## DISCUSSION

All subjects rapidly became familiarized with the system and the tests on their one-and-only encounter. The rebreathing trial—which for convenience was contained within the same setup and protocol—could easily be isolated and conducted in the laboratory to test candidates with no diving experience. From a practical standpoint alone, the system satisfied the requirements of a rather simple test applicable to novice divers—one which could be repeated after an interval of months or years, and one which could serve as a good 1-ATA control for future studies of pressure. From a theoretical standpoint, some points follow regarding interpretation of the study results.

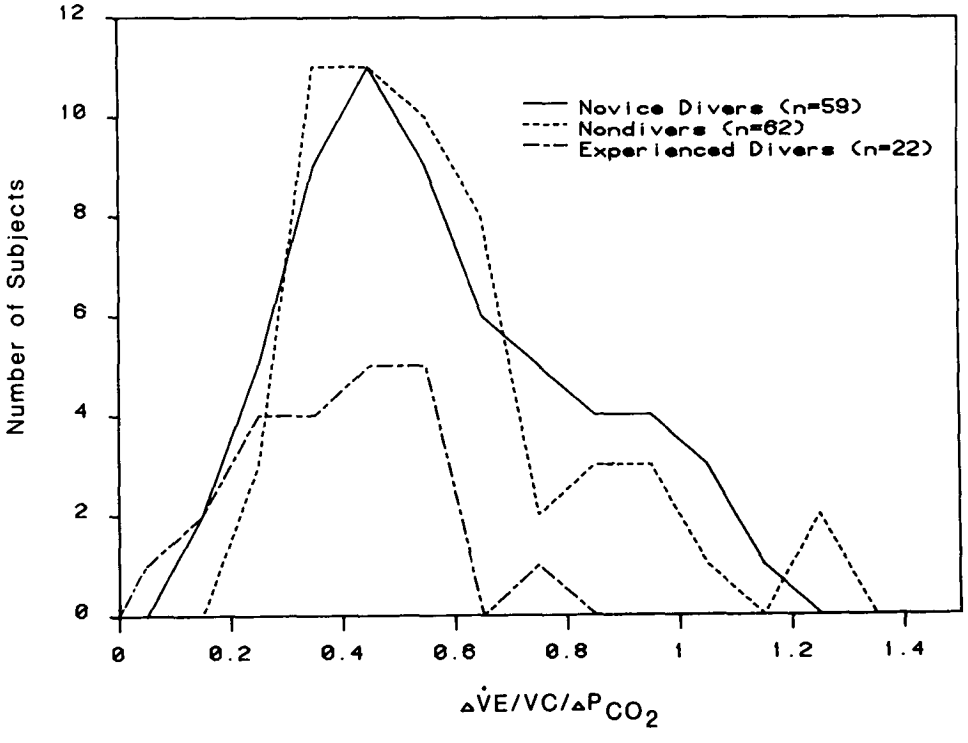



Fig. 3. Frequency distribution of $CO_2$ responses of novice divers from this series; distributions of nondivers and experienced divers from Ref. 7.

## $CO_2$ Retention

The seemingly arbitrary choice of the 600 kp m/min rate of exercise is justified because it involves a mean $V_{O_2}$ of 1.8 L/min STPD (estimated from the baseline slope of the bag-in-a-box volume trace during closed-system exercise breathing), which is near the mean value reported for sustained underwater swimming (1). In addition, it was found adequate for demonstrating $CO_2$ retention in experienced divers (5). The similarity of exercise ventilatory pattern and rate and of end-tidal $P_{CO_2}$ during exercise in novice divers and nondivers implies that $CO_2$ retention is largely a later acquisition. (This implication disregards the possibility of the canceling of an existing difference by the effects of submersion.) Another interpretation could be that only a small group of our sample of novice divers will become experienced divers. This group may already possess a degree of $CO_2$ retention that at this stage is masked by the rest of the sample. Nevertheless, diving experience, a time-dependent variable, must also be involved in $CO_2$ retention because extreme cases of this retention are common in experienced divers and lacking in novice divers.

## Ventilatory Response to $CO_2$

Regarding ventilatory response to $CO_2$, our novice divers adhered to the nondiver mean and distribution patterns, a fact that weakens the possibility of this ventilatory response as an inherent trait prevalent among diving candidates. Since the ventilatory response to $CO_2$ is less subject to conditioned modification, and since experienced divers seem to comprise a subgroup of the normal population regarding this response (7), an ongoing selection favoring the low responders might well be the more likely explanation for this phenomenon.

## Individual Correlation of $CO_2$ Retention and Ventilatory Response to $CO_2$

The weak correlation with existing retention tendency does not favor the ventilatory response to $CO_2$ as an adequate screening test for $CO_2$ retention. It is possible that a better correlation will develop at the end of this study when selection and full evolution of $CO_2$ retention have occurred. A recent outside study on 19 experienced divers (11), however, could not clearly distinguish $CO_2$ retainers in terms of their ventilatory response to $CO_2$.

In summary, at this stage of our longitudinal study, we can conclude that the ability to retain $CO_2$ takes time to develop and that a good test to screen diving candidates for future degree of $CO_2$ retention has not been found. In the likelihood that such a test is not forthcoming soon, more stress should be placed on diver training with the aim of suppressing the tendency for $CO_2$ retention. Testing for the ventilatory response to $CO_2$ should have independent value in closed-circuit diving candidates. Although it may take several years to establish a clear cause-effect relationship between diving accidents or near-accidents and low response to $CO_2$, we recommend that all diving candidates be tested and that very low responders be excluded from engaging in such diving.

## Acknowledgments

We thank Israel Shreger of INHI for his excellent technical help, and Maureen Miller, Ellen Hughes, and Janet Gaines of HMPC, NMRI for their expert editorial services.

This study was supported by the Israeli Naval Hyperbaric Institute. Dr. D. Kerem is currently participating in hyperbaric research conducted at the Hyperbaric medicine Program Center, Naval Medical Research Institute, USA.

## References

1. Lanphier EH, Camporesi EM. Respiration and exercise. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving. 3rd ed. San Pedro, CA: Best Publishing 1982:99–156.
2. Lanphier EH. Nitrogen-oxygen mixture physiology: Phase 3. Washington, DC: U.S. Navy Experimental Diving Unit, 1956. (Research report 2-56).

3. Goff LG, Brubach HF, Specht H. Measurements of respiratory responses and work efficiency of underwater swimmers utilizing improved instrumentation. J Appl Physiol 1957;10:197–202.
4. Lally DA, Zechman FW, Tracy RA. Ventilatory responses to exercise in divers and nondivers. Respir Physiol 1974;20:117–129.
5. Kerem D, Melamed Y, Moran A. Alveolar $P_{CO_2}$ during rest and exercise in divers and non-divers breathing $O_2$ at 1 ATA. Undersea Biomed Res 1980;7:17–26.
6. Florio JT, Morrison JB, Butt WS. Breathing pattern and ventilatory response to carbon dioxide in divers. J Appl Physiol: Respir Environ Exercise Physiol 1979;46:1076–1080.
7. Sherman D, Eilender E, Shefer A, Kerem D. Ventilatory and occlusion-pressure responses to hypercapnia in divers and non-divers. Undersea Biomed Res 1980;7:61–74.
8. Barlow HB, MacIntosh FC. Shallow water black-out. Alverstoke: Royal Naval Physiological Laboratory, 1944. (RNPL report 44/125 UPS 48[a])
9. Lanphier EH, Morin RA, Canty JA, Miller JN. Pressure capability of 170 atm for physiological research at Buffalo. Med Sport 1971;24:238–240.
10. Middleton JR. Unmanned evaluation of six closed-circuit oxygen rebreathers. Panama City, FL: U.S. Navy Experimental Diving Unit, 1982. (NEDU research report 3-82)
11. Hashimoto A, Daskalovic I, Reddan WG, Lanphier EH. Detection and modification of $CO_2$ retention in divers. Undersea Biomed Res 1981;8(Suppl):47.

# MIXING OF INSPIRATE IN THE LUNG WHILE BREATHING DENSE GASES, AEROSOLS, OR LIQUIDS

*H. D. Van Liew and G. L. Peer*

Our purpose is to gain perspective on the mechanisms of gas mixing in high-density, low-diffusivity environments by review of a limited number of observations with humans or animals in diverse situations. The experiments all involve measurements of inspired and expired concentrations of materials that are inert and essentially insoluble, therefore it is possible to separate mixing within the lung from gas-to-blood transport.

In normal breathing, inspired air must mix with resident gas in order for $O_2$ molecules to travel from where they are delivered by inspiratory bulk flow to capillaries in the alveolar walls; molecular diffusion is so effective that convective mixing processes play little or no role. Molecules or particles may have low diffusivity either because they are large (aerosols), or because they are immersed in a dense medium (gases in dense-gas atmospheres (1) or molecules in liquids). One can conceive of a continuum of gas-exchange possibilities from an altitude environment of 0.2 ATA of pure $O_2$, through normal air, through dense hyperbaric environments, to the extreme of gas exchange supported by dissolved $O_2$ in a medium in which diffusivity is orders of magnitude less than in a gas medium, as when degassed lungs are ventilated by water or other fluids (2–7). Half-micron aerosol particles suspended in normal air are essentially nondiffusible markers in a normal medium (8–11).

## Alveolar Gas and Deadspace

The first gas expired from a person's lung is the same as the inspirate because it resided in the central airways at end-inspiration. Figure 1 shows results of a simulation of diffusion of $O_2$ in a lung-shaped vessel in a normal air environment. In the computer model, each of 23 generations of branching

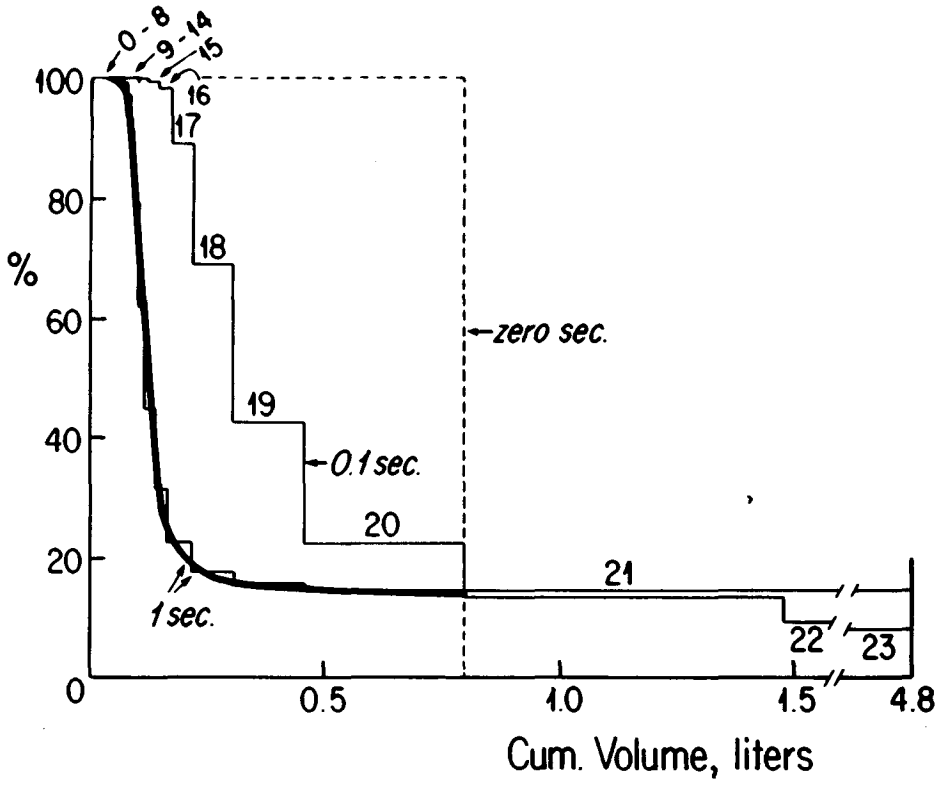



Fig. 1. Profiles of concentration (relative to inspired concentration) vs. depth in the lung airways (expressed as cumulative volume from mouth to the airway in question). For this computer simulation, indicator gas was originally in generations 1 through 20 (*dashed rectangle*). *Stairstep shapes* show concentrations in each generation (generation numbers *above the steps*). *Solid curve* is a simulation of the 1.0-s time interval by other authors (13).

is represented as a compartment having dimensions which conform to one generation according to the morphometric equations of Weibel (12). The figure simulates the case that an indicator gas could be introduced, without mixing, into the central 20 generations of airways. After only 0.1 s, it is clear that gas has diffused from generations 17 through 20 into higher-numbered generations, and after 1.0 s, the profile shows unmixed indicator only in the central *deadspace* generations and a flat *alveolar plateau* throughout all the rest of the lung volume.

Figure 1 illustrates the major manifestation of diffusion in the lung: diffusion moves inspired gas into the well-mixed gas that resides in the peripheral airspaces. Wherever the interface between inspirate and residual gas is located, and despite the fact that it may be blurred by mixing processes that occur during inspiration, diffusion will remove gas from the central airways

and thereby move the interface into the central airways. Peripheral airways are short and have large, summed cross-sectional areas which aid diffusion and cause the flattening of the concentration along the peripheral airway path. In contrast, central airways have long lengths and small summed cross-sectional area. In 1 or 2 s, the interface reaches a level where these hindrances to diffusion are so great that the mouthward progression becomes negligible. An unrealistic aspect of the Fig. 1 simulation, that there is neither convective nor diffusive mixing during inspiration, makes little difference to the final outcome. A second unrealistic aspect of the simulation, that there is no provision for differences of gas distribution between parallel pathways in the lung, also makes little difference for our present discussion since concentration differences between paths in healthy individuals are small compared to differences between unmixed deadspace gas and mixed alveolar gas.

## Impaired Diffusion

Molecular diffusivity determines the speed of the mouthward movement of the interface; if diffusivity were one tenth of normal, the profile for 1.0 s would resemble the 0.1-s profile of Fig. 1. In the times of normal breathing, low diffusivity will slow the mouthward movement of the interface and, because more of the gas remains unmixed in central airways, lower the level of the flat part in the peripheral airways. The logical limit when diffusivity is very low would be a front of inspirate at the generation where volume is big enough to hold the inspirate (the *dashed rectangle* in Fig. 1).

Figure 2 shows concentration patterns during exhalation after single half-litre inhalations of marker substances. Concentrations of the substances, in percent of the concentration that was inhaled, are plotted against volume that has been exhaled at any time during the breath.

The *solid "normal" curve* resembles an upside-down plot of $CO_2$ vs. volume or time and closely resembles the intra-lung profile of Fig. 1, as expected if gas from peripheral airspaces follows that from central airways during exhalation. The curve is representative of data obtained in normal people in normal environments with all gases (14), including a light gas, helium (He), and a heavy gas, sulphur hexafluoride ($SE_6$—a synthetic gas with molecular weight of 144). Concentration soon falls to an essentially flat alveolar plateau at a value of about 10% (because the 0.35 litre [L] of test gas that reaches the alveolar regions mixes with about 3.5 L of gas in the subject's functional residual capacity (FRC)). The plateau continues unchanged when the person exhales his expiratory reserve volume (to the *right of the vertical line* at 0.5 L).

When subjects breathed a mixture which contained small amounts of foreign gases in a hyperbaric chamber (14), we found that exhaled patterns of He and $SF_6$ at 9.5 ATA (*curves labelled He and $SF_6$* in Fig. 2) were markedly different from their behavior at normal pressure and from the behavior of each other. Neither gas fell to the alveolar plateau as rapidly as in the normal case, and $SF_6$ concentration continued to fall throughout the breath.

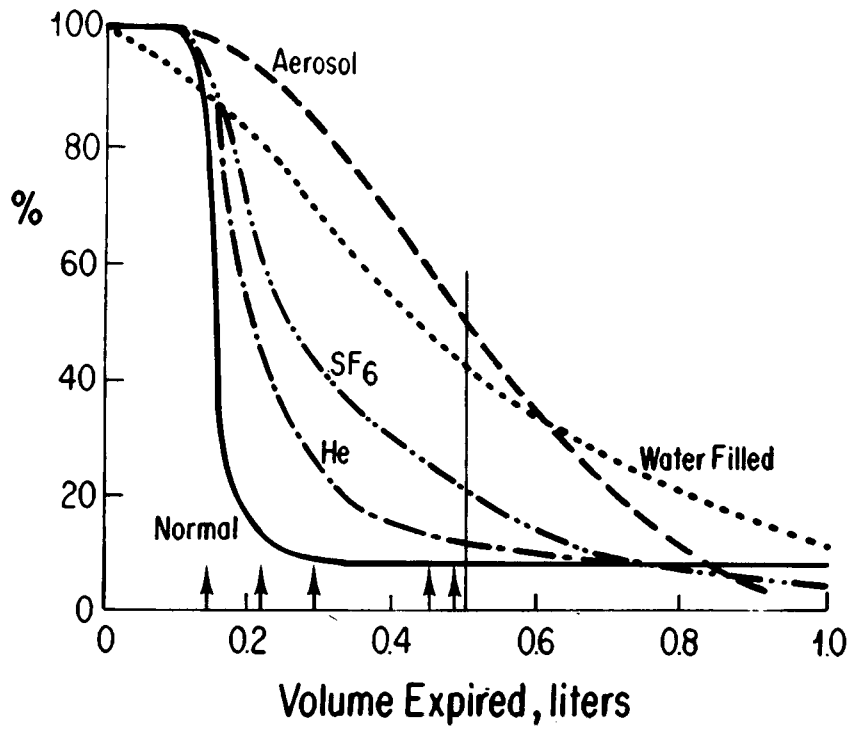



Fig. 2. Exhaled patterns of marker substances which have entered the lung in a single inhalation; the plot shows concentrations of the markers measured at the mouth as the exhalation proceeds. Data abstracted from Refs. 6, 10, and 14.

The *dotted Water Filled curve* is from an experiment in which single inhalations of various marker substances dissolved in saline were pumped into excised dog lungs that had been filled with saline (6). For the figure here, data on mixing of labelled albumen has been scaled to make an appropriate comparison with the human experiments. The pattern for the marker material in the water-filled lung is reminiscent of the gas data, but the transition between inhaled concentration (100%) and alveolar concentration extends over the entire range of the exhalation; concentration is between 40 and 50% when the tidal volume has been exhaled (*vertical line* at 0.5 L).

The *dashed curve* shows the pattern obtained when men at normal pressure take one breath of air which contains aerosol particles of 0.5 microns diameter (10). It is almost the same as the pattern for the water-filled lung; aerosol reaches 50% concentration almost exactly at the *vertical line,* where the lung is back to its normal resting volume.

## Deadspace

In the *Fowler* method for estimation of deadspace, a line is dropped through the midpoint of the fall from inhaled level to the alveolar plateau (15).

*Small vertical arrows* on the horizontal axis of Fig. 2 show results of such a procedure. Deadspaces for the normal, He, and $SF_6$ curves are 150, 220, 300 mL, respectively, and deadspaces for the water-filled and aerosol curves are nearly equal to the inhaled volume.

Figure 2 allows the conclusion that poor mixing caused by low diffusivity is manifested at the beginning of the expiration as if there were an enlarged deadspace. In the water-filled lung and with the aerosol there is some exchange of the material with the lung residuum since not all the inspirate was recovered when the volume is back to where the inspiration started (*vertical line*). For the water-filled and aerosol cases, much of the marker material was not irreversibly mixed in the lung; most of the inhaled material could be retrieved by a deep exhalation.

## Volume-Dependence

Normally, the amount of inspirate that is retained in the lung depends strongly on the volume of the breath relative to the functional residual capacity (FRC) in which the inspirate becomes diluted (16). Consider the case of perfect mixing, except for the deadspace, between one breath of inspirate and the residual lung contents. The absolute amount of some foreign indicator material left in the lung, after the mixing and after expiration (which removes part of the mixture), can be derived from simple mass-balance formulas

$$A_I = C_I V_A = C_M(V_A + FRC) \qquad (1)$$

in which $A_I$ is amount of the indicator inspired into the alveolar spaces, $C_I$ is inspired concentration of the indicator material, $C_M$ is mixed concentration of the indicator material (the flat part on the concentration vs. volume plot), $V_A$ is the inspired volume (corrected for deadspace) of the mixture that contains the indicator, and FRC is the volume of the lung before the breath was taken. The amount of indicator, A, that remains in the lung after the breath, relative to $A_I$, is

$$A/A_I = C_M FRC/C_I V_A = FRC/(V_A + FRC) \qquad (2)$$

Thus both $V_A$ and FRC have strong influence on retention.

*Equation 2* does not apply when diffusivity is very low since, as seen in Fig. 2, there is no longer a flat part of the concentration-vs.-volume curve which reflects the concentration in the FRC after the breath. The amount mixed becomes much more dependent on blurring of the deadspace/alveolar gas interface caused by convective processes (17). The convective processes undoubtedly occur during breathing of normal gases, but are inconsequential because their contributions are masked by diffusive mixing.

## Fate of Inspired Material

It is of interest to account for the total amount of an inert gas or other material which is inspired. Some remains unmixed in the deadspace and some

becomes mixed with the residual gas which was present before the inspiration. Some of the material leaves the lung during exhalation: that in the deadspace and that in the portion of the mixed volume which becomes the alveolar plateau. Only the portion of the material that is retained in the FRC is actually *exchanged* during the breath.

Figure 3 illustrates how various-sized breaths of pure indicator material would be partitioned in the ideal case that mixing between inspirate and FRC were perfect except for an unchanging deadspace. None of the material would be retained in the FRC when breath size is less than the deadspace, and small breaths differ from larger ones in that less of the total material is exhaled as the alveolar plateau. In normal breathing, inspired gases behave much like this ideal case (18).

The partitioning changes when diffusivity of the inspired material is low. Figure 4 shows retention data plotted on the Fig. 3 coordinate system after appropriate scaling for differences of volumes and for the fact that the inspirate was not pure indicator material. Points for retention of helium and $SF_6$

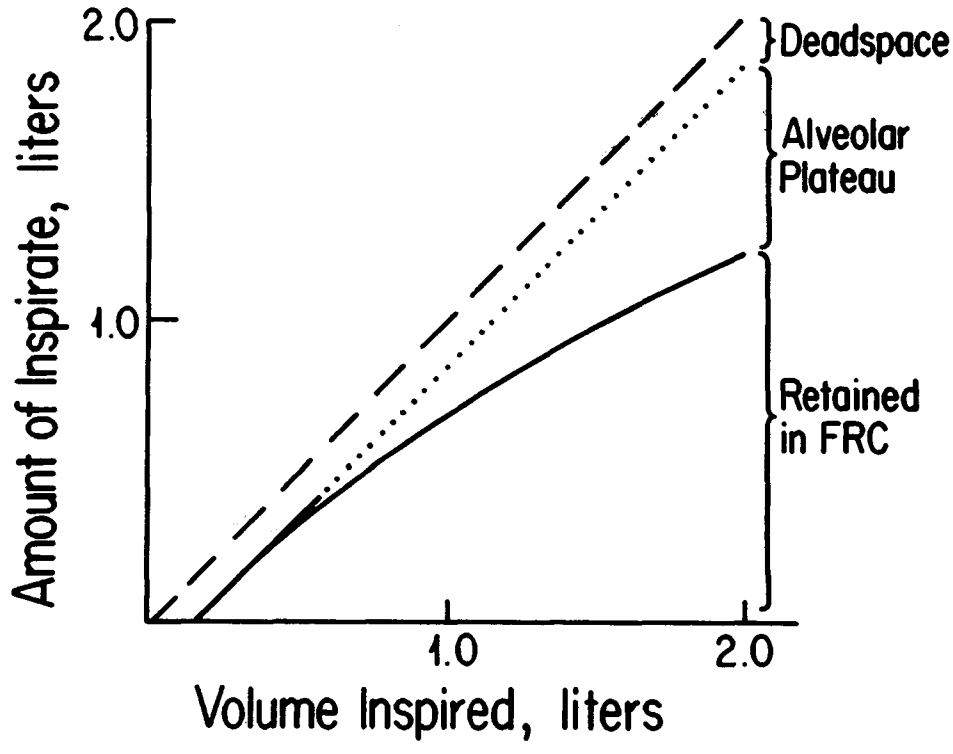



Fig. 3. Partioning of inspirate. Theoretical amounts of inspirate found in each category are plotted *above each other* as functions of volume inspired. Lung assumed to have a deadspace of 150 mL and FRC of 3.5 L.

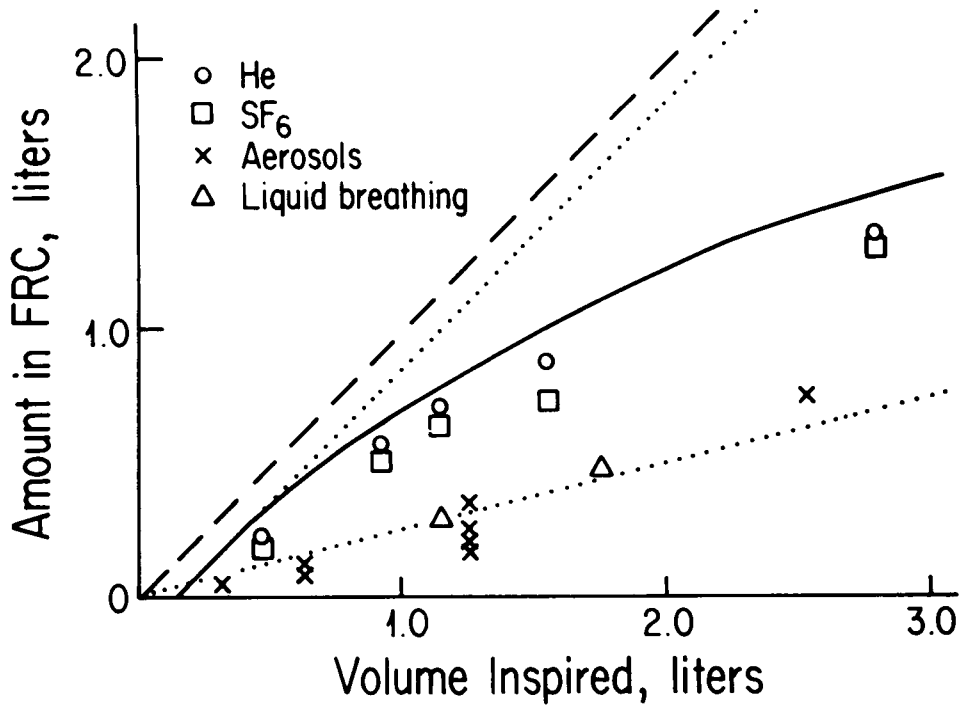



Fig. 4. Amounts of various kinds of inspired materials that remain in the FRC as a function of lung volume. *Axes* are similar to those in Fig. 3 and the *curves* of Fig. 3 are reproduced. For aerosol data, amounts in the FRC and inspired volumes have been scaled for comparison with a person having 3.5 L FRC. Water-breathing data have been scaled appropriately to allow comparison between excised dog lungs and a person with 3.5-L FRC. *Lowest dotted line* shows locus of points for 25% of the inspirate retained in the FRC.

from subjects who breathe at 9.5 ATA (14) are close to the solid theoretical curve, but $SF_6$ is always below helium. Points for half-micron aerosols at 1 ATA (10) and for liquid breathing (6) shows retentions of about 25% of the inspirate (*lower dotted line*), well below the theoretical curve.

## Mixing from Deadspace to FRC

Is convection ever more important than diffusion for mixing of ordinary gases in a normal environment? We did a few experiments (unpublished) to extend the finding that gas exchange occurs even when the volume of inspired gas is less than the volume of the deadspace (19). Three subjects at normal pressure took small breaths (38–550 mL), starting from FRC, of an air-like mixture (5% each of He, Ne, Ar, and $SF_6$; 21% $O_2$, remainder $N_2$). They either exhaled immediately to residual volume (RV) or held the breath for 10 or 30 s and then exhaled to RV. The subjects breathed into a bag-in-box

arrangement to monitor volume, and gas concentrations were continuously measured at the mouth with a mass spectrometer. Data recorded on a polygraph were read by eye. Twenty nonbreathhold and 11 breathhold breaths were analyzed by studying the pattern of concentration vs. expired volume and of concentration in the FRC (estimated from mean concentration in the expiratory reserve volume).

In all cases the patterns resembled the aerosol and liquid-breathing examples of Fig. 2 in that the fall of concentration from inspired level to plateau level spread over a volume. In breaths below 130 mL, the concentration was about 50% of the inspired concentration when a volume equal to the inspired volume had been exhaled; in larger breaths, concentration was below 50%. When volume inspired was below 100 mL, concentration in the FRC was zero; apparently despite spread of the front over a volume in the airway tree, the penetration was not deep enough for diffusion to spread the inspirate on throughout the rest of the lung volume in the manner that was illustrated in Fig. 1. There were no important differences in concentrations of the various indicator gases, either with or without breathholding, an indication that the gas probably entered the FRC during inspiration and the subsequent breathhold by convective means.

Figure 5 summarizes the results. Concentration of Ne (neon) in the FRC (*left axis*) is plotted against volume inspired. There were significant amounts of inhaled gas remaining in the FRC except at the very lowest inspired volumes, and the amount increased with a breathhold. The *axis at the right* shows the approximate amounts of inspirate retained in the FRC for comparison with Figs. 3 and 4; the axis was obtained by multiplying scaled concentration by FRC volume.

## Mixing in Central Airways

If it is true that diffusive mixing is virtually nil in the water-filled lungs and for the heavy aerosol particles, characteristics of convective processes can be studied with these low-diffusion cases. Aerosol data suggest that time increases in importance when mixing is predominantly convective. Figure 6 shows data for aerosol retention in the FRC (11) obtained in various combinations of large and small breaths and fast and slow inhalations. When replotted as a function of the duration of inhalation, variability due to differences of volume and speed of inhalation reduced to the simple straight-line functions seen; retention of larger particles exceeded that of small ones, but the two sizes appear to have the same *intercept* on the *vertical axis*.

From data of Fig. 5, we can estimate the rate of transfer of inspirate from central airways to the rest of the lung. Vertical difference between the immediate exhalation and breathhold curves is 61 mL (*read right axis*), except at very low volumes, so for the 10-s breathhold, rate was 6 mL/s for these small breaths between 0 and 500 mL. This must be considered a low estimate; data for 30 s were similar to that for 10 s, suggesting that the mixing process was essentially complete in less than 10 s. Data from Sikand et al. (20) on change

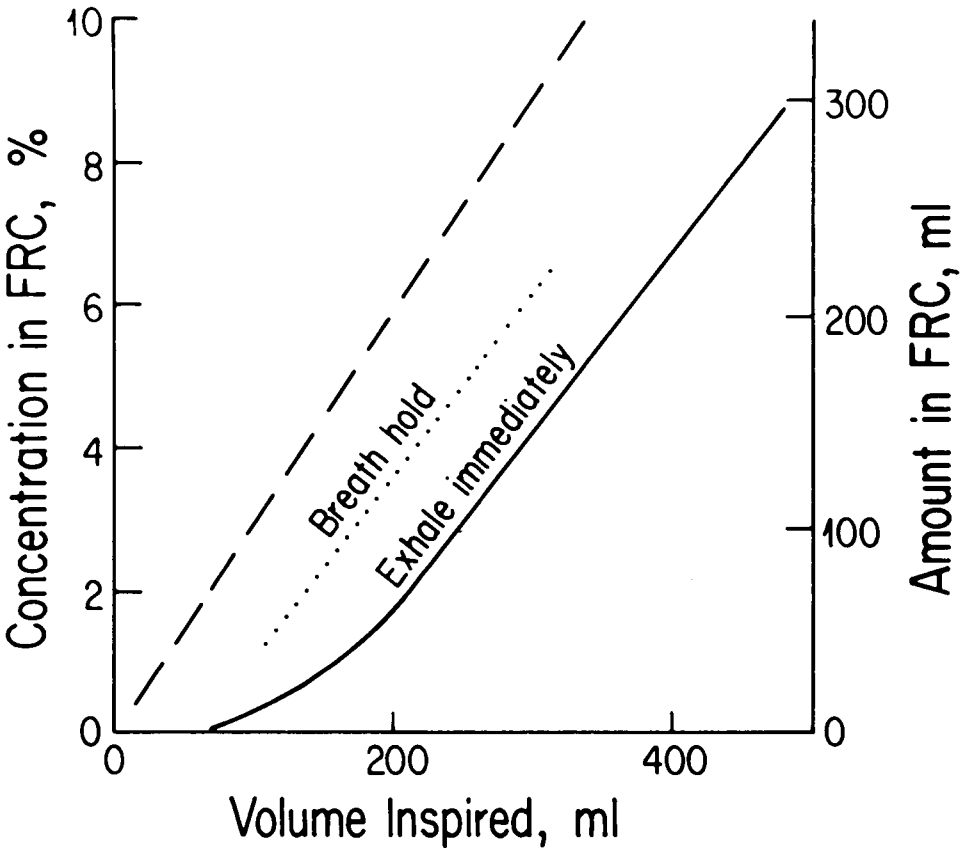



Fig. 5. Concentration of a foreign gas, neon (Ne), in the FRC after small breaths of varying size. *Left vertical axis* is concentration relative to inspired concentration; *right vertical axis* is estimate of amount of inspirate that was in the FRC. *Solid curve* is based on 20 breaths without breathholds; *dotted line* is based on 11 breaths with 10- and 30-s breathholds. *Dashed line* shows the impossible perfect-mix case in which all the inspirate would be retained in the FRC.

of deadspace yields a comparable value of 12 mL/s for 1-L breaths based on 4 s of breathholding. These values can be compared with rates derived from aerosol data where transfer is predominantly due to convective processes. In Fig. 6 for 0.5-, 1.0-, and 2.0-L breaths, the rates are 12, 25, and 50 mL/s, respectively. Finally, these rates can be compared with the average rate of transfer of gas into the FRC that occurs in normal ventilation of the alveoli. An alveolar ventilation of 4 L/min, typical for a person at rest, converts to 67 mL/s. Thus mixing from central airways to the FRC occurs at a rate that is equivalent to 10% or more of ventilation required for metabolic needs. However, with gases in our study and Sikand's the transfer soon ceased; exchange could be sustained only if there were continual replenishment of

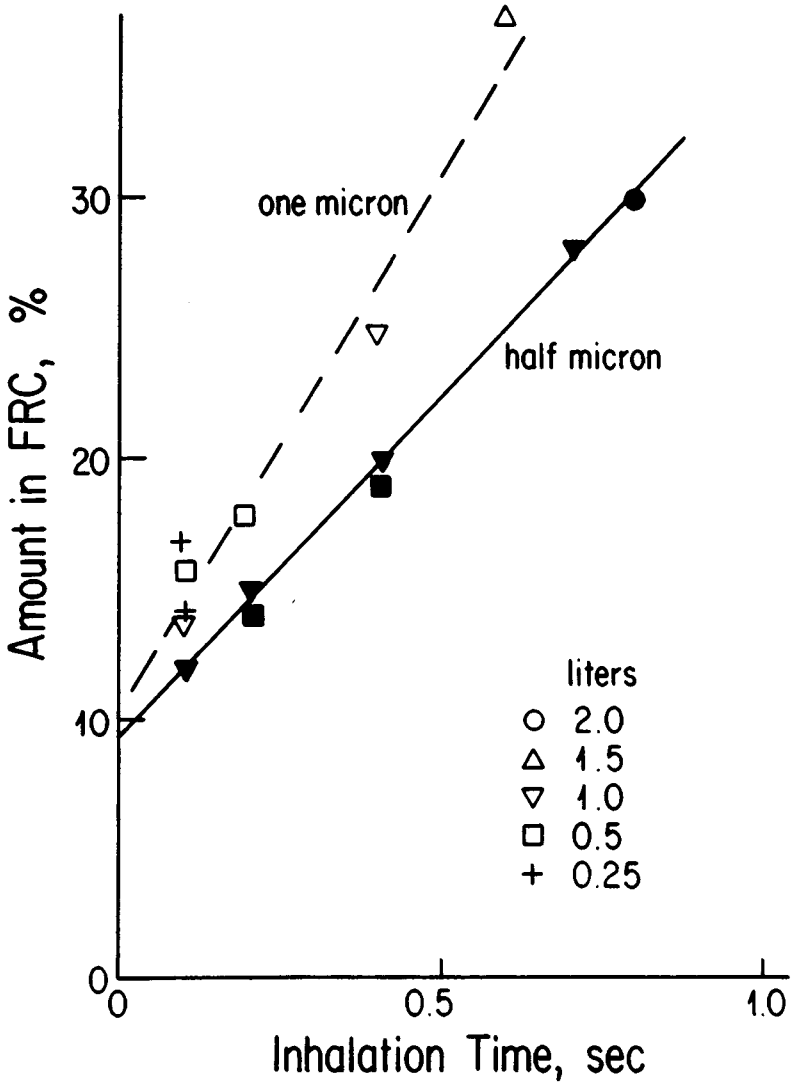



Fig. 6. Time dependence of aerosol transfer from inspirate to FRC (replotted data of Taulbee, Yu, and Heyder, Ref. 11). Percentages of the inspired amounts of half-micron and one-micron particles that remain in the FRC after the breath are plotted as a function of the duration of inhalation.

inspirate at an airway that was deep enough to allow mixing to the rest of the lung.

## SUMMARY

1) Inspired material remains in the lung after a breath because of mixing by two time-dependent processes: diffusion and convection.

2) Normally, diffusive mixing is so rapid that time and convective mixing are practically irrelevant; for any breath duration, only the small volume of gas in the central-airway deadspace remains unmixed, so amount of inspired gas that remains in the FRC after the breath depends almost exclusively on volumes of the breath and the FRC.

3) In diverse situations in which diffusive mixing is poor, exchanges between inspired material and resident material in the lung have similar characteristics; a larger-than-normal portion of the breath at the beginning of expiration is unmixed or poorly mixed, as if the deadspace were enlarged.

4) When diffusion is impaired, mixing between the inhaled and resident material can occur by convective mixing; time for mixing becomes an important issue and volumes of inspirate and FRC decrease in importance.

## Acknowledgment

Supported in part by HLBI Grant Po1-HL-14414.

## References

1. Van Liew HD, Paganelli CV, Sponholtz DK. Estimation of gas-phase diffusivities in hyperbaric environments. Undersea Biomed Res 1982;9:175–181.
2. Kylstra JA. Liquid breathing. Undersea Biomed Res 1974;1:259–269.
3. Kylstra JA, Schoenfisch WH, Herron JM, Blenkarn GD. Gas exchange in saline-filled lungs of man. J Appl Physiol 1973;35:136–142.
4. Kylstra JA, Paganelli CV, Lanphier EH. Pulmonary gas exchange in dogs ventilated with hyperbarically oxygenated liquid. J Appl Physiol 1966;21:177–184.
5. Matthews WH, Balzer RH, Shelburne JD, Pratt PC, Kylstra JA. Steady-state gas exchange in normothermic, anesthetized, liquid-ventilated dogs. Undersea Biomed Res 1978;5:341–354.
6. West JB, Dollery CT, Matthews CME, Zardini P. Distribution of blood flow and ventilation in saline-filled lung. J Appl Physiol 1965;20:1107–1117.
7. West JB, Maloney JE, Castle BL. Effect of stratified inequality of blood flow on gas exchange in lqiuid-filled lungs. J Appl Physiol 1972;32:357–361.
8. Altshuler B, Palmes ED, Yarmus L, Nelson N. Intrapulmonary mixing of gases studied with aerosols. J Appl Physiol 1959;14:321–327.
9. Cumming G, Horsfield K, Jones JG, Muir DCF. The influence of gaseous diffusion on the alveolar plateau at different lung volumes. Respir Physiol 1967;2:386–398.
10. Muir DCF. Distribution of aerosol particles in exhaled air. J Appl Physiol 1967;23:210–214.
11. Taulbee DB, Yu PC, Heyder J. Aerosol transport in the human lung from analysis of single breaths. J Appl Physiol: Respir Environ Exercise Physiol 1978;44:803–812.

12. Weibel ER. Morphometry of the human lung. Berlin: Springer-Verlag, 1963.
13. LaForce RC, Lewis BM. Diffusional transport in the human lung. J Appl Physiol 1970;28:291–298.
14. Van Liew HD, Thalmann ED, Sponholtz DK. Hindrance to diffusive gas mixing in the lung in hyperbaric environments. J Appl Physiol: Respir Environ Exercise Physiol 1981;51:243–247.
15. West JB. Respiratory physiology—the essentials. 2nd ed. Baltimore: Williams & Wilkins, 1979, p. 18.
16. Van Liew HD, Sponholtz DK. Effectiveness of a breath during exercise in a hyperbaric environment. Undersea Biomed Res 1981;8:147–161.
17. Scherer PW, Haselton FR. Convective exchange in oscillatory flow through bronchial-tree models. J Appl Physiol: Respir Environ Exercise Physiol 1982;53:1023–1033.
18. Van Liew HD, Giglia JS, Lapennas GN. Concentration of an inert, insoluble gas in the FRC after a single breath. Fed Proc 1983;42:1349.
19. Briscoe WA, Forster RE, Comroe JH Jr. Alveolar ventilation at very low tidal volumes. J Appl Physiol 1954;7:27–30.
20. Sikand RS, Magnussen H, Scheid P, Piiper J. Convective and diffusive gas mixing in human lungs: Experiments and model analysis. J Appl Physiol 1976;40:362–371.

# INVITED REVIEW: CONCEPTS OF VENTILATORY AND RESPIRATORY GAS HOMEOSTASIS IN SIMULATED UNDERSEA EXPOSURE

*R. Gelfand*

From the beginning of research on ventilatory effects of exposure to the undersea environment, emphasis has been on decrements and limitations of ventilation and metabolic gas exchange during muscular exercise and in pulmonary function studies related to maximum ventilatory capacity (1–18). This is natural, since the purpose of operational diving is to do useful work, and increase in pulmonary flow resistance and decrease in maximum ventilatory capacity were recognized early in diving research as probable factors limiting work-depth capability in dense atmospheres. In contrast, relatively few studies have been concerned principally with effects of undersea-related respiratory stress on ventilatory and respiratory gas homeostasis (1,7,10,19–25), or with respiratory $CO_2$ reactivity (3,11,21,26–29), in states of rest.

With the advent of prolonged-exposure diving of all types and saturation diving in particular (Fig. 1), intermittent periods of physical exertion are interposed with more extensive periods of time in states of light activity, rest, and sleep. Just as ventilatory insufficiency must occur with combinations of exercise level and gas density, which result in intolerable increase of arterial $P_{CO_2}$, it is inevitable that ventilatory insufficiency and failure must occur even in states of rest when respiratory $CO_2$ reactivity is virtually abolished by high levels of inspired gas density (28,29). It is important to recognize that ventilation during physical exertion involves stimuli to breathing that are not available at rest. As depicted in Fig. 2, stimuli to ventilation vary with state of physical activation. Should ventilatory inadequacy develop during work, retreat to a state of lesser exertion or rest with decreased ventilatory requirement is usually possible and can be prompt. In contrast, relief of ventilatory

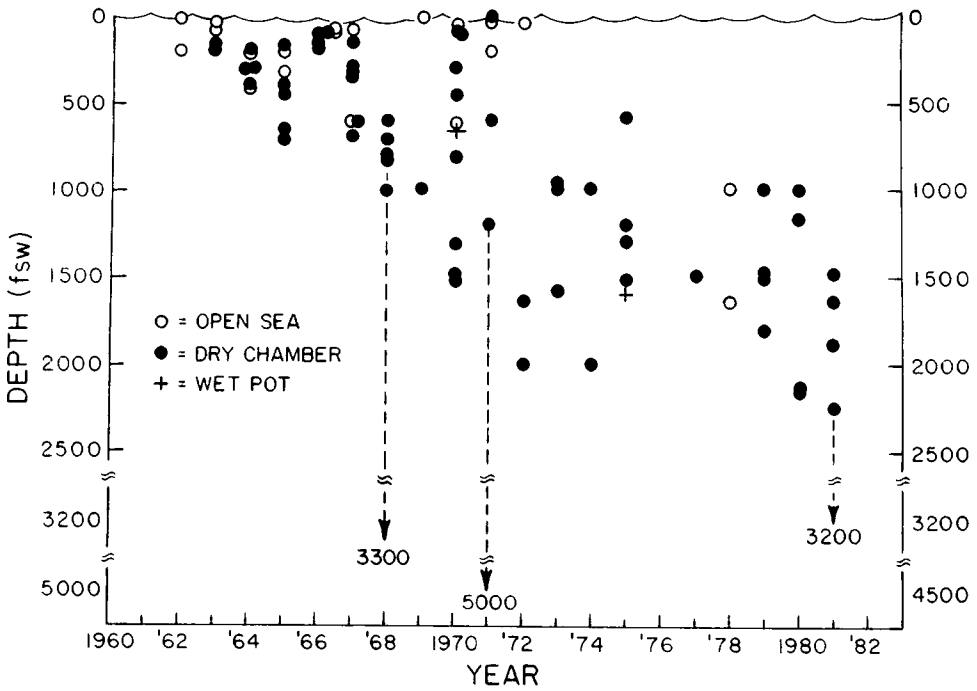



Fig. 1. Evolution of human saturation pressure exposures in the laboratory and open sea (operational diving excluded). *Dashed vertical lines* indicate "double simulations," i.e., dry laboratory chamber simulation of descent under water *and* simulation of He-$O_2$ gas density at pressures greater than actual exposure pressure through use of inert gas higher in molecular weight than helium. Depths indicated *below the dashed lines* are density equivalents of normoxic He-$O_2$ obtained in 1968 by use of Ne-$O_2$ (8,15); in 1971 by use of Crude Ne-$O_2$ (12,14,29); in 1981 by use of He-$N_2$-$O_2$ (33,34). Adapted from Lambertsen et al. (42).

difficulty occurring in a rest state must be gradual, possibly limited to the slow process of decompression from a saturation exposure (30).

## INTERACTION OF FACTORS IN LIMITATION OF EXERCISE CAPABILITY

The nature and relative quantitative roles of ventilatory stimuli elicited by muscular exercise remain in dispute among physiologists (31,32). Whatever their origins, their net effect is to adjust ventilation to metabolic need during exertion. However, the effectiveness of these stimuli in driving ventilation to satisfy metabolic needs can be reduced in simulated and real undersea exposures. Several different symptoms have been associated with limitation of exercise in dry chambers (Fig. 3, *top section*). Nitrogen narcosis (12), high gas density (12), and high ambient pressure (33,34) have each been considered *dominant* factors in cessation of rhythmic leg exercise in dry chambers.

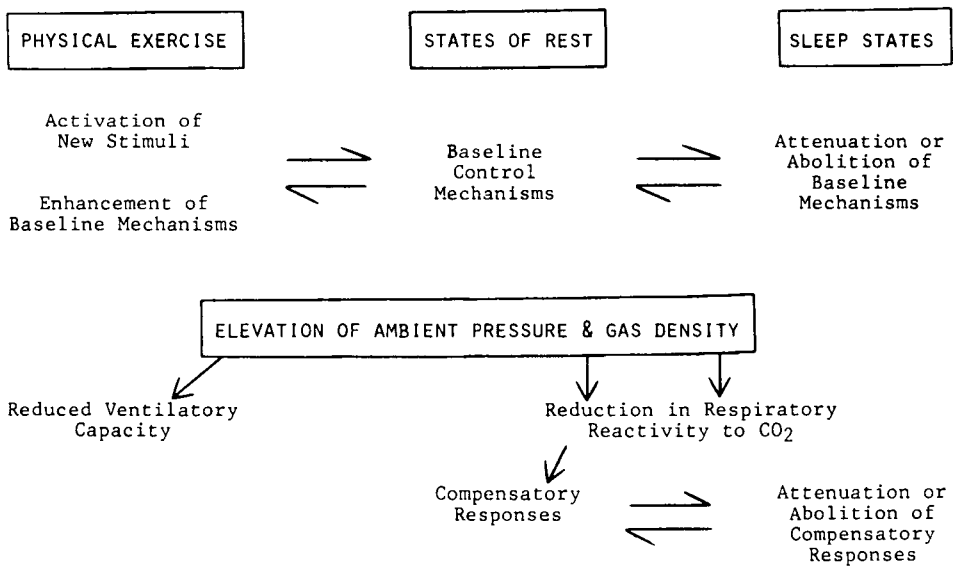



Fig. 2. Stimuli to ventilation differ in kind and in degree according to states of physical activation. Elevation of pulmonary gas-flow resistive loading by high gas density reduces maximum ventilatory capacity available during physical exertion, while exercise stimuli to breathe remain fully available. Transition from exercise to rest reduces ventilatory requirement, but respiratory $CO_2$ reactivity is attenuated. Transition to sleep states further reduces ventilation and respiratory reactivity to $CO_2$. Sleep also attenuates or abolishes favorable compensatory response to the loading by high gas density.

However, in no case were these factors present alone (Fig. 3), and interaction with secondary factors must have been involved as well in the genesis of the limiting symptoms.

Furthermore, useful work does not usually involve continuous rhythmic leg exercise on bicycle ergometers. Intermittent work at useful intensities should be possible for healthy men even when peak pulmonary ventilatory capacity is reduced by increase of pulmonary gas-flow resistance. As shown in the *bottom section* of Fig. 3, *nonrhythmic whole-body work* on a simulated oil wellhead in a wet chamber was completed without symptoms (35), whereas dyspnea was reported at comparable exposure conditions in two other wet-chamber "dives" during rhythmic leg exercise (17,18).

Additional factors that may affect tolerance to physical exertion include static lung loading (36), individual differences in physical conditioning, previous experience with exposure conditions, apparatus gas-flow resistance, and apparatus peak-flow characteristics.

## FACTORS CHALLENGING RESPIRATORY HOMEOSTASIS IN REST STATES AT ELEVATED PRESSURES AND GAS DENSITIES

Factors that can influence the effectiveness of respiratory homeostatic mechanisms involved in maintenance of oxygenation and acid-base balance

| INTERACTING FACTORS | GAS INSPIRED | SIM. DEPTH FSW | DENSITY G/L | CHAMBER DRY/WET | EXERTION TYPE | LIMITING SYMPTOM |
|---|---|---|---|---|---|---|
| Narcosis | | | | | | |
| Density $CO_2$ | $N_2O_2$ | 400 | 14.5 | dry | rhythmic leg | muscular incoordination |
| Density | | | | | | |
| $CO_2$ | $Ne/He/O_2$ | 1,200 | 25.2 | dry | rhythmic leg | breathlessness |
| Pressure | | | | | | |
| Density Narcosis | $He/N_2/O_2$ | 2,132 | 16.1 | dry | rhythmic leg | dyspnea |
| Pressure | | | | | | |
| Density | $He/O_2$ | 1,400 | 7.3 | wet | rhythmic leg | dyspnea |
| Pressure | | | | | | |
| Density | $He/O_2$ | 1,600 | 7.7 | wet | rhythmic leg | dyspnea |
| Pressure | | | | | | |
| Density | $He/O_2$ | 1,610 | 8.9 | wet | non rhythmic whole body | none |

Fig. 3. Interaction of factors limiting physical exertion in simulated undersea exposure. Experiments represented (*from top to bottom*) can be found in Refs. 11, 12, 33, 18, 17, and 35.

during states of rest in the undersea environment are summarized in Fig. 4. Increase in gas density and the associated increase in pulmonary gas-flow resistance are consequences of compression. Hydrostatic pressure influences on excitable tissues may include respiratory structures in the central nervous system (CNS). Nitrogen narcosis is inevitable during compressed air and $N_2$-$O_2$ diving, and is a consequence of the use of $N_2$ as an additive in He-$O_2$ diving. Sustained low-level hyperoxia is frequently used during compression and at stable high pressure. It is used to a greater degree during decompression and in therapy of decompression sickness at any pressure. Acute hypoxia can be accidental or a consequence of ventilatory insufficiency from any cause.

Although a single dominant stress such as high gas density may not itself result in respiratory difficulty during a particular exposure, interaction with others may precipitate it (30). Such difficulty may appear to occur at random, because not only are there individual differences in response to identical stresses, but actual tolerance limits may be duration-related, varying even in the course of a day due to changing states of wakefulness and fatigue. Sleep itself may be deleterious in extreme exposure conditions, when compensatory reserve is already severely diminished, and compensatory responses to elevated pressure and pulmonary gas-flow resistance may be attenuated or abolished.

## INFLUENCES OF HIGH GAS DENSITIES ON THE VENTILATORY APPARATUS

### Pulmonary Gas-Flow Resistance

Pulmonary gas flow occurs in response to changes in intrapulmonary pressure acting on pulmonary impedance. Cyclic flow during ventilation is a

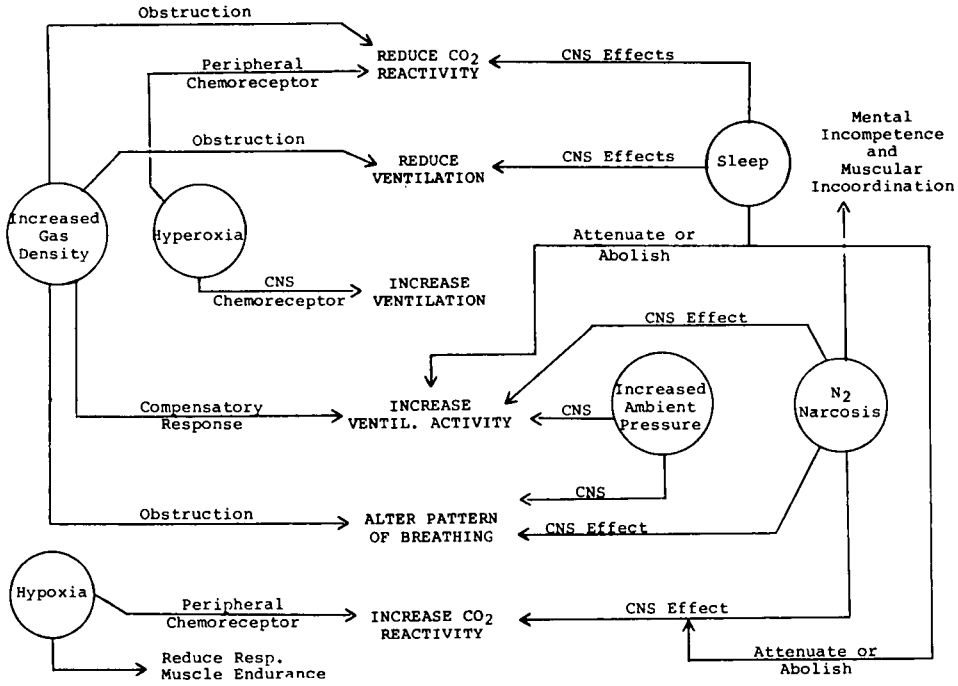



Fig. 4. Factors affecting respiratory homeostatic capability in rest states during simulated undersea exposure. Interaction of responses to several factors makes identification and quantitation of individual relationships difficult.

complex function of flow rate, airway geometry, lung volume, gas properties (density, viscosity), and flow direction (37). Increase of ambient pressure, while it raises gas density, has virtually no effect on viscosity of a particular gas (38), but change of gas composition can. Density alone appears to suffice in explaining decrements of maximal flows in studies of forced pulmonary maneuvers over ranges of raised ambient pressure. Thus, there was no discernible effect of gas viscosity on pulmonary mechanics when gases with different viscosity were used during tests of pulmonary function (8,14,15) and exercise tolerance (12) over wide ranges of gas density. Under the conditions of those experiments, turbulent flow can be considered to persist throughout all airways (and during the entire breathing cycle). However, as will be seen in a later section, although gas density alone may be the appropriate index of pulmonary gas-flow resistance when ventilation is at high levels as in maximal voluntary maneuvers and during vigorous exercise, the reciprocal of kinematic viscosity (ratio density/viscosity) appears to be the valid index during lower ventilatory flows associated with inhalation of $CO_2$ and during $CO_2$-free breathing.

Respiratory resistance appears to be a linear function of gas density at low flow rates of 1 L/s and 2 L/s to densities of 10 g/L (*see* Ref. 39 for a

summary and list of citations) and to 25 g/L (14). However, respiratory resistance is a nonlinear function of gas density at high flow rates of maximum voluntary ventilation (unpublished observations, *Predictive Studies III*, Ref. 12).

## Work of Breathing

As pulmonary gas-flow resistance increases due to elevation of gas density, so should the work of breathing increase. However, a distinction must be made between the specific work required to move a unit volume of gas and the actual total work of breathing over time. Two studies illustrate this point. The oxygen cost of breathing a mixture of 7% $CO_2$/73% $SF_6$/20% $O_2$ was about 50% higher than when 7% $CO_2$/air was breathed at essentially the same ventilation (40). As expected, more energy was consumed in respiring the denser gas mixture. In a different study (41), the total work of breathing *decreased* at 3 and 6 ATA air compared to 1 ATA air both during maximal exercise and the 15-s maximum voluntary ventilation maneuver, while, consistent with the earlier study (40), the work per litre of gas respired increased (*see* Fig. 5).

Figure 5 shows work of breathing during maximal exercise as measured by Hesser et al. (41) expressed analytically and extrapolated by computation to 25 g/L gas density. The extrapolation predicts work of breathing to be less at

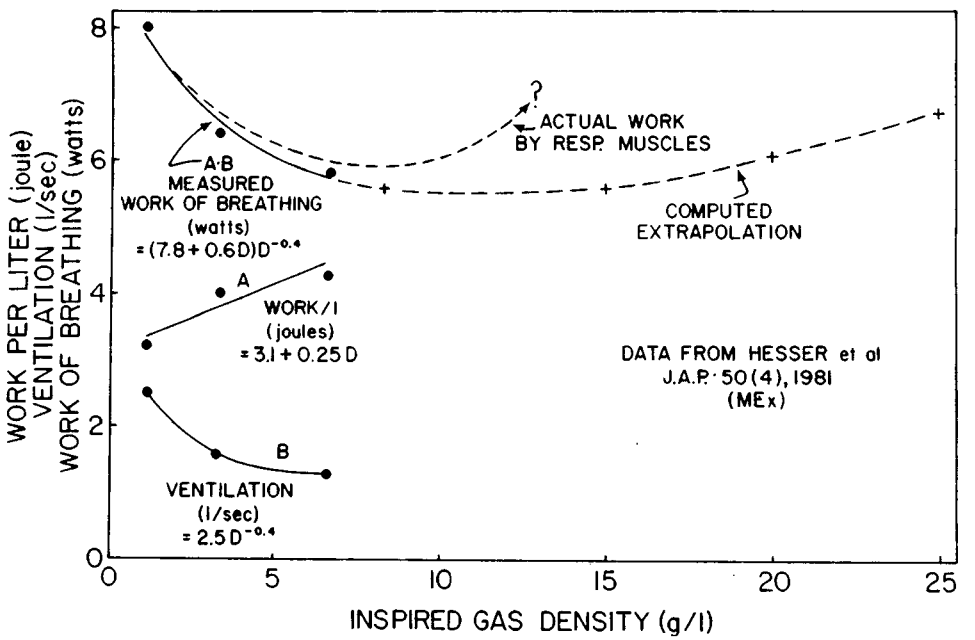



Fig. 5. Extrapolation of work of breathing to high gas densities based on measurements at lower gas densities.

25g/L than at 1 ATA. This is misleading, however, since (as noted by Hesser et al., Ref. 41) the actual total work done by the respiratory muscles is greater than that which was measured. In particular, distortion of the chest wall, which may be exacerbated by high levels of pulmonary gas-flow resistance, can dramatically increase total work of breathing (M. Goldman, personal communication to J. M. Clark, *see* Ref. 42).

### Respiratory Muscle Fatigue

The role of fatigue of respiratory muscles in ventilatory limitation and respiratory failure of pulmonary disease has received increased attention in recent years (43,44). Progress has been made in defining conditions that lead to fatigue in terms of variables such as transdiaphragmatic pressure swing and the ratio inspiration time to total cycle time (45). Also important are findings that: a) the frequency spectrum of the diaphragmatic electromyogram changes shape in a characteristic manner when it is fatigued; b) this change first develops before actual failure of the diaphragm as a pressure generator (46); c) hypoxia (inspired $P_{O_2}$ = 13%) reduces endurance time of inspiratory muscles (47); and c) ventilatory muscle endurance may be enhanced by training (48). There can be little question that such findings are relevant to ventilatory tolerance at high inspired gas densities.

### Pulmonary Gas Exchange

Observation of postural changes in goats led to the suggestion that oxygen uptake during inhalation of normoxic He-$O_2$ is impaired at pressures above 50 ATA (1600 fsw) (49). The observation was not verified either in mice at pressures to 122 ATA (50) or in man during vigorous exercise at lower pressure but at twice the gas density (12). Pulmonary gas exchange involves multiple phenomena in a system of complex geometry, with interactions of increased density and altered diffusivity that are not yet completely worked out (51,52). Artificial enhancement of intrapulmonary gas mixing by use of high-frequency oscillation (53) of airway gas is under investigation for potential application to the undersea environment (54).

## EFFECTS OF UNDERSEA-RELATED RESPIRATORY STRESS ON VENTILATORY CONTROL IN MAN

Resistive loading of the ventilatory system can be perceived subjectively (55), and it elicits a compensatory response in the form of increase in central inspiratory activity (56). Such loading reduces the slope of the ventilatory response to $CO_2$, whether due to pulmonary obstructive disease, external resistors, or elevated gas density. Despite apparent similarity in effect on $CO_2$ reactivity, there are differences between the three types of loading. In obstructive pulmonary disease, narrowed airways at specific sites cause increased

resistance. External resistance imposed on normal man differs from that of disease processes because the site of obstruction is discrete and is external to the unobstructed airways. Airway pressure distributions would differ from those in disease states. Loading by dense gas differs from both of the preceding situations in that increased resistance and alteration from normal pressure distribution occurs throughout the airways. Consistent with the latter, patterns of change in respiratory muscle activity during inspiratory or expiratory loading of cats by external resistors at 1 ATA differed from changes induced by loading with dense gas to densities of 16.1 g/L (57).

Despite masking of effects on ventilatory parameters during $CO_2$-free breathing by natural fluctuations, by chemical and neural feedback mechanisms, and by compensatory responses, small but consistent changes in function even during $CO_2$-free breathing have been detected. Dramatic influences on ventilatory responses to $CO_2$ have been observed. These are summarized in separate sections to follow. Since hyperoxia is employed widely in all aspects of diving, effects of hyperoxia on control of breathing are summarized below.

## Effects of Hyperoxia on Control of Breathing

Hyperoxia reduces $CO_2$-free ventilation and the slope of the ventilatory response to $CO_2$ by reducing the electrical activity of the peripheral chemoreceptors (58). At the same time, saturation of hemoglobin by $O_2$ requires that elimination of metabolic $CO_2$ from brain tissue be by its solution in plasma. The result is elevation in the tissue to plasma $CO_2$ gradient; CNS $P_{CO_2}$ rises and ventilatory activity of CNS chemoreceptors is increased. The net result of the interaction between reduced peripheral-related $CO_2$ drive and increase of CNS-related drive in the stable state following $O_2$ administration (more evident at 3 ATA $O_2$ but observable at 1 ATA as well) is a small increase in ventilation coupled with a decrease in $Pa_{CO_2}$ and a reduction in $CO_2$ reactivity (58).

These effects of hyperoxia are considered important here because not only are raised levels of inspired $O_2$ currently employed in all aspects of diving, but its use may well increase as methods of extending tolerance to $O_2$ are developed (58,59).

## Ventilation at Rest during $CO_2$-Free Breathing

*Low levels of raised pressure (to 10 ATA).* Analysis of data from several experiments with compressed air conducted at low levels of raised ambient pressure have led to several conclusions concerning the effects of density, $N_2$ narcosis, and pressure itself on ventilation and $CO_2$ retention. With data obtained at 1 to 4 ATA in compressed air, separate effects of gas density, hyperoxia, and narcosis were identified (20). Conclusions were that ventilation was reduced both by increased density and by hyperoxia, but not by $N_2$ narcosis. Absence of a $N_2$ narcosis effect as a ventilatory depressant was also a conclusion of studies with compressed air to 7.8 ATA (24).

It is natural to associate significant narcosis with depression of ventilation. However, although the degrees of $N_2$ narcosis in the cited experiments may have affected mental function, effects on ventilation, if any, were apparently too mild to be detected. Furthermore, change in ventilation during $CO_2$-free breathing is not particularly sensitive for detection of ventilatory effects of respiratory-active agents.

*Higher levels of raised pressure (above 10 ATA): non-narcotic gases.* We have recently reported (25) an analysis of ventilatory parameters and respiratory gas tensions obtained during $CO_2$-free breathing in several studies as components of *Predictive Studies III* (12) and *Predictive Studies IV* (42). Data at stable pressures obtained after slow compressions and during decompression from saturation involved $N_2$-$O_2$ to 13 ATA (14.5 g/L inspired gas density), Ne-He-$O_2$ to 37 ATA (25.2 g/L), as well as He-$O_2$

The nature of the responses observed with the non-narcotic gases suggested at least a dual effect of raised pressure on ventilation (25). It was concluded that the observations could be explained by an effect of pressure acting to progressively *increase* respiratory activity, opposing the ventilation-reducing effect of progressive increase in pulmonary gas-flow resistance. It was possible to estimate by a graphical analysis that the effect of pressure on ventilation, in the absence of increasing pulmonary flow resistance, would resemble an *S*-shaped dose-response curve, with little effect below 10 ATA.

These observations on ventilation and $P_{ET_{CO_2}}$ are actually consistent with results obtained over ranges of raised He-$O_2$ pressures in a number of other laboratories. An incline in $P_{CO_2}$ and decline in ventilation occurred for pressures from 1 to 10 ATA (1), while similar but small changes were seen from 1 to 19 ATA (7). At still higher pressures, the pattern was reversed: ventilation at the higher pressures was greater than or was not different from ventilation at low pressures, while $P_{CO_2}$ at the elevated pressures was less than it was low pressure (10,19,22). Alterations in metabolic rate, which can interfere with interpretation of data in rest states (25), may help to explain simultaneous increase in ventilation and $Pa_{CO_2}$ in another study at 1–8.5 ATA (23).

*Rapid compression.* Ventilatory parameters have rarely been measured during actual compression. Compression in the range 1–10 ATA with air at 66 fsw/min resulted in transitory $P_{ET_{CO_2}}$ increase of 4–8 Torr at 1–5 ATA and of 1–3 Torr at 5–10 ATA (1), apparently due to compression-related influx of gas to the lungs, an effect that must decrease with pressure increase (25). Compressions at higher pressures with He-$O_2$ (8 fsw/min from 600 to 800 fsw; 5 fsw/min from 1100 to 1200 fsw; 20 fsw/min from 1400 to 1500 fsw) were associated with small, transitory alterations in ventilatory and respiratory gas levels, as compared to pre- and postcompression values (25). These were attributed to mildly increased levels of physical activity associated with experimental procedures during compression, rather than with compression-related gas flow into the lungs, which would be negligible at high pressures. In those circumstances of rapid compression and high pressure, neither bulk gas move-

ment into the lungs nor acute increase in pulmonary flow resistance caused hypercapnia. Neither did hypocapnia result from general excitement of the experimental circumstances or from effects of acute exposure to rapid increase in hydrostatic pressure on respiratory structures in the CNS (25). The observed signs and reported symptoms typically associated with rapid compressions to high pressures (42,60) can therefore be associated with effects of pressure itself on excitable cells, rather than due to an interaction of pressure with consequences of altered respiratory homeostasis (25).

*Nitrogen narcosis.* Data for the narcotic gas $N_2$ at 1–13 ATA (25) are consistent with the earlier reports at lower pressures that $N_2$ did not depress ventilation or cause $CO_2$ retention (20,24). In fact, the limited data available for $CO_2$-free breathing of $N_2$-$O_2$ to 13 ATA are consistent with increase in $N_2$ partial pressure causing a mild increment in ventilatory activity, opposing the ventilation reducing effect of gas density increase (25). Data on $CO_2$ reactivity consistent with this conclusion are described below.

## Ventilatory Response to $CO_2$

Changes in slope, position, or both, of the ventilatory response to $CO_2$ are far more sensitive in detecting ventilatory depression or stimulation by respiratory-active agents than are changes in ventilatory parameters during $CO_2$-free breathing.

*Interaction with nitrogen narcosis.* Because narcotics and anesthetics generally have depressant effects on ventilatory stimulation by $CO_2$, it is natural to expect that inert gases such as $N_2$, which at elevated partial pressures depress mental function, would also have depressant effects on $CO_2$ "reactivity" (beyond the effect of elevated density on pulmonary gas-flow resistance). The results already cited for $CO_2$-free breathing suggest that moderate depressant effects of $N_2$ on mental functions are not expressed as depression of spontaneous ventilatory activity. The limited available evidence also indicates that $N_2$ at a partial pressure, which is moderately narcotic to mental function, does not have a depressant effect on chemosensory reactivity to $CO_2$ (21,28). It may even be that the opposite is true, i.e., $N_2$ levels to at least 10 atm may actually produce a mild increase in respiratory activity (25,28), perhaps analogous to $N_2O$, which also appears to increase respiratory activity while it depresses mental function. However, it remains possible that $N_2$ narcosis at still higher pressures may depress respiratory functions.

*Interaction with flow resistance and pressure.* The effect of increased pulmonary gas-flow resistance over a wide range of inspired gas densities on respiratory $CO_2$-reactivity in the absence of complicating interactions with $N_2$ narcosis or hyperoxia is shown in Fig. 6*A*. The slope of the ventilatory response to $CO_2$ ($CO_2$ reactivity) was always less (at the same density) when He-$O_2$ was breathed than was the case with the more viscous Crude Ne-$O_2$

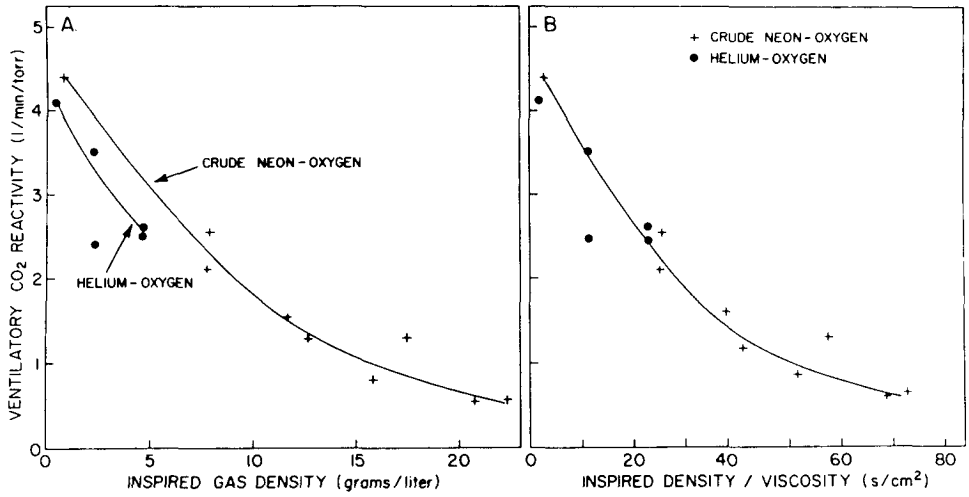



Fig. 6. Respiratory $CO_2$ reactivity as functions of inspired gas density (*A*) and of reciprocal of kinematic viscosity (density/viscosity) (*B*) for non-narcotic gases. The data are individual values for two subjects from *Predictive Studies III* (12,28,29), pooled by scaling one subject's data to match that of the other subject at lowest gas density. Oxygenation was at the natural level. Exposures to Crude Ne-$O_2$ were of 30–40 min duration.

(28). This difference vanished when the ratio density/viscosity was used as the independent variable (Fig. 6*B*). On this basis and on theoretical grounds as well (37), the ratio density/viscosity has been considered the valid index of pulmonary gas-flow resistance when both gas composition and density are altered (25,28). Apparently, during maximal or near maximal ventilatory efforts, as in maximal pulmonary function test maneuvers or during vigorous exercise, turbulent flow is dominant in all airways throughout the ventilatory cycle. For lesser efforts, as in $CO_2$-free breathing at-rest, or during stimulation by $CO_2$, there must be significant spatial and temporal variations of both viscous and turbulent flow regimens in various airways as pulmonary flow cycles through zero (laminar flow dominant) and through peak values (turbulent flow dominant). Significant laminar flow is also more likely during the more sinusoidal-like flow pattern of quiet or moderately stimulated breathing than during the square-wave type of flow pattern (61) of strenuous breathing efforts. Dominance of turbulent flow during voluntary hyperventilation and a mixture of laminar and turbulent flows during tidal breathing was also a conclusion reached in an earlier study by other investigators (8).

The $CO_2$ reactivity was reduced by a factor of 6 over the gas density/viscosity range studied (2.6 to 70 s/cm$^2$) during acute exposures to dense normoxic gas (28). Also important was the observation that the *linear range* of ventilatory response to $CO_2$ was considerably reduced from normal (28) (Figs. 7 and 8) when the ventilatory response to $CO_2$ became zero. Presumably, increase in density/viscosity to higher levels will further reduce reactivity and

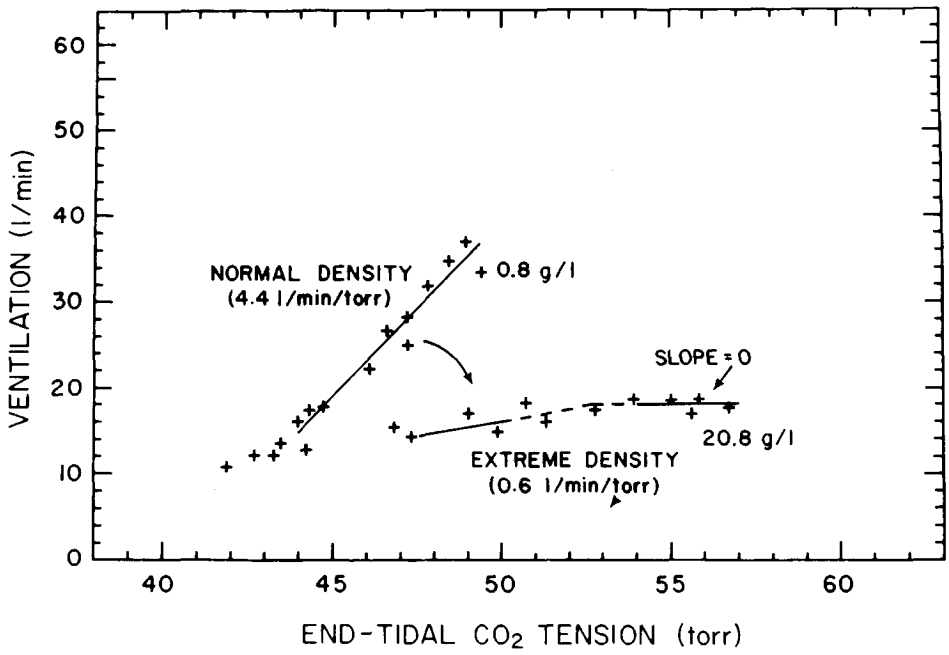



Fig. 7. Respiratory response to $CO_2$ at "normal" and at extremely high pulmonary gas-flow resistance, for one subject of *Predictive Studies III*, breathing Crude Ne-$O_2$. Not only is the respiratory reactivity markedly reduced (here by a factor of 6), but the slope actually goes to zero when end-tidal $P_{CO_2}$ is about 52 Torr. Results for the other subject were similar (28).

virtually abolish linear response range. This plateauing of ventilatory $CO_2$ reactivity represents complete abolition of the ventilatory homeostatic response to both endogenous and exogenous $CO_2$ loading.

*Limitation of ventilatory tolerance to undersea exposure in states of rest.* The virtual abolition of ventilatory response to $CO_2$ shown in Figs. 7 and 8 occurred during acute exposure to normoxic dense gas in the fully awake state (28). As illustrated further in Fig. 9, ventilation and end-tidal $P_{CO_2}$ during $CO_2$-free breathing may remain in the normal range even while $CO_2$ reactivity and its linear range, already severely diminished by high pulmonary gas-flow resistance, may be further reduced by hyperoxia (58) and by sleep (62). Sleep (63) and narcosis (64) can attenuate or abolish favorable compensatory responses to flow-resistive loading. Should $CO_2$ reactivity and its linear range become sufficiently small, then the circular chain of events shown in Fig. 9, if initiated by increased $CO_2$ loading for any reason, could lead to progressive ventilatory insufficiency and respiratory failure.

It is in this context that the difference in viscosity between neon and helium assumes practical importance. The *density* equivalent of normoxic He/

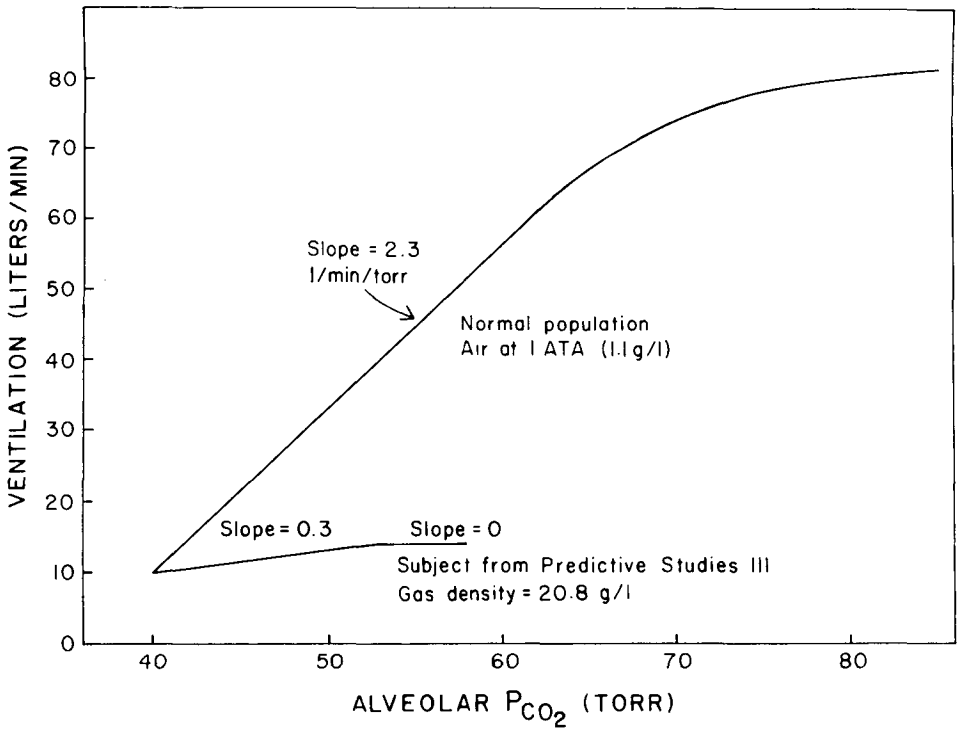



Fig. 8. Slope and linear range of respiratory response to $CO_2$ for normal population at natural density and for subject from *Predictive Studies III* at extremely high gas density. When slope is zero, any increase in inspired $CO_2$ tension produces an equal increase in arterial $CO_2$ tension. Any increase in rate of metabolic $CO_2$ production produces a rise in arterial $CO_2$ tension, which is a function of the level of ventilation and the horizontal distance between isometabolic rate curves on a plot of alveolar ventilation vs. arterial $CO_2$ tension. The *curve* for the normal population is adapted from Lambertsen (In: *Drill's Pharmacology in medicine*, ed. 4. New York: McGraw-Hill, 1971). The response for high gas density is adapted from Gelfand et al. (28).

$O_2$ for 20.8 g/L of Crude Ne-$O_2$ is about 4500 fsw. However, the *density/viscosity* equivalent of 20.8 g/L of Crude Ne-$O_2$ is considerably less in depth, approximately 3000 fsw (29).

*Effects on pattern of breathing.* Resistive loading is generally acknowledged to reduce respiratory frequency and increase tidal volume. These effects have been seen in loading by increase of gas density (7), although respiratory influences of high pressure and of $N_2$ narcosis may counter them (25). In contrast to the modest effects during $CO_2$-free breathing at rest, elevation of gas density has marked effects on both tidal volume and respiratory frequency responses to $CO_2$ (28).

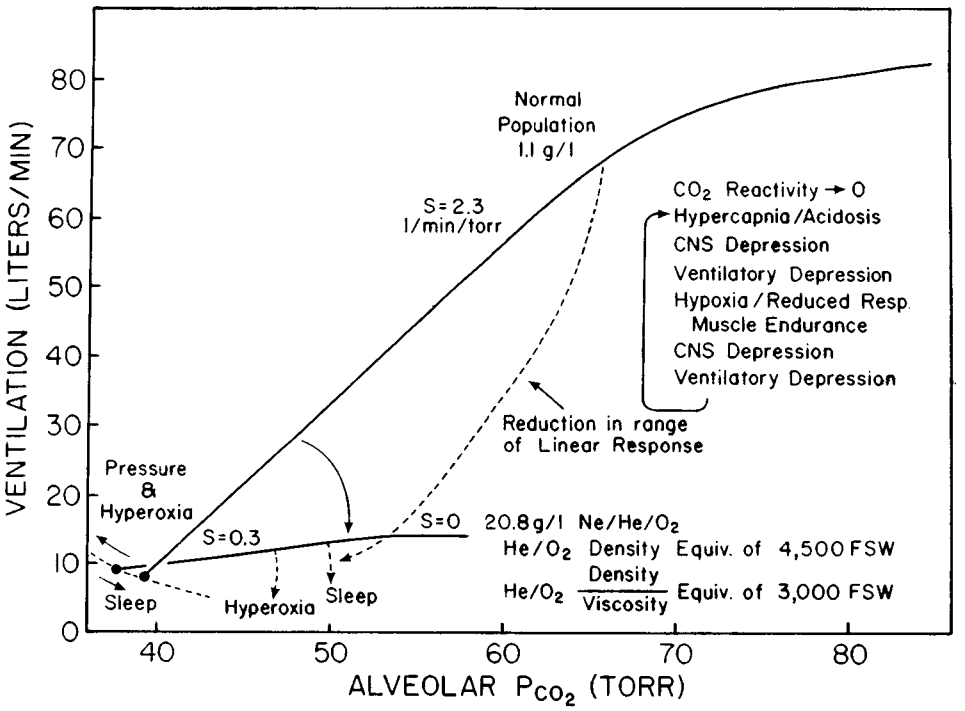



Fig. 9. Prediction of ventilatory insufficiency and respiratory failure in rest states at high inspired gas density and ambient pressure.

## ANIMAL MODELS FOR INVESTIGATION OF RESPIRATORY CONTROL AT HIGH PRESSURES AND GAS DENSITIES

Available data on the effects of exposure to elevated ambient pressures and gas densities on the control of breathing at rest have largely originated from observations on animals and measurements on small groups of men. Full definition of the dose-response effects of such exposure in appropriate sample sizes, investigation of tolerance limits, and investigation of their underlying mechanisms, will require quantitative measurements on appropriate animal models as well.

### Visual Observations of Animals

Visual observation of ventilatory activity in mice to 122 ATA (4000 fsw) (50); in goats to 151 ATA (almost 5000 fsw) (49); and in miniature pigs and

goats to 127 ATA (4200 fsw) (65) revealed both survival and varying degrees of ventilatory difficulties.

## Measurements in Animals

Unique experiments in nine awake cats (instrumented with a body plethysmograph and electromyograph electrodes) to He-$O_2$ pressures of 100 ATA (3300 fsw) showed no symptoms of respiratory distress at stable pressures (57). During compressions, however, V, VT, and f increased transiently, most prominently at 2600 to 3300 fsw, sometimes accompanied by panting.

The authors reported no change in f or $TI/T_{tot}$ at stable elevated pressures, while VI, VT and VT/TI were significantly higher at pressure equivalents greater than at 980 fsw than 1 ATA (57). The latter observation is compatible with our observation of interaction of pressure and density on ventilatory activity (25). Other observations on the instrumented cats (57) include alteration in the characteristic shape of the electrical activity of the diaphragm during inspiration, and the appearance of electrical activity of respiratory muscles during expiration at simulated depths greater than 980 fsw.

Other methods include investigation with rats in a hyperbaric whole-body plethysmograph (68), and an approach toward use of a larger animal (the goat) by the development of a counterweighted respiratory/metabolic helmet system with gas-tight seals at the goat's horns and neck (66). An external loop with recirculation blower, $CO_2$ scrubber, and heat exchanger, and a bag-in-box for measurement of ventilatory parameters complete the system. Initial results have demonstrated a) that the ventilatory response of goats to hyperoxia (both during $CO_2$-free breathing and in response to $CO_2$) is quantitatively similar to those previously reported for man (67), and b) that the decrement in $CO_2$ reactivity related to increase in pulmonary gas-flow resistance (by $SF_6$-$O_2$ at 1 ATA) is essentially the same as in man breathing $N_2$-$O_2$ at the same density/viscosity ratio (unpublished observations).

Clearly, investigation of human limits of respiratory tolerance to high ambient pressures and inspired gas densities can benefit greatly from prior quantitative measurements on appropriate animal models.

## Acknowledgment

Supported in part by Grant HL 28602-02 from the National Heart, Lung and Blood Institute of the Department of Health and Human Services.

## References

Literature cited is more extensive on some topics than on others. It is not exhaustive on any topic.

1. Albano G, Principles and observations on the physiology of the scuba diver. Chap. IV. Hyperbaric Respiration. Translated from Italian. (1970). Washington, DC: Superintendant of Documents, 1970. (ONR report DR-150).

2. Bühlmann AA. Respiratory resistance with hyperbaric gas mixtures. In: Lambertsen CJ, Greenbaum LJ Jr, eds. Proceedings second symposium on underwater physiology. Washington, D.C.: National Academy of Sciences-National Research Council, 1963:98–107.
3. Hamilton RW Jr. Physiological responses at rest and in exercise during saturation at 20 atmospheres of He-$O_2$. In: Lambertsen CJ, ed. Underwater physiology. Proceedings of the third symposium on underwater physiology. Baltimore: Williams & Wilkins, 1967:361–374.
4. Lanphier EH. Interactions of factors limiting performance at high pressures. In: Lambertsen CJ, ed. Underwater physiology. Proceedings of the third symposium on underwater physiology. Baltimore: Williams & Wilkins, 1967:375–385.
5. Wood LDH, Bryan AC. Mechanical limitations of exercise ventilation at increased ambient pressure. In: Lambertsen CJ, ed. Underwater physiology. Proceedings of the fourth symposium on underwater physiology. New York, London: Academic Press, 1971:307–316.
6. Miller JN, Wangensteen OD, Lanphier EH. Ventilatory limitations on exertion at depth. In: Lambertsen CJ, ed. Underwater physiology. Proceedings of the fourth symposium on underwater physiology. New York, London: Academic Pres, 1971:317–323.
7. Bradley ME, Anthonisen NR, Vorosmarti J, Linaweaver PG. Respiratory and cardiac responses to exercise in subjects breathing helium-oxygen mixtures at pressures from sea level to 19.2 atmospheres. In: Lambertsen CJ, ed. Underwater physiology. Proceedings of the fourth symposium on underwater physiology. New York, London: Academic Press, 1971:325–337.
8. Anthonisen NR, Bradley ME, Vorosmarti J, Linaweaver PG. Mechanics of breathing with helium-oxygen and neon-oxygen mixtures in deep saturation diving. In: Lambertsen CJ, ed. Underwater physiology. Proceedings of the fourth symposium on underwater physiology. New York, London: Academic Press, 1971:339–345.
9. Salzano J, Rausche DC, Saltzman HA. Cardio-respiratory responses to exercise at a simulated sea water depth of 1,000 feet. J Appl Physiol 1970;28:34–41.
10. Schaefer KE, Carey CR, Dougherty J Jr. Pulmonary gas exchange and urinary electrolyte excretion during saturation-excursion diving to pressures equivalent to 800 and 1000 feet of sea water. Aerosp Med 1970;41:856–864.
11. Lambertsen CJ, Gelfand R, Lever MJ, Bodammer G, Takano N, Reed TA, Dickson JG, Watson PT. Respiration and gas exchange during a 14-day continuous exposure to 5.2% $O_2$ in $N_2$ at pressure equivalent to 100 fsw (4 ATA). Aerosp med 1973;44:844–849.
12. Lambertsen CJ, Gelfand R, Peterson R, Strauss R, Wright WB, Dickson JG Jr, Puglia C, Hamilton RW Jr. Human tolerance to He, Ne, and $N_2$ at respiratory gas densities equivalent to He-$O_2$ breathing at depths of 1200, 2000, 3000, 4000, and 5000 feet of sea water. Predictive Studies III. Aviat Space Environ Med 1977;48:843–855.
13. Linnarson D, Fagraeus L. Maximal work performance in hyperbaric air. In: Lambertsen CJ, ed. Underwater physiology V. Proceedings of the fifth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1976:35–48.
14. Peterson RE, Wright WB. Pulmonary mechanical functions in man breathing dense gas mixtures at high ambient pressures. Predictive Studies III. In: Lambertsen CJ, ed. Underwater physiology V. Proceedings of the fifth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1976:67–77.
15. Vorosmarti J, Bradley ME, Anthonisen NR. The effects of increased gas density on pulmonary mechanics. Undersea Biomed Res 1975;2:1–10.
16. Anthonisen NR, Utz G, Kryger MH, Urbanetti JS. Exercise tolerance at 4 and 6 ATA. Undersea Biomed Res 1976;3:95–102.
17. Spaur WH, Raymond W, Knott MM, Crothers JC, Braithwaite WR, Thalmann ED, Uddin DF. Dyspnea in divers at 49.5 ATA: mechanical, not chemical in origin. Undersea Biomed Res 1977;4:182–198.
18. Dwyer J, Saltzman HA, O'Bryan R. Maximal physical-work capacity of man at 43.4 ATA. Undersea Biomed Res 1977;4:359–372.
19. Broussole N, Chouteau J, Hyacinthe R, LePechon J, Burnet H, Battesti A, Cresson D, Imbert G. Respiratory function during a simulated saturation dive to 51 ATA (500 meters) with a helium-oxygen mixture. In: Lambertsen CJ, ed. Underwater physiology V. Proceedings of the fifth symposium on

underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1976:79–89.
20. Hesser CM, Holmgren B. Effects of raised barometric pressures on respiration in man. Acta Physiol Scand 1959;47:28–43.
21. Linnarson D, Hesser CM. Dissociated ventilatory and central respiratory responses to $CO_2$ at raised $N_2$ pressure. J Appl Physiol: Respir Environ Exercise Physiol 1978;45:756–761.
22. Morrison JB, Florio JT. Respiratory function during a simulated saturation dive to 1,500 feet. J Appl Physiol 1971;30:724–732.
23. Saltzman HA, Salzano JV, Blenkarn GD, Kylstra JA. Effects of pressure on ventilation and gas exchange in man. J Appl Physiol 1971;30:443–449.
24. Lanphier EH. Influence of increased ambient pressure upon alveolar ventilation. In: Lambertsen CJ, Greenbaum LJ Jr, eds. Proceedings second symposium on underwater physiology. Washington, D.C.: National Academy of Sciences-National Research Council, 1963:124–133.
25. Gelfand R, Lambertsen CJ, Strauss R, Clark JM, Puglia CD. Human respiration at rest in rapid compression and at high pressures and gas densities. J Appl Physiol: Respir Environ Exercise Physiol 1983;54:290–303.
26. Fagraeus L, Hesser CM. Ventilatory response to $CO_2$ in hyperbaric environments. Acta Physiol Scand 1970;80:19A–20A.
27. Wood LDH, Bryan AC. Respiratory sensitivity to $CO_2$ at increased ambient pressure Physiologist 1970;13:348.
28. Gelfand R, Lambertsen CJ, Peterson RE. Human respiratory control at high ambient pressures and inspired gas densities. J Appl Physiol: Respir Environ Exercise Physiol 1980;48:528–539.
29. Gelfand R, Peterson RE. The effects on $CO_2$ reactivity of breathing crude neon, helium, and nitrogen at high pressure. In: Lambertsen CJ, ed. Underwater physiology V. Proceedings of the fifth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1976:603–615.
30. Lambertsen CJ. Prediction of physiological limits to human undersea activity and extension of tolerance to high pressure. In Obai F, Benedek G, eds. Advanced physiological science, vol. 18. Environmental physiology, 1981:143–164.
31. Lambertsen CJ. Physical, chemical, and nervous interactions in respiratory control. In: Mountcastle VB, ed. Medical physiology, vol II. St. Louis: C.V. Mosby, 1980:1873–1900.
32. Whipp BJ. The control of exercise hyperpnea. In: Hornbein TF, ed. Regulation of breathing, Pt. II. New York: Marcel Dekker, 1981:1069–1139.
33. Salzano JV, Stolp BW, Moon RE, Camporesi EM. Exercise at 47 and 66 ATA. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:181–196.
34. Bennett PB, Coggin R, Roby J. Control of HPNS in humans during rapid compression with trimix to 650 m (2132 ft). Undersea Biomed Res 1981;8:85–100.
35. Lambertsen CJ, Greene KM, Overlock R, Clark JM. Practical underwater work performance at pressures to 1200 and 1600 fsw. In: Lambertsen CJ, Gelfand R, Clark JM, eds. Predictive studies IV. Work capability and physiological effects of He-$O_2$ excursion to pressures of 400-800-1200-1600 fsw Philadelphia: Institute for Environmental Medicine, 1978. (report 78-1)
36. Sponholtz DK, Thalmann ED, Lundgren CEG. The effects of static lung loading on cardiorespiratory function in the completely submerged diver. Undersea Biomed Res 1977;4:A37.
37. Drazen JM, Loring SH, Ingram RH Jr. Distribution of pulmonary resistance: effects of gas density, viscosity and flow rate. J Appl Physiol 1976;41:388–395.
38. Hirschfelder JO, Curtiss CF, Bird RB. The transport properties of dense gases, chap 9. In: Molecular theory of gases and liquids. New York: John Wiley, 1954:611–667.
39. Clarke JR, Jaeger MJ, Zumrick JL, O'Bryan R, Spaur WH. Respiratory resistance from 1 to 46 ATA measured with the interrupter technique. J Appl Physiol: Respir Environ Exercise Physiol 1982;52:549–555.
40. Glauser SC, Glauser EM, Rusy BF. Gas density and the work of breathing. Respir Physiol 1967;2:344–350.

41. Hesser CM, Linnarson D, Fagraeus L. Pulmonary mechanics and work of breathing at maximal ventilation and raised air pressure. J Appl Physiol: Respir Environ Exercise Physiol 1981;50:747–753.
42. Lambertsen CJ, Gelfand R, Clark JM, eds. Predictive studies IV. Work capability and physiological effects of He-$O_2$ excursions to pressures of 400-800-1200-1600-fsw. Philadelphia: Institute for Environmental Medicine, 1978. (report 78-1)
43. Sharp JT. Respiratory muscles: a review of old and newer concepts. Lung 1980;157:185–199.
44. Smidt V. Respiratory muscle fatigue as limitation for exercise. Prog Respir Res 1979;11:215–223.
45. Bellemare F, Grassino A. Effect of pressure and timing of contraction on human diaphragm failure. J Appl Physiol: Respir Environ Exercise Physiol 1982;53:1190–1195.
46. Gross D, Grassino A, Ross WRD, Macklem PT. Electromyogram pattern of diaphragmatic fatigue. J Appl Physiol: Respir Environ Exercise Physiol 1979;46:1–7.
47. Jardim J, Farkas G, Prefaut C, Thomas D, Macklem PT, Roussos Ch. The failing inspiratory muscles under normoxic and hypoxic conditions. Am Rev Respir Dis 1981;124:274–279.
48. Keens TG, Krastins IRB, Wanamaker EM, Levison H, Crozier DN, Bryan AC. Ventilatory muscle endurance training in normal subjects and patients with cystic fibrosis. Am Rev Respir Dis 1977;116:853–860.
49. Chouteau J. Respiratory gas exchange in animals during exposure to extreme ambient pressures. In: Lambertsen CJ. Underwater physiology IV. Proceedings of the fourth symposium on underwater physiology. New York: Academic Press, 1971:385–397.
50. MacInnis J, Dickson JG, Lambertsen CJ. Exposure of mice to a helium-oxygen atmosphere at pressure to 122 atmospheres. J Appl Physiol 1967;22:694–698.
51. Christopherson SK, Hlastala MP. Pulmonary gas exchange during altered density gas breathing. J Appl Physiol: Respir Environ Exercise Physiol 1982;52:221–225.
52. Van Liew HD, Paganelli CV. Relations among gas-phase diffusivity, density, and pressure in gas mixtures. Undersea Biomed Res 1982;9(Suppl):34.
53. Bohn DJ, Miyasaka K, Marchak BE, Thompson WK, Froese AB, Bryan AC. Ventilation by high-frequency oscillation. J Appl Physiol: Respir Environ Exercise Physiol 1980;48:710–716.
54. Clarke JR, Homer LD. Density dependance of high frequency ventilation. Undersea Biomed Res 1982;9(Suppl):34.
55. Wiley RL, and Zechman FW. Perception of added airflow resistance in humans. Respir Physiol 1966/67;2:73–87.
56. Lopata M, Onal E, Evanich MJ, Lourenco RV. Respiratory neuromuscular response to $CO_2$ rebreathing with inspiratory flow resistance in humans. Respir Physiol 1980;39:95–110.
57. Imbert G, Jammes Y, Naraki N, Duflot JC, Grimaud C. Ventilation, pattern of breathing, and activity of respiratory muscles in awake cats during oxygen-helium simulated dives (1000 msw). In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:273–282.
58. Lambertsen CJ. Effects of hyperoxia on organs and tissues. In Robin ED, ed. Extrapulmonary manifestations of respiratory disease, chap 8. New York: Marcel Dekker, 1978:239–303.
59. Hendricks PL, Hall DA, Hunter WH Jr, Haley PJ. Extension of pulmonary $O_2$ tolerance in man at 2 ATA by intermittent $O_2$ exposure. J Appl Physiol: Respir Environ Exercise Physiol 1977;42:593–599.
60. Hunter WL Jr, Bennett PB. The causes, mechanisms, and prevention of the high pressure nervous syndrome. Undersea Biomed Res 1974;1:1–28.
61. Grodins FS, Yamashiro Y. Optimization of the mammalian respiratory gas transport system. In: Mullins LJ, ed. Annual review of biophysics and bioengineering. Palo Alto, CA: Ann Rev vol 2, 1973;115–130.
62. Bülow K. Respiration and wakefulness in man. Acta Physiol Scand Suppl 1963;59:1–110.
63. Iber C, Berssenbrugge A, Skatrud JB, Dempsey JA. Ventilatory adaptions to resistive loading during wakefulness and non-REM sleep. J Appl Physiol: Respir Environ Exercise Physiol 1982;52:607–614.
64. Santiago TV, Goldblatt K, Winters K, Pugliese AC, Edelman NH. Respiratory consequences of methadone: the response to added resistance to breathing. Am Rev Resp Dis 1980;122:623–628.
65. Parc J, Michaud A, LeChuiton J, Michael A, Barthelemy L, Balouet G. Very deep diving experiments on miniature pigs. In: Lambertsen CJ, ed. Underwater physiology V. Proceedings of the fifth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1976:251–261.

66. Gelfand R, O'Toole M, Nace D, Lambertsen CJ. Closed circuit recirculation respiratory system with sealed helmet for ventilatory metabolic measurements in unanesthetized goats. Fed Proc 1982;41:1380.
67. Gelfand R, O'Toole M, Salatiello J. Mock D, Lambertsen CJ. Effects of $O_2$ at 1 ATA on ventilatory control of awake goats. Fed Proc 1983;42:1013.
68. Lin YC, Shida KK, Respicio BL. Hyperbaric whole-body plethysmograph for rodents. Undersea Biomed Res 1983;10:135–145.

# Part VII

## MOLECULAR AND CELLULAR EFFECTS OF PRESSURE

# THE EFFECT OF PRESSURE ON THE CELL MEMBRANE CHANGES INDUCED BY SOME CALCIUM BLOCKERS

*T. K. Akers, M. Ereth, and P. M. Rowles*

Two basic types of plasmalemma calcium channels are thought to be responsible for the response to vascular smooth muscle membrane signals. These channels are thought to reflect the processes of pharmaco-mechanical and electrical-mechanical coupling. Early work has shown that calcium blockers act selectively on the slow inward calcium current in cardiac muscles (1). The agents that cause this kind of pharmaco-mechanical block include substances from a wide variety of drug classes. These include procaine, sodium nitroprusside, trifluoperazine, dilantin, and indomethacin and verapamil. All of these agents at high concentrations exhibit other pharmacological actions such as receptor blocking, sodium channel blocking, and local anesthetic properties.

In our laboratory recent studies on the effect of procaine on the surface ultrastructure of chick blastoderm indicate increases in microappendages. Others have reported the development during cytokinesis of microvilli and microappendages along the line of division of the cell membrane in many different animals (2–4). Microappendages of the plasmalemma can be observed in three major forms: microvilli, blebs, and ruffles. These structures are now recognized to be plasmalemmal extensions prevalent on the free surface of most cells (5). They are indicative of internal cell activity. Microvilli are small surface extensions of approximately 0.1μm in diameter and of variable length. These structures are found on various absorptive areas such as the small intestine and the proximal tubules of kidneys, as well as the free surfaces of other cells. Microtubules as internal structures aligned parallel to the longitudinal axis of a microvilli also have been reported. This has not been confirmed in all areas.

Studies in other labs have reported the formation of microappendages associated directly with changes in concentration of the extra mitochondrial calcium ions in hepatocytes (6).

Our immediate aim in the present study was to obtain scanning electron micrographs (SEM) to examine the alterations of the surface structures of free surfaces of the chick blastoderm to describe the effect of a) calcium blockers and local anesthetics, b) helium-oxygen and air pressure, and c) cholinergic transmitters and blockers.

## METHODS

Fertile Rhode Island Red and White Leghorn pullet eggs were incubated under constant temperature (38°C) and humidity (55%). Embryos were examined after 48-72 h of incubation and staged according to the Hamburger-Hamilton series (Stages 9 to 14) (7). Blastoderms were removed by the paper ring method of Low (5) and explanted into isotonic Hank's saline (300 m0sm; 26°C). The controls had no further treatment. Other embryos were explanted into Hank's saline containing either 1 mM procaine HCl, Digitalis 1 mM/L, Acetylcholine 1 mM/L, Atropine 1 mM/L, or d-tubocurare 1 mM/L. These all were exposed for 30 min at 1 atm air.

In addition, nondrug-treated embryos were exposed to either 100 msw (metres sea water) air or 500 msw He-$0_2$ for 30 min. Three groups of procaine-treated embryos were also exposed to 500 msw He-$0_2$ for the last 15 min of drug treatment.

At the end of the incubation time the blastoderms were fixed with 2% gluteraldehyde in Hank's saline at their experimental pressures. They were then decompressed in 1 h if necessary and postfixed for 30 min with 2% osmium tetroxide in 0.72 N sodium cacodylate buffer, rinsed and dehydrated in acetone, and critical point dried.

Blastoderms were then mounted vertical side up and coated with palladium-gold and examined in a JEOL JSM 35 scanning electron microscope.

## RESULTS

The blastoderm cell layer of the early chick embryo has been shown to possess a large number of microappendages in complex arrays (5).

Control embryos incubated in Hank's saline and exposed to 1 atm of air for 30 min had consistent characteristic contours of cell surface.

The endodermal cells presented bulging surfaces due to underlying yolk granules previously phagocytosed, similar in appearance to micrographs appearing in the literature. These surfaces were covered with microvilli, blebs, and pinocytotic pits (*see* Figs. 1 & 2).

Exposure of Hank's-saline-treated tissue to 500 msw helium for 15 min, followed by fixation at pressure, yields surfaces that have considerably less

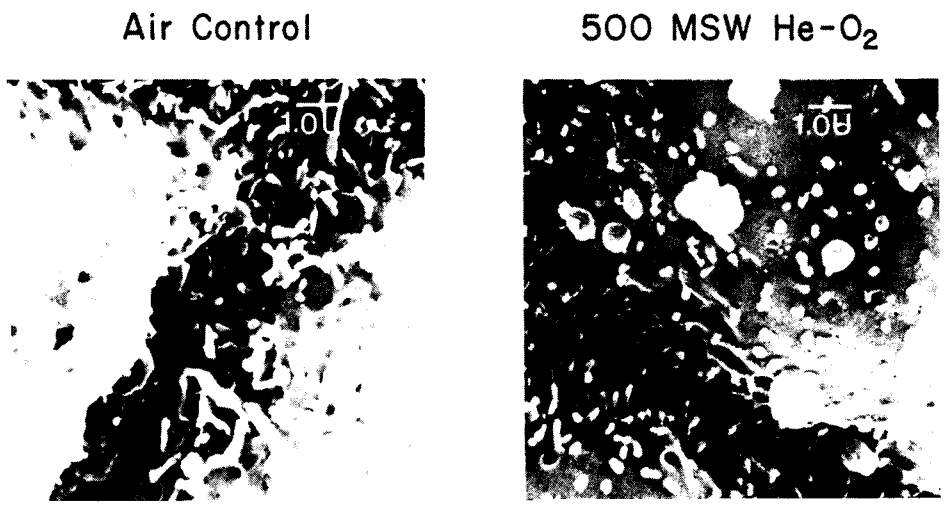



Fig. 1. Scanning electron micrographs (SEM) of normal chick blastoderm exposed to 1 atm air compared with 30-min exposure to 500 msw He-$O_2$.

microvilli and blebs but still have numerous pits indicating intracellular activity. In contrast, those cells exposed to 1 atm air presented surfaces with only a few pits but large numbers of microvilli and ruffles (Fig. 1).

Endodermal cells of embryos exposed to procaine (1 mM/L) for 30 min produced long microvilli (Fig. 2). Pressurization of procaine-treated cells to 500 msw helium induced bulbous terminations on the microvilli (see Fig. 3).

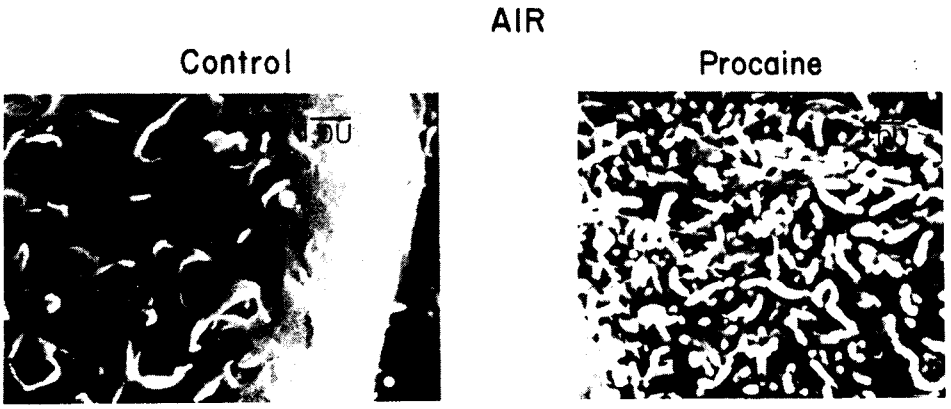



Fig. 2. Control blastoderm compared to 30-min exposure to 1 mM/L procaine.

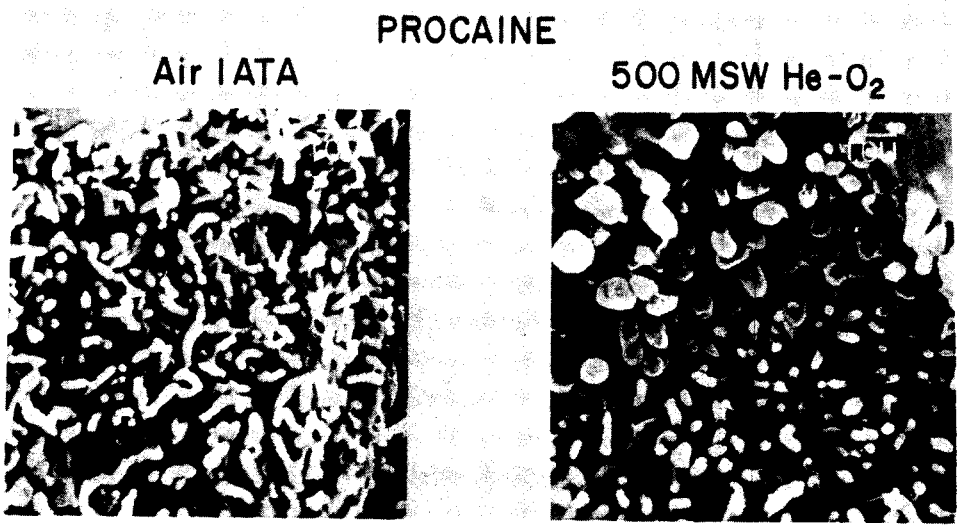



Fig. 3. Comparison of the effect of 1 atm air and 500 msw He-$O_2$ on 1 mM/L procaine-treated blastoderm.

Compression with 100 msw air produced large areas devoid of microvilli in cells which appeared to have crypts on their surface (see Fig. 4).

In our study embryos treated with Hank's saline and exposed to air for 30 min before fixation presented bulging surfaces containing yolk granules. These surfaces were covered with short microvilli, blebs, and also pinocytotic pits. Procaine treatment 1 mM/L caused a large number of long microvilli, while digitoxin treatment 1 mM/L caused a denser network of microvilli to form. Other substances tested in our laboratory that induced microvilli growth included dilantin, ketamine, pentobarbital, and ouabain. Other substances tested, which included acetylcholine, pilocarpine, atropine, alpha and beta blockers, and norepinephrine, did not produce the same type of plasmalemmal changes.

An interesting highlight occurred in the series of cholinergic substances. Acetylcholine induced fine microvilli and ruffles very similar but less dense than the controls (Fig. 5). However, atropine and curare produced a reduction of microappendages and formed crypts (Fig. 6).

## DISCUSSION

This study shows that procaine anesthetic induced changes in surface-fixed structure of the endodermal cell. Microvilli increase in length and number in response to anesthetic action following 30-min exposure. Lengthened microvilli are the predominant morphological change induced by procaine. Helium pressure reduces the packing and length of anesthetic-induced microvilli and at 500 msw actually changes the microvilli to blebs. Com-

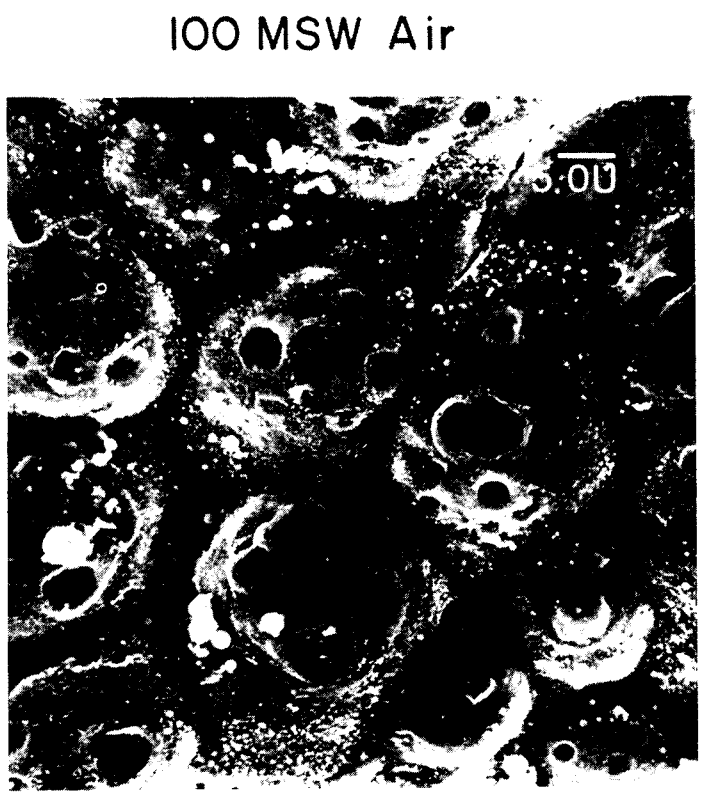



Fig. 4. The effect of 30-min treatment of 100 msw air on normal blastoderm.

pressed air at 100 msw produces a smoothing out of the surfaces and, in most cases, crypting—which possibly indicates membrane stability.

Microappendages of blastoderm cells have been shown to be very labile in response to a variety of physiological stresses (8–10) as well as experimental conditions (11,12).

Sheetz and co-workers (13–15) state that membranes behave as bilayer couplets which respond independently to membrane perturbations. They have shown that erythrocytes and lymphocytes display microappendage growth under the influence of local anesthetics and imply that a selective expansion of the outer or inner bilayer of the membrane is the causative factor for the morphological change.

If anesthetics produce an expansion of the membrane as manifested by the increase in microvilli length and number, then the reduction in length and changes in shape seen during pressurization with helium (that is, the reversal of growth of microvilli) may be why helium pressurization reverses the anesthetic effect.

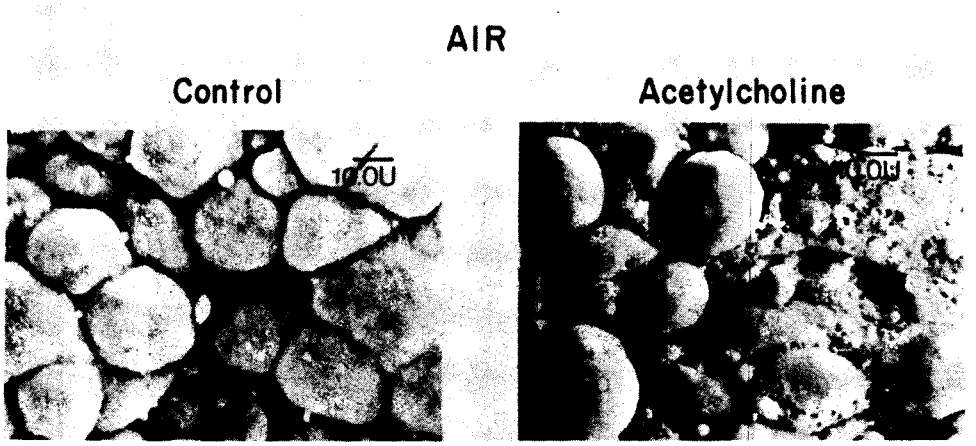



Fig. 5. Comparison of the effect of acetylcholine 1 mM/L and normal.

However, the similarity seen between the effect of procaine and other known calcium blockers in our experiments indicates that perhaps we have not considered the role of calcium transport in the various theories of anesthesia.

The loss of microvilli growth and pinocytotic pits seen during compressed air treatment to 100 msw may indicate stabilization of the membrane and loss of membrane activity.

Nitrogen narcosis produces impairment of recent memory, which appears to be mediated by cholinergic transmission. The similarity of action of cholinergic blockers (atropine and curare) on cell surfaces and the action of 100

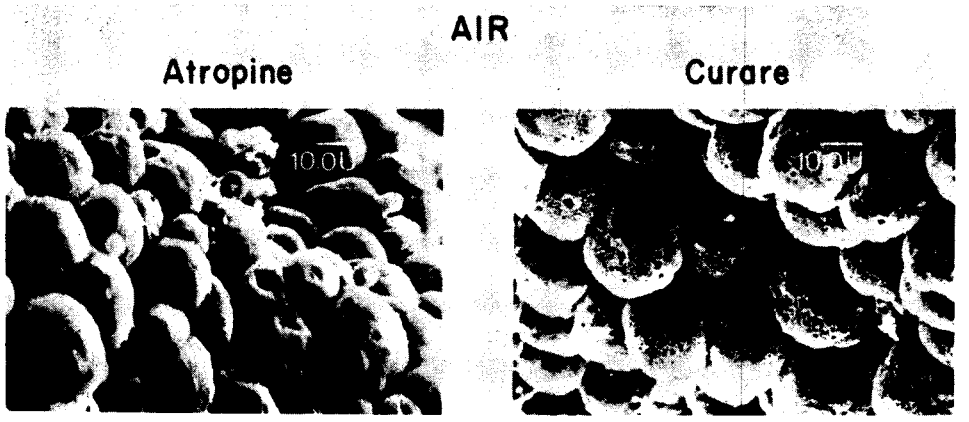



Fig. 6. The effects of 30-min treatment of atropine 1 mM/L and curare 1 mM/L on normal blastoderm.

msw air on cell surfaces implies a possible relationship of nitrogen narcosis to blocked cholinergic transmission by changes on cell surfaces.

## SUMMARY

1) Normal blastoderm have ruffles, blebs, microvilli, and pinocytotic pits.
2) Pressure (500 msw $He-O_2$) reduces all of the usual features.
3) Procaine enhances microvilli formation.
4) Pressure changes procaine-induced microvilli to bleb forms.
5) Digitalis causes meshlike long microvilli.
6) Epinephrine causes long microvilli at cell edges.
7) Acetylcholine decreases ruffling.
8) Atropine and curare induce pitting.
9) 100 msw air induce pitting.

## CONCLUSIONS

1) Seeing similar characteristic plasmalemma changes under high pressure helium and exposure to calcium blockers, one may extrapolate that a mechanism responsible for pressure reversal of anesthesia and also for the role of helium in high pressure neurological syndrome may be in part due to changes in calcium transport across cell membranes.

2) The similarities of the effect of 100 msw air and cholinergic blockers suggest that part of nitrogen narcosis may involve cholinergic transmission.

## References

1. Fleckenstein A. Calcium and the heart. Harris P, Opie L, eds. London and New York: Academic Press, 1971:135–188.
2. Abercrombie M, Heaysman JEM, Cegrum SM. The locomotion of fibroblast in culture. II. Ruffling. Exp Cell Res 1970;60:437–450.
3. Abercrombie M. The locomotion of fibroblast in culture. III. Electron microscopy of the leading lamella. Exp Cell Res 1971;67:359–382.
4. Porter K, Prescott D, Frye J. Changes in the surface morphology of Chinese hamster ovary cells during the cell cycle. J Cell Biol 1973;57:851–836.
5. Harri JE, Low FN. Microappendages on the ventral surface of the primitive streak chick embryo. Am J Anat 1974;141:569–574.
6. Jewell SA, Bellowo G, Thor H, Onenius S. Science 1982;217:1257–2159.
7. Hamilton HH. Lillie's development of the chick. 3rd ed. New York: Holt, Rinehart, and Winston, 1952.
8. Low FN, Developing boundary membranes in the chick embryo. Anat Res 1967;159:231–238.
9. England MA, Cowper SU. Wound healing in the early chick embryo studied by scanning electron microscope. Anat Embryol 1977;152:1–14.
10. Wakely J, England MA. Development of the chick embryo endoderm studied by SEM. Anat Embryol 1978;153:167–178.

11. Stagno PA, Low FN. Effects of cytochalasin B on the fine structure of organized endodermal cells of the early chick embryo. Am J Anat 1978;151:159–171.
12. Litke LL, Low FN. Scanning electron microscopy of yolk absorption in early chick embryos. Am J Anat 1975;142:527–531.
13. Sheetz MP, Singer SJ. Biological membranes as bilayer couples. Proc Nat Acad Sci USA 1974;71:4457–4461.
14. Scheetz MP, Singer SJ. Equilibrium and kinetic effects of drugs on the shapes of human erythrocytes. J Cell Biol 1976;70:247–251.
15. Sheetz MP, Painter RG, Singer J. Biological membranes as bilayer couples. III. Compensatory shape changes induced in membranes. J Cell Biol 1976;78:193–203.

# γ-AMINOBUTYRIC ACID TRANSMISSION AND THE HIGH PRESSURE NEUROLOGICAL SYNDROME

*A. R. Bichard and H. J. Little*

The use of drugs as tools to study the high pressure neurological syndrome (HPNS) has until recently been somewhat limited: the main reason has been the long-established connection between high pressure and general anesthetics, which has diverted interest away from the possibility of specific mechanisms. General anesthetics were the first drugs known to affect the HPNS and have for many years remained the only major group of drugs that significantly postponed the effects of pressure. The first evidence that more specific effects might be involved in the actions of both pressure and anesthetics came from the studies showing that not all anesthetics postponed the HPNS (1,2). Of the intravenous anesthetics, ketamine and althesin were the most effective, thiopentone was ineffective, and propanidid and methohexitone actually exacerbated the symptoms in rats (2)—but the anesthetic effects of all are pressure reversible.

In 1974, Hunter and Bennett (3) suggested that because the potency of the anesthetic gases nitrogen, hydrogen, and nitrous oxide against the tremor phase of the HPNS was twice as great as their potency against convulsions, clinical anticonvulsants might act synergistically with anesthetics to raise all the thresholds equally. A few years later it was noted that the anticonvulsant barbiturate phenobarbitone was more effective (in absolute terms and relative to anesthetic potency) than the hypnotic barbiturate pentobarbitone in postponing HPNS convulsions in mice (4). Further studies of the effects of clinically used anticonvulsants led the first move away from anesthetics in HPNS drug research, but it was shown that, in general, nonanesthetic anticonvulsants were not useful in preventing the HPNS. No particular correlation between the clinical use of the different antiepileptics and their effects on the HPNS was observed, as drugs useful against a wide range of different types of epilepsy

were all ineffective (5). Drugs useful against human petit mal/absence seizures, ethosuximide (250 mg/kg$^{-1}$) and trimethadione (250 mg/kg$^{-1}$, i.p. to rats), were ineffective. Drugs useful against grand mal, status epilepticus and psychomotor epilepsy, phenytoin (25 mg/kg$^{-1}$) and carbamzepine (100 mg/kg$^{-1}$), were also ineffective and have even been shown to lower HPNS thresholds (5,6). Some correlation may be seen with the effectiveness of phenytoin against maximal seizures (animals) and grand mal (humans) when it was shown to protect against the tonic type II HPNS seizures of Brauer's laboratory (60 mg/kg$^{-1}$) while lowering tremor and type I clonic seizures (6). Brauer et al. (6) also showed that phenobarbitone and trimethadione selectively postponed type II seizures, and these results were confirmed by another group (7). The benzodiazepines clonazepam (0.8–1.6 mg/kg$^{-1}$) and diazepam (8 mg/kg$^{-1}$, i.p. to rats), useful against all types of epilepsey especially petit mal, were very effective against the HPNS (5). It was not certain whether this was a specific effect, as "anesthetic" effects occurred at higher doses and, indeed, the effects of the benzodiazepines were complicated by their vehicle of propylene glycol/ethanol, which has been shown to induce an anesthetic-like state (8) and postpone the HPNS (9).

In 1979 we decided to study the involvement of γ-aminobutyric acid (GABA) transmission in the HPNS. The project was designed to investigate both increased inhibitory transmission as a means of specifically postponing the symptoms and in vitro studies of underlying changes. γ-Aminobutyric acid was chosen because its functions have been very widely investigated at normal pressure and because increased GABA transmission may be involved in the specific action of anticonvulsants, such as the benzodiazepines, and decreased GABA transmission is thought to be the mechanism of several experimental convulsants.

It is possible to increase GABA transmission in the central nervous system (CNS) by drug action at several different sites, such as decreased breakdown of GABA or increased postsynaptic action. No drug is totally selective in its effects, however; to clarify the involvement of GABA, we chose a selection of drugs that act by different mechanisms. The drugs used were amino-oxyacetic acid (AOAA), which inhibits GABA-transaminase; 2,4-diaminobutyric acid (DABA), which blocks the reuptake of GABA; sodium valproate, which potentiates the postsynaptic actions of GABA and decreases its breakdown (10); flurazepam, a water-soluble benzodiazepine, which acts on benzodiazepine receptors at the GABA-receptor-ionophore complex; and muscimol, a GABA agonist.

All of the drugs tested, except muscimol, were found to provide protection in mice against the HPNS in mice (11). The rate of compression used was 3 atm/min$^{-1}$ of helium, and both tremor and convulsion thresholds were raised. Sodium valproate and flurazepam were particularly effective (Figs. 1 and 2); they raised the pressure thresholds for the onset of the signs by up to 75%. Amino-oxyacetic acid and DABA did not provide as much protection but gave significant increases in pressure thresholds of up to 27% (Figs. 1 and

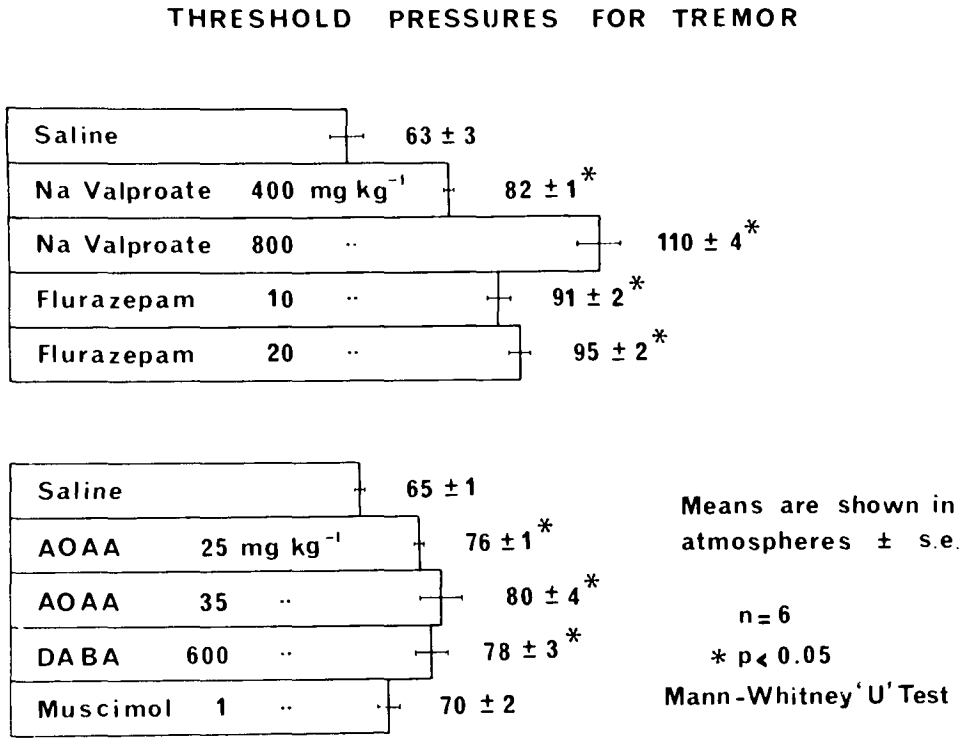



Fig. 1. Threshold pressure for the onset of coarse tremor in mice. The first group of drugs were given (i.p.) immediately before compression, the second group 1 h before. $n$ = 5–6. (Data reproduced with permission from Bichard and Little, Ref. 11.)

2). Muscimol did not cause any significant changes either when given by the intraperitoneal route or intracerebroventricularily.

The doses of the drugs used were those previously established to be effective against convulsions due to the GABA antagonist bicuculline. After obtaining the results in the pressure studies, we carried out a detailed comparison of the effectiveness of the anticonvulsants on bicuculline convulsions, with their actions on pressure convulsions. We used intravenous infusions of bicuculline; doses and time schedules for the drug administration and body temperature control were the same as those for the pressure studies. The orders of effectiveness of the drugs against bicuculline and against pressure convulsions were very similar, including the poor anticonvulsant properties of muscimol (11). Two main differences were noted: First, the drugs seemed to be less effective, in all, against pressure convulsions—although we would hesitate to draw a direct quantitative comparison between the application of pressure and the infusion of a convulsant drug. Second, there appeared to be a limit to the extent to which the drugs, particularly sodium valproate and

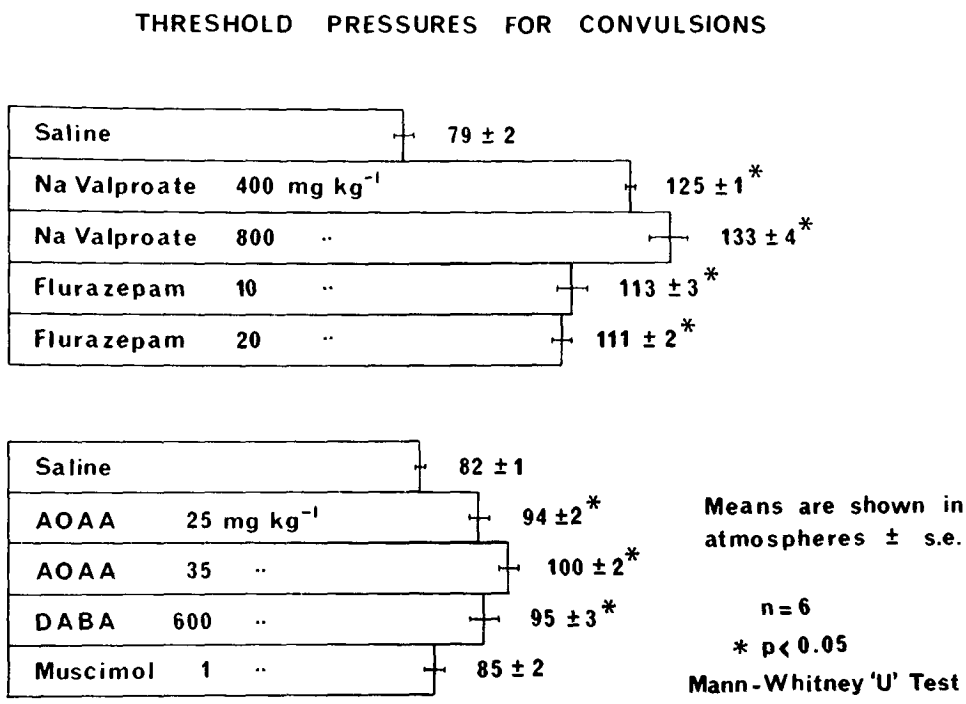



Fig. 2. Threshold pressures for the onset of full convulsions in mice. Treatments as for Fig. 1. $n$ = 5–6. (Data reproduced with permission from Bichard and Little, Ref. 11.)

flurazepam, could protect against pressure convulsions, because the higher doses did not raise thresholds much further than the lower doses.

These results showed that drugs which potentiate GABA transmission were clearly effective in protecting against the effects of pressure. The similarity of their potencies in this respect with their anticonvulsant activities against bicuculline suggested that their actions on GABA transmission were the reason for their protective effects, but we did not consider, as we will explain, that this necessarily indicated that changes in GABA transmission were the cause of the HPNS signs.

There remained, however, the possibility that nonspecific "general anesthetic" properties of the compounds contributed to their effects at pressure. At the doses used the compounds did not cause any overt effects on the behavior of the mice, except some ataxia with the higher dose of valproate and the muscimol, and righting reflexes were not lost, but at higher doses these compounds are known to have sedative effects. This problem was solved by the timely appearance of the benzodiazepine antagonist Ro 15-1788, which binds to benzodiazepine receptors and prevents the in vivo and in vitro actions

of these drugs (12,13). We tested this drug against the effect of flurazepam on bicuculline convulsions and showed that it completely prevented the anticonvulsant effects, so we investigated its actions on the protective effect of flurazepam on the HPNS (14). The results are summarized in Fig. 3 and show that Ro 15-1788 completely prevented the effects of flurazepam on the HPNS in mice. Ro 15-1788 did not have any effect on either tremor or convulsions when given alone, a finding which suggests that changes in endogenous benzodiazepine ligands, whose existence has been recently postulated (15), do not contribute to the effects of pressure.

The actions of Ro 15-1788 showed that the effects of flurazepam in raising both tremor and convulsion thresholds were mediated by its action at benzodiazepine receptors. These receptors are found in many areas of the CNS and are thought to mediate the effects of the benzodiazepines by causing increases in the effects of GABA at the postsynaptic membrane (16,17). To our knowledge this is the first time that the effects of a drug on the HPNS have been prevented by a specific antagonist. This action distinguishes the effects of flurazepam from those of the general anesthetics, because Ro

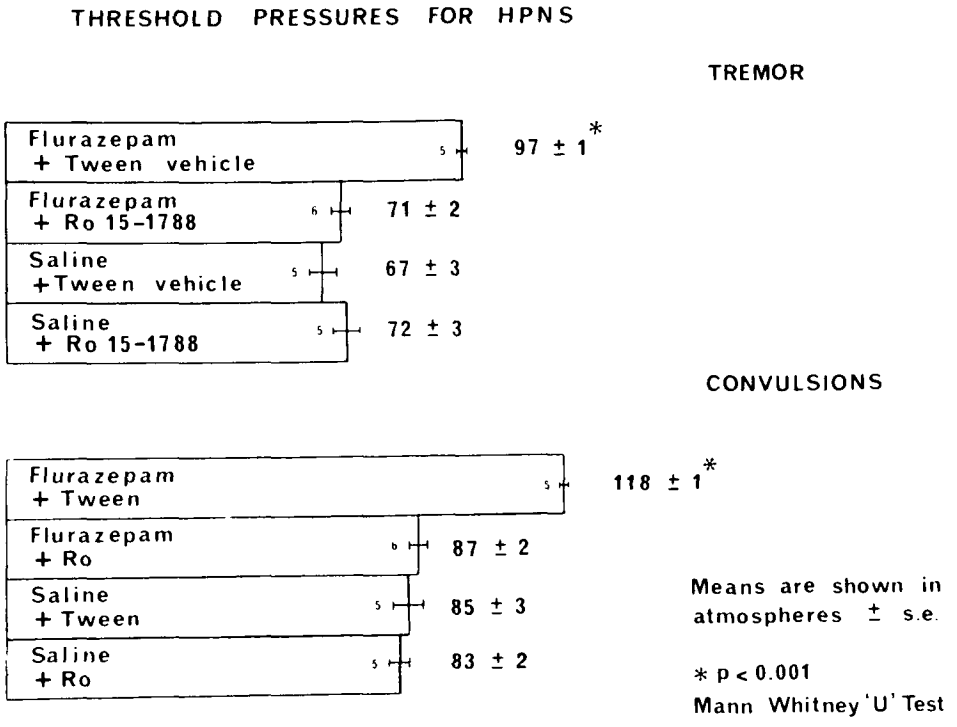



Fig. 3. Threshold pressures for the onset of coarse tremor and of full convulsions in mice. Drugs were injected immediately before compression. Ro 15-1788, 20 mg/kg$^{-1}$, was suspended in Tween vehicle, which was given to the corresponding control animals.

15-1788 does not affect the actions of the latter drugs. We considered using GABA antagonists such as bicuculline or picrotoxin against the actions of the other protective drugs, but there would have been problems in the interpretation of these studies because these antagonists cause convulsions when given alone. It would, therefore, have been difficult to distinguish between decreases in the effects of the anticonvulsants and increases in central excitability from the intrinsic effects of the convulsants.

The effectivensss of the anticonvulsants in protecting against the HPNS raised the possibility that some, or all, of the effects of pressure might result from decreases in central GABA transmission. The results from the studies in vivo described previously do not provide any evidence that this is the case as these drugs are anticonvulsant in many types of seizures. The parallel effects of the drugs on tremor and on convulsion thresholds provide evidence that the effects of the drugs on these symptoms may have a common origin but not necessarily that the symptoms have a common underlying cause.

We carried out some preliminary studies on the effects of GABA antagonists on the HPNS (18) but realized that the effects of convulsant drugs on the syndrome, even at subconvulsive doses, would be meaningless. A synergistic effect with pressure could mean either that pressure was having a depressant effect on that particular transmitter system, which was additive with that of the drug, or that that particular transmitter system was intact and unaffected at pressure so that the effects of the drug could similarly be exerted. Similarily, a lack of effect of a convulsive drug might suggest that pressure was not acting on the same system as the drug or, alternatively, that pressure had depressed the system or systems on which the drug had its effects so that drug action could no longer be seen.

Therefore, to study this problem, we turned to in vitro studies to examine the effects of pressure on GABA transmission directly. Changes in GABA transmission at pressure could occur at many sites on GABA neurones and we thought it was important to study these sites to locate any changes. We have so far studied two components of GABA transmission at pressure in vitro: a) the actions of GABA on the postsynaptic membrane, and b) the release of $^{3}$H-GABA from the spinal cord.

For the former studies we used the rat superior cervical ganglion because this preparation contains GABA receptors closely resembling those in the CNS but the neurones apparently do not use GABA as a transmitter, so the effects on the membrane potential can be studied without interference from effects on presynaptic GABA. Apparatus that enabled extracellular recording of the effects of exogenously applied drugs was designed; details of the apparatus have been given elsewhere (19), so the results will be briefly summarized. Helium pressure of up to 130 atm did not cause any changes in the responses of the tissue to GABA; the depression of nicotinic responses seen was in agreement with earlier results of Kendig et al. (20). We also have found that the responses of this preparation to GABA which had been potentiated by ketamine or pentobaritone remained unaltered by this pressure of helium. These studies do not therefore provide any evidence that pressure depresses the

postsynaptic effects of pressure. In view of these findings and our in vivo results, we suggest that general anesthetics which protect against the HPNS may do so in part by their effects on GABA transmission.

To investigate the effects of pressure on the release process for GABA from the presynaptic terminals, we have used the isolated frog spinal cord. The method of Collins (21) was adapted for use inside a pressure chamber. The isolated, hemisected spinal cord was incubated in 0.5 μCi $^{3}$H-GABA (specific activity 58 Ci/mmol) for 1 h, with 1.0 μCi $^{14}$C-urea (specific activity 57 mCi/mmol) as a marker for nonspecific release. The tissue was then superfused with Ringer solution (containing 10-5M aminooxyacetic acid to inhibit GABA degradation) at 0.15 mL min$^{-1}$ and samples of perfusate collected every 10 min. After 60 min washout the apparatus was transferred to the pressure chamber. The chamber was flushed with 95% $O_2$, 5% $CO_2$ for 1 min at the beginning of each experiment. Both spontaneous and electrically stimulated GABA release were studied in separate experiments and two experimental schedules were used. Experiments without pressure showed that the amount of evoked release was reproducible when two stimulation periods were used 70 min apart. Helium pressure was applied either before or between the stimulation periods at 5 atm min$^{-1}$. In control experiments, 1 atm helium was added to simulate the small, rapid temperature rise that occurred on the initial addition of helium (2–3°C over a few seconds, returning to normal within 2 min). During the remainder of the experiments the temperature was controlled to 21°C ± 1°C. Electrical stimulation was applied to the rostral end of the cord at 50 Hz and 4 mA for 2 min. The concentrations of $^{3}$H-GABA in the fractions were measured by liquid scintillation counting and the results were expressed as sample disintegrations per minute (d.p.m.) as a percentage of tissue d.p.m. Each experiment was repeated a minium of four times.

The spontaneous release of GABA was not changed significantly by the application of either 50 or 100 atm helium (Table I). The effects of pressure on $^{14}$C-urea washout were studied in separate experiments as a control for tissue damage. No change in urea efflux was seen at 50 or 100 atm helium (Table I). A change in spontaneous GABA release was seen, however, when helium pressure was applied after electrical stimulation (to be discussed further).

The electrically evoked release of GABA was unchanged when helium pressure of 50 atm was applied between the two periods of stimulation (Table II). The washout of urea was not changed by the electrical stimulation at normal or high pressures when compared with the efflux immediately preceding the electrical stimulation. There were no significant differences between the urea efflux during stimulation at 50 atm helium when compared with that at 1 atm helium or when no helium was added.

A significant effect of pressure was seen, however, in the spontaneous GABA release when helium was added after the electrical stimulation (Table III). This effect is in contrast to the lack of change in spontaneous release when no electrical stimulation had been applied before compression. A small increase in urea washout was also seen at this time, but as this was also seen

TABLE I

Ratios of Spontaneous Release before Addition of Helium to Amount of Release 10 min after Compression

| Helium Pressure | $^3$H-GABA | $^3$H-urea |
|---|---|---|
| 1 | 1 | 0.83 |
| 50 | 1.04 | 1.1 |
| 100 | 1.56 | 1.17 |

Pressures refer to the helium added on top of the initial 1 atm of 95% $O_2$, 5% $CO_2$, 40 min after the tissues had been transferred to the pressure chamber. No significant changes were seen in spontaneous GABA release or in urea washout after addition of helium.

when only 1 atm helium was added it is likely to have been caused by temperature rather than pressure changes.

To study further the interaction between stimulation and compression, we repeated the experiments using a different schedule. The results in Tables IV and V show the changes when pressure was applied before the two stimulation periods. The GABA release during the stimulation was significantly increased at 100 atm helium, but not at 50 atm, when compared with the evoked release at 1 atm. At both 50 and 100 atm, however, the release was also increased after the stimulation periods. Using this schedule at both 50 and 100 atm, we found no significant changes in urea washout.

The calcium dependence of the changes seen was investigated and the results are given in Table VI. In the absence of calcium the electrically evoked release of GABA at 100 atm helium was signficantly lower than in normal

TABLE II

Ratios of Evoked Release during First Electrical Stimulation to Evoked Release during Second Stimulation

| Helium Pressure | $^3$H-GABA | $^{14}$C-urea |
|---|---|---|
| 0 | 0.97 | 0.70 |
| 1 | 1.00 | 0.91 |
| 50 | 0.98 | 0.83 |

Pressures refer to the helium added on top of the initial 1 atm of 95% $O_2$, 5% $CO_2$, 40 min after the tissues had been transferred to the pressure chamber. Helium pressure was applied between the first and second periods of stimulation, over 10 min, and held at that pressure for the remainder of the experiment. No significant changes were seen in either the amount of GABA release or the urea washout after the helium pressure was applied.

TABLE III

Ratios of the Spontaneous Release Measured 20 min after the Application of Helium Compared with the Release Immediately Preceding the Compression

| Helium Pressure | $^3$H-GABA | $^{14}$C-urea |
|---|---|---|
| 0 | 0.87 | 0.85 |
| 1 | 1.55 | 1.32 |
| 50 | 2.45* | 1.55† |

Release ratios at the corresponding times when no helium was added are included for comparison. Pressures refer to the helium added on top of the initial 1 atm of 95% $O_2$, 5% $CO_2$, 40 min after the tissues had been transferred to the pressure chamber. *GABA release was significantly ($P = 0.05$) increased after addition of 50 atm helium compared with either 0 or 1 atm helium. †Urea washout was increased ($P = 0.05$) at 50 atm helium compared with no helium added, but the difference between 1 and 50 atm was not significant.

Ringer solution, but the spontaneous release after the stimulation was similar in both solutions.

In summary, therefore, our results showed that helium pressure caused increases in spontaneous GABA release only if the preparations had been previously subjected to electrical stimulation. The immediately evoked release on stimulation was increased only by 100 atm helium (not by 50 atm) and this latter change, but not the former, was dependent on the presence of calcium in the medium. The lack of changes in urea washout under these conditions suggested that the changes seen resulted from effects on the GABA release

TABLE IV

Ratios of Evoked Release after Addition of Both 1 atm and High Helium Pressure

| Helium Pressure | $^3$H-GABA | | $^{14}$C-urea | |
|---|---|---|---|---|
| | First Stimulation | Second Stimulation | First Stimulation | Second Stimulation |
| 1 | 1 | 1 | 1 | 1 |
| 50 | 0.94 | 0.99 | 0.89 | 1.01 |
| 100 | 2.25* | 1.16 | 1.02 | 1.12 |

Pressures refer to the helium added on top of the initial 1 atm of 95% $O_2$, 5% $CO_2$, 40 min after the tissues had been transferred to the pressure chamber. Electrical stimulation did not alter $^{14}$C-urea efflux and no changes in washout were seen between the different pressures of helium. *The evoked release of GABA was significantly ($P = 0.01$) increased during the first stimulation period at 100 atm, when compared with the evoked release at 1 atm, first stimulation. Helium pressure was applied before both stimulation periods.

TABLE V

Ratios of Spontaneous Efflux after Addition of High Helium Pressures Compared with Efflux after 1 atm Helium, Measured 10 min after Each Electrical Stimulation Period

| Helium Pressure | $^3$H-GABA | | $^{14}$C-urea | |
|---|---|---|---|---|
| | First Stimulation | Second Stimulation | First Stimulation | Second Stimulation |
| 1 | 1 | 1 | 1 | 1 |
| 50 | 1.51 | 1.55* | 1.11 | 1.14 |
| 100 | 2.01* | 1.81* | 1.20 | 1.02 |

Pressures refer to the helium added on top of the initial 1 atm of 95% $O_2$, 5% $CO_2$, 40 min after the tissues had been transferred to the pressure chamber. *The spontaneous release of GABA during the time period following electrical stimulation was significantly ($P = 0.05$) increased by the application of 50 or 100 atm helium, before both periods of stimulation. No significant changes in urea washout were seen at these times.

process rather than nonspecific changes in membrane permeability. It does not appear, therefore, that the hyperexcitability seen at high pressure can be attributed to a lowering of the amount of GABA released, because all the changes seen involved increases. There remains the possibility that pressure may cause changes in GABA synthesis, however, and it would be necessary to study this process separately—for example, by measuring glutamic and decarboxylase activity at pressure. These results are consistent in direction, but not in detail, with those reported for acetylcholine release (22), where we found a

TABLE VI

Ratios of Evoked Release in Normal Ringer Solution to Evoked Release in Calcium-Free Medium, at 100 atm Helium

| $^3$H-GABA | | $^{14}$C-urea | |
|---|---|---|---|
| First Stimulation | Second Stimulation | First Stimulation | Second Stimulation |
| 0.49* | 0.77 | 1.13 | 1.03 |
| Corresponding Ratios 10 min after Stimulation | | | |
| 1.2 | 1.15 | 1.14 | 1.03 |

Pressures refer to the helium added on top of the initial 1 atm of 95% $O_2$, 5% $CO_2$, 40 min after the tissues had been transferred to the pressure chamber. *In the calcium-free medium the evoked release at 100 atm was significantly ($P = 0.05$) lower than in normal Ringer solution, but no significant changes were seen in the delayed increase in GABA release following the stimulation or, at either time, in urea washout.

small increase in spontaneous acetylcholine output but not in electrically evoked release at pressure.

The effects of pressure described in other preparations might account for, or contribute to, the change seen in GABA release. The most likely of these is repetitive firing, reported by Kendig et al. (23) and Grundfest (24). This firing might explain the increases seen following electrical stimulation; such increases were absent when no stimulation had been applied.

Other effects that might contribute to increased transmitter release are inhibition of active sodium transport and changes in intracellular calcium distribution (25).

In conclusion, the results we have obtained from in vitro studies not do support the concept that the HPNS is caused by decrease in GABA transmission, although it is recognized that this may occur at sites which have not yet been investigated.

We would like to speculate that neuronal hyperexcitability in many systems may be caused by pressure and that for some reason yet unknown the overall balance in vivo results in the hyperexcitability syndrome.

## Acknowledgments

A. R. Bichard was a Medical Research Council Scholar during this work. We would like to thank Dr. Haefely of Hoffman-la-Roche for gifts of Ro 15-1788.

## References

1. Wardley-Smith B, Halsey MJ. Prevention of HPNS: the possible use of structural isomers of anesthetics. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:337–343.
2. Green CJ, Halsey MJ, Wardley-Smith B. Possible protection against some of the physiological effects of high pressure. J Physiol 1977;267:46P–47P.
3. Hunter WL Jr, Bennett PB. The causes, mechanisms, and prevention of the high pressure nervous syndrome. Undersea Biomed Res 1974;1:1–28.
4. Beaver RW, Brauer RW, Lahser S. Interaction of central nervous system effects of high pressures with barbiturates. J Appl Physiol 1977;43:221–229.
5. Halsey MJ, Wardley-Smith B. The high pressure neurological syndrome: do anti-convulsants prevent it? Br J Pharmacol 1980;72:502–503P.
6. Brauer RW, Mansfield JR, Beaver RW, Gillen HW. Stages in development of high pressure neurological syndrome in the mouse. J Appl Physiol 1979;46:756–765.
7. Rowland-James P, Wilson MW, Miller KW. Pharmacological evidence for multiple sites of action of pressure in mice. Undersea Biomed Res 1981;8:1–11.
8. Lehman AJ, Newman HW. Propyleneglycol: rate of metabolism, absorption, and excretion with a method for estimation in body fluids. J Pharmacol Exp Ther 1937;60:312–322.
9. Jackson N. Investigations into the effect of drugs and hyperbaric pressures on mice. Part II dissertation thesis, B.Sc. Chemistry. Oxford: Oxford University, 1978.
10. Chapman A, Keane PE, Meldrum BS, Simiand J, Vernieres JC. Mechanism of anticonvulsant action of valproate. Prog Neurobiol (Oxf) 1983;19:315–359.
11. Bichard AR, Little HJ. Drugs that increase γ-aminobutyric acid transmission protect against the high pressure neurological syndrome. Br J Pharmacol 1982;76:447–452.

12. Hunkeler W, Mohler H, Pieri L, Polc P, Bonetti EP, Cumun R, Schaffner R, Haefely W. Selective antagonist of benzodiazepines. Nature 1981;290:514–516.
13. Nutt DJ, Cowen PJ, Little HJ. Unusual functional interactions of benzodiazepine antagonists. Nature 1982;295:436–438.
14. Bichard AR, Little HJ. The benzodiazepine antagonist, Ro 15-1788, prevents the effects of flurazepam on the high pressure neurological syndrome. Neuropharmacology 1982;21:877–880.
15. Mohler H. Benzodiazepine receptors: are there endogenous ligands in the brain? Trends Pharmacol Sci 1981:116–119.
16. Squires RF, Braestrup C. Benzodiazepine receptors in rat brain. Nature 1977;266:732–734.
17. Haefely W. General pharmacology and neuropharmacology of benzodiazepine derivatives. Vol. 55/II. In: Hoffmeister F, Stille G, eds. Handbook of experimental pharmacology. Berlin, Heidelberg, New York: Springer Verlay, 1981:13–262.
18. Bichard AR. Gamma-aminobutyric acid transmission and the high pressure neurological syndrome. PhD Thesis. Oxford: Oxford University, 1983.
19. Little HJ. Effects of ketamine and of high pressure on the responses to γ-aminobutyric acid of the rat superior cervical ganglion *in vitro*. Br J Pharmacol 1982;77:209–216.
20. Kendig JJ, Trudell JR, Cohen EN. Effects of pressure and anaesthetics on conduction and synaptic transmission. J Pharmacol Exp Ther 1975;195:216–224.
21. Collins GGS. The spontaneous and electrically evoked release of $^{3}$H-GABA from the isolated hemisected frog spinal cord. Brain Res 1974;66:121–127.
22. Little HJ, Paton WDM. The effects of high pressure helium and nitrogen on the release of acetylcholine from the guinea pig ileum. Bri J Pharmacol 1979;67:221–228.
23. Kendig JJ, Schneider JM, Cohen EN. Pressure, temperature, and repetitive impulse generation in crustacean axons. J Appl Physiol 1978;45:742–746.
24. Grundfest H. Effects of hydrostatic pressures on the excitability, the recovery, and the potential sequence of frog nerve. Cold Spring Harbor Symp Quant Biol 1936;4:179–187.
25. Goldinger JM, Kang BS, Morin RA, Paganelli CV, Hong SK. Effect of hydrostatic pressure on active transport, metabolism, and the Donnan equilibrium in human erythrocytes. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:589–599.

# AN EVALUATION OF MEPHENESIN AND RELATED DRUGS IN CONTROLLING THE EFFECTS OF HIGH PRESSURE ON ANIMALS

*F. Bowser-Riley, N. M. Lashbrook, W. D. M. Paton, and E. B. Smith*

The high pressure neurological syndrome (HPNS) has been described in man (1) and in experimental animals (2), and although the signs and symptoms have been well documented there still remains no clear picture of how pressure gives rise to the observed effects. In animals the stimulant action of pressure is manifested not only as convulsions and intense motor activity but also by the appearance of pronounced tremors and a variety of stereotyped movements. These manifestations, when considered along with the relative ineffectiveness of classical antiepileptics (3) in relieving the effects of pressure, indicate that pressure acts at a subcortical rather than at a cortical level within the central nervous system (CNS). Neuroanatomical studies have confirmed that pressure has a subcortical site of action, and have suggested, in common with other subcortically induced convulsive states, that HPNS may result from a failure of central inhibition (4,5).

The aim of the present study is to investigate the role of subcortical inhibitory pathways in the genesis of HPNS using a group of centrally acting muscle relaxants comprising the propandiols: mephenesin, methocarbamol, meprobamate, and carisoprodol. The properties of these compounds have been summarized by Smith (6). Their mode of action is generally accepted to be mediated at a subcortical level via polysynaptic pathways. Although not normally regarded as anticonvulsants, they are known to be effective antagonists of a variety of induced seizure states. While their effectiveness as muscle relaxants is limited by their short duration of action they have been well characterized clinically and are virtually free of anesthetic or narcotic effects.

## METHODS

All experiments were performed on male mice (CD1 strain; Charles River) weighing between 20 and 25 g. Drugs were administered intraperitoneally and obtained from the following sources: a) *Muscle relaxants*; Carisoprodol (N-isopropyl-2-methyl-2-propyl-1,3-propandiol dicarbamate: Pharmax); Mephenesin (3-(o-tolyoxy)-1,2-propandiol: Sigma); Meprobamate (2-methyl-2-propyl-1,3-propandiol: Pharmax); Methocarbamol (3-(o-methoxyphenoxy)-1,2-propandiol-1-carbamate: A.H.Robins); Orphenadrine citrate (N,N-dimethyl-2-(o-methyl-$\alpha$-phenylbenzyloxy) ethylamine: Riker); Styramate (1-phenyl-1,2,ethandiol 2-carbamate: Armour); b) *Convulsants*; Strychnine Hydrochloride (BP: Burroughs-Wellcome); Picrotoxin (Sigma); Metrazol (pentamethylentetrazol: Bilhuber-Knoll).

Drugs were dissolved in either saline or 1% ethanol-saline solution at 37°C and injected 15 min before pressurization. Controls received the appropriate drug vehicles. Doses of the muscle relaxants were calculated to give equivalent clinical effects on the basis of a 15-min loss in righting reflex. Strychnine, picrotoxin, and metrazol were given in subconvulsive doses, calculated as the highest dose compatible with the failure to elicit convulsions in groups of 12 mice exposed to a $P_{O_2}$ of 1 atm for 1 h within the pressure vessel.

The procedure employed to assess the actions of these compounds on HPNS comprised the pressurization with helium (rate 3 atm/min: $P_{O_2}$ 1 atm) of batches of 6 mice in a 251 pressure vessel equipped with $CO_2$ scrubber, temperature sensors, and internal heating control. Animal temperature (monitored by rectal thermistor) was maintained between 36 and 37°C. Continuous video recordings of animal behavior were made during compression; these recordings allowed subsequent playback for analysis under conditions in which observer bias was controlled. Assessment of drug action was made from the ability of the drug to alter the following behavioral end-points associated with HPNS: a) *fine tremors*, intermittent twitching of the neck and back muscles often associated with the adoption of a hunched posture; b) *coarse tremor*, shivering of the whole body with difficulty in coordinated movement; c) *convulsions*, seizures involving total loss of righting reflex; d) *death*.

## RESULTS

The actions of the centrally acting muscle relaxants on HPNS are shown in Table I and their relative effectiveness is shown in Fig. 1. It was found that mephenesin, a phenyl substituted propandiol, significantly increased the pressure of onset for all four behavioral end-points associated with HPNS. The mono-carbamate analogue of mephenesin, methocarbamol, although less potent, afforded a similar degree of protection. The action of mephenesin and

TABLE I

HPNS Thresholds (atm ± SEM) for CD1 Mice Treated with Mephenesin-Like Muscle Relaxants

| Drug | Fine Tremor | Coarse Tremor | Convulsions | Death | Number |
|---|---|---|---|---|---|
| Vehicle Control | 35 ± 1 | 72 ± 1 | 83 ± 1 | 118 ± 2 | 15 |
| Mephensin (260 mg/kg) | 87 ± 3 | 110 ± 2 | 120 ± 2 | 160 ± 3 | 10 |
| Methocarbamol (500 mg/kg) | 86 ± 4 | 102 ± 2 | 116 ± 2 | 167 ± 5 | 6 |
| Mephenesin (130) + Methocarbamol (250) | 82 ±2 | 102 ± 1 | 115 ± 2 | 153 ± 6 | 11 |
| Meprobamate (200 mg/kg) | 58 ± 2* | => Continuous Convulsions | | 167 ± 1 | 12 |
| Styramate (250 mg/kg) | 65 ± 1 | 86 ± 1 | 120 ± 1 | 171 ± 6 | 6 |
| Carisoprodol (200 mg/kg) | 42 ± 2 | 75 ± 1 | 90 ± 4 | 139 ± 4 | 6 |
| Orphenadrine (50 mg/kg) | 40 ± 2 | 63 ± 1 | 73 ± 1 | 168 ± 7 | 6 |

* Onset of severe tremors leading to continuous convulsions.

methocarbamol together appeared to be simply additive, indicative of a common site of action. The relative effectiveness of mephenesin and methocarbamol on the different components of the HPNS (Fig. 1) clearly shows their protective action is most marked on the early stages of HPNS, a finding which could reflect the short duration of action of these compounds. Yet this may not be the case because the ethandiol derivative, styramate, a more potent and longer-acting muscle relaxant (7) postponed the convulsions to the same extent as mephenesin, but was much less effective in controlling tremors. Two additional muscle relaxants, meprobamate and carisoprodol, were tested for their effectiveness against HPNS. Both are aliphatic dicarbamate derivatives of mephenesin and, like styramate, are more potent and longer acting than mephenesin in muscle relaxation (8). However, meprobamate and carisoprodol gave two sets of very different results. Meprobamate postponed the onset of HPNS from a control value of 35 to 58 atm and elevated the death threshold to 177 atm, but, in addition, drastically changed the nature of the response. This change was characterized by the sudden onset of severe tremors culminating in violent continuous myoclonic seizures. In contrast, carisoprodol, while marginally increasing the threshold for fine tremor, had no discernible effect on either the nature or the onset pressures of the other end-points of HPNS.

The action of these compounds on HPNS is comparable to their actions in opposing other induced seizure states (6). Mephenesin and methocarbamol are

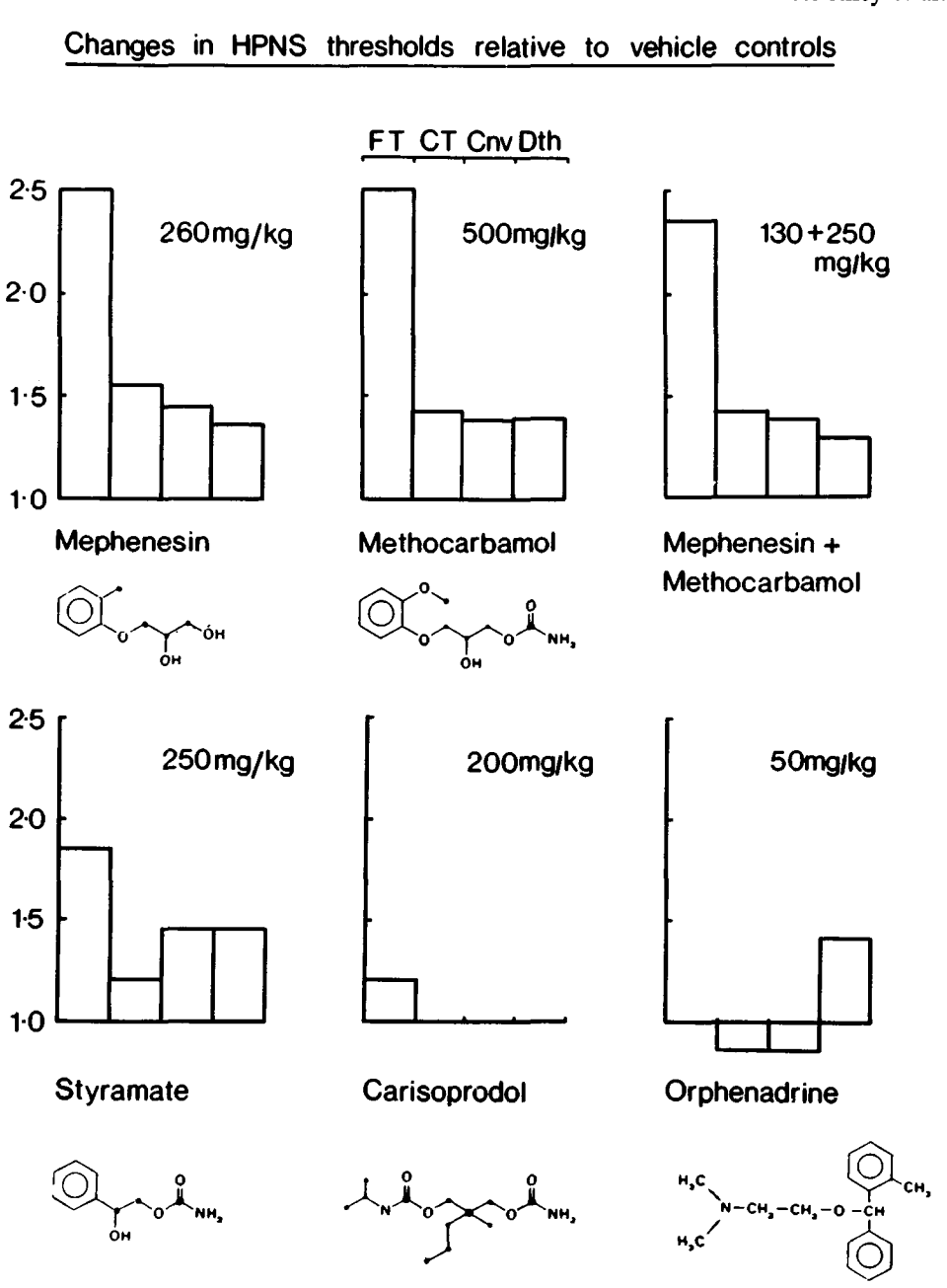



Fig. 1. Relative changes in HPNS thresholds for the actions of some mephenesin-related muscle relaxants and antispasmodics compared to the appropriate drug vehicle control. Only statistically significant changes ($P<1\%$) are shown. FT: fine tremors; CT: coarse tremors; Cnv: convulsions; Dth: death.

both strong antagonists of the convulsions and lethality of strychnine. On the other hand, although meprobamate gives good protection against the lethality, it is a poor antagonist of strychnine convulsions and carisoprodol has very little effect on either aspect of strychnine toxicity. With metrazol-induced seizures, the order of effectiveness is reversed: carisoprodol and meprobamate afford better protection than either mephenesin or methocarbamol. Additional experiments with the related antispasmodic orphenadrine, which is ineffective against strychnine seizures (9), demonstrated that this compound, like carisoprodol, failed to give protection to HPNS. Indeed, the converse was the case, orphenadrine actually reduced the onset pressures for both coarse tremors and convulsions (see Fig. 1).

To test the apparent relationship between the differential actions of the muscle relaxants on HPNS with their ability to antagonize chemically induced seizures, we tested subconvulsive doses of strychnine, picrotoxin, and metrazol on the responses to pressure. The results of these experiments are shown in Table II and their relative effectiveness on the different components of HPNS are summarized in Fig. 2. Strychnine proved to be the most potent and while it was not possible to distinguish the fine tremor induced by pressure from those resulting from the actions of strychnine alone, the thresholds for coarse tremor and convulsions were dramatically reduced. The nature of the convulsions observed were characteristic of HPNS and were clearly distinguishable from the tonic extensor spasms provoked by convulsive doses of strychnine. Furthermore, unlike strychnine, where the convulsive episode is closely followed by death, the lethal effects of pressure, although enhanced, were clearly separated from the convulsions. Picrotoxin moderately lowered the threshold for fine tremors, markedly lowered the thresholds for coarse tremors and convulsions, and gave rise to a small but not significant increase in the death threshold. Metrazol, although least potent, similarly lowered the

TABLE II

HPNS Thresholds (atm ± SEM) for CD1 Mice treated with Convulsants

| Drug | Fine Tremor | Coarse Tremor | Convulsions | Death | Number |
|---|---|---|---|---|---|
| Vehicle Control | 36 ± 1 | 72 ± 1 | 88 ± 1 | 131 ± 2 | 36 |
| Strychnine (1 mg/kg) | ND | 39 ± 1 | 49 ± 3 | 105 ± 6 | 6 |
| Picrotoxin (3 mg/kg) | 28 ± 1 | 47 ± 3 | 57 ± 6 | 149 ± 9 | 6 |
| Metrazol (40 mg/kg) | 33 ± 2 | 56 ± 1 | 59 ± 3 | 137 ± 11 | 6 |
| Untreated Control | 35 ± 1 | 73 ± 1 | 87 ± 1 | 129 ± 4 | 14 |

ND: not determined. *See text.*

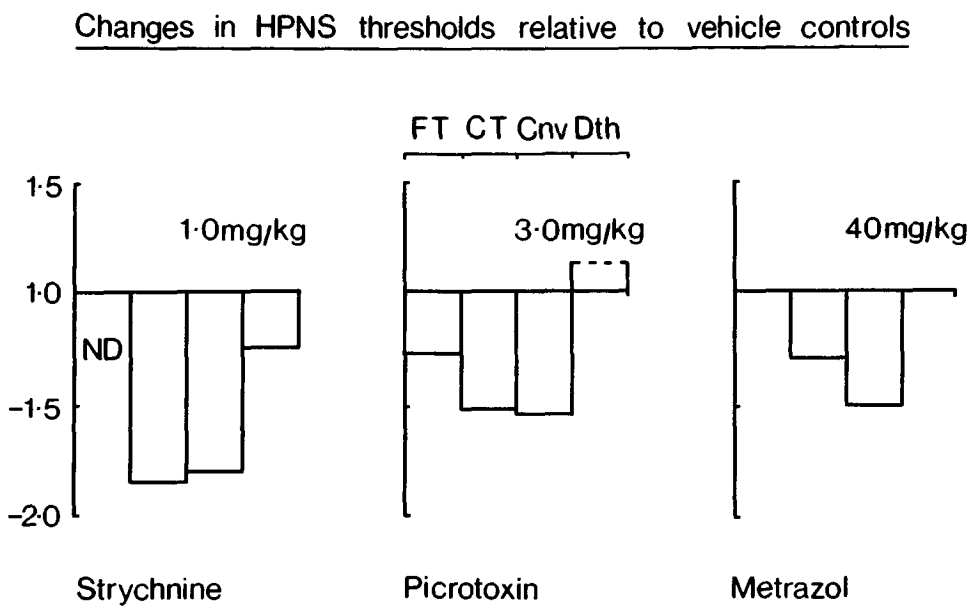



Fig. 2. Relative changes in HPNS thresholds for the actions of three convulsants compared to the appropriate vehicle control. Only statistically significant changes ($P<1\%$) are shown. FT: fine tremors; CT: coarse tremors; Cnv: convulsions; Dth: death. ND not determined (*see text*).

thresholds for coarse tremors and convulsions but failed to have any effect on the thresholds for fine tremors and death.

## DISCUSSION

The results of this investigation have shown that although the propandiols all exhibit muscle relaxant activity they appear to differ in their effects on the response to pressure. The aromatic propandiols, mephenesin and methocarbamol, give a remarkable degree of protection against HPNS. Their relative potency against HPNS is very close to that required to produce muscle relaxation, methocarbamol being approximately one-half that of mephenesin; this finding suggests that their anti-HPNS properties may arise as a result of muscle relaxation masking the symptoms of HPNS. However, the findings that the more potent muscle relaxants, meprobamate and carisoprodol, are relatively ineffective against the actions of pressure indicates that the anti-HPNS actions of mephenesin and methocarbamol are related to some mechanism other than their ability to produce muscle relaxation.

The clear division of action between the aromatic and aliphatic propandiols in opposing the effect of pressure appears to be related to their relative ability to antagonize seizures induced by strychnine. Mephenesin is a potent

antagonist of strychnine (10), an action which, like its effect against pressure, has been shown to be independent of its ability to produce muscle relaxation (11). The finding that strychnine potentiaties HPNS suggests that the anti-HPNS and antistrychnine action of the propandiols may share a similar mechanism. It is well established that strychnine acts by blocking the inhibitory processes mediated by glycine (12). Thus, it may be that the convulsive effects of high pressures are a result of its action on those inhibitory processes mediated by glycine.

The findings that both metrazol and pictrotoxin potentiate HPNS, though to a lesser extent than strychnine, indicate that the action of pressure is not expressed exclusively via an action on these pathways. The action of these convulsants is thought to be mediated via γ-aminobutyric acid (GABA) (13), and it has been established that GABA-potentiating agents (e.g., Sodium Valproate and Flurazepam) have anti-HPNS action (14). Previous work (15,16) has shown that inhibitory processses mediated by noradrenaline also play a part in determining the overall response to pressure. The relative contributions of these pharmacological mechanisms to the overall response to pressure remains to be determined.

We believe that further investigation with the mephenesin-like propandiols may present not only important evidence as to the site of action of pressure within the CNS but also improved methods of controlling the disabling effects of pressure and avoiding the narcotic problems concomitant with the current use of anesthetics.

## Acknowledgments

Supported by ONR Contract No.0014-77-0-0078. We wish to thank the following for their kind donation of drugs: Armour Pharmaceuticals (Styramate); Pharmax Research (Carisoprodol and Meprobamate); and A. H. Robins Co., Ltd. (Methocarbamol).

## References

1. Bennet PB. The high pressure nervous syndrome: man. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving and compressed air work, 2nd ed. London: Baillière Tindall, 1975:248–263.
2. Brauer RW. The high pressure nervous syndrome: animals. In Miller JG, ed. The pharmacology and clinical usefulness of carisoprodol. Detroit: Wayne State University Press, 1959:25.
3. Halsey MJ, Wardley-Smith B. The high pressure neurological syndrome: Do anti-convulsants prevent it? Br J Pharmacol 1980;72:502–503.
4. Bowser-Riley F, Paton WDM, Smith EB. Experiments to investigate the neural origin of the high pressure neurological syndrome. Br J Pharmacol 1981;74:820.
5. Kaufmann PG, Bennett PB, Farmer JC Jr. Differential effects of pressure on the mammalian central nervous system. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:371–379.
6. Smith CM. Relaxants of the skeletal muscle. In: Root WS, Hofmann FG, eds. Physiological pharmacology vol 11. New York, London: Academic Press, 1965:2–73.

7. Witkin LB, Spitaletta P, Galdi F, O'Keefe E. Some neuropharmacologic effects of four mephenesin-like agents in mice. Toxicol Appl Pharmacol 1960;2:264–269.
8. Berger FM. The pharmacological properties of 2-methyl-2-N-propyl-1, 3-propandiol dicarbamate (Miltown), a new interneuronal blocking agent. J Pharmacol Exp Ther 1954;112:413.
9. Cronheim G. Pharmacologic observations with orphenadrine hydrochloride. J Pharmacol Exp Ther 1958;122:16A.
10. Berger FM. The mode of action of myanesin. Br J Pharmacol 1947;2:241–250.
11. Berger FM. Central depressant and anticonvulsant properties of glycerol ethers isomeric with mephenesin. J Pharmacol Exp Ther 1952;105:450–457.
12. Eccles JC. Presynaptic inhibition in the spinal cord. In: Eccles JC, Schade JP, eds. Progress in brain research, vol 12: Physiology of spinal neurons. Amsterdam: Elsevier Publishing, 1964:65–91.
13. In: Glaser GH, Penny JK, Woodbury DM, eds. Antiepileptic drugs: Mechanism of action. New York: Raven Press, 1980:249–304.
14. Bichard AR, Little HJ. The involvement of GABA in the high pressure neurological syndrome (HPNS). Br J Pharmacol 1981;74:221P.
15. Bowser-Riley F, Dobbie JA, Paton WDM, Smith EB. A possible role for monoaminergic inhibition in the high pressure neurological syndrome. Undersea Biomed Res 1982;9(Suppl):32–33.
16. Daniels S, Green AR, Koblin DD, Lister RG, Little HJ, Paton WDM, Bowser-Riley F, Shaw SG, Smith EB. Pharmacological investigation of the high pressure neurological syndrome: Brain monoamine concentrations. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:329–336.

# ALTERATIONS IN PROTEIN METABOLISM IN MAN DURING DIVES TO A MAXIMUM OF 660 MSW AND OBSERVATIONS UPON ASSOCIATED DISTURBANCES OF LIVER FUNCTION, MUSCLE FUNCTION, AND THYROID HORMONE METABOLISM

*G. R. Doran and M. P. Garrard*

Divers operating at depths below 200 msw are known to develop a critical state of fatigue, which arises irrespective of the other major hazard of compression, the high pressure neurological syndrome (HPNS). As the operational depth requirements of saturation diving increase, concern is growing because of the shorter allowable lock-out times, which inevitably lead to impairment of diver performance and efficiency when compared with shallow-dive capabilities. The etiology of hyperbaric fatigue is most likely multifactorial, including such problems as hypohydration, laborious respiration in dense atmospheres, and HPNS, as well as muscular inefficiency and hyperreflexia.

This whole spectrum, collectively identifiable as *compression sickness*, would lead clinicians to classify the condition as a systemic affliction.

This paper presents clinical biochemical evidence of disturbed liver and skeletal muscle function, together with altered thyroid hormone metabolism seemingly invoked by exposure to very high pressures; it provides supportive evidence for previously reported alterations in protein metabolism (1).

## THE DIVE PROGRAMS

The work concerns itself with observations upon, and the analysis of samples taken from, the same two divers (coded *Red* and *Blue*) who undertook two separate chamber dives at Alverstoke in 1980 and 1981.

*Dive 12B* (1980) was to a "depth" of 660 msw with divers breathing a "Trimix" atmosphere ($He/N_2$ 10%/$O_2$; 0.4 bar). Compression was spread over 3½ days; divers remained at "bottom" (67-bar pressure) for 36 h and were then decompressed to "surface" over the next 35 days.

*Dive BISAT 81A* (1981) was to a depth of 540 msw with divers breathing a heliox mixture ($0_2$; 0.4 bar). Compression took 6 days; divers remained at bottom (55-bar pressure) for 6 days and were then decompressed over the following 23 days.

## METABOLIC, BIOCHEMICAL, AND CLINICAL CHEMICAL TESTS PERFORMED

Although these tests were initially aimed at investigating nitrogen balance, thyroid function, and liver function, it will be seen that the spectrum of investigations widened on the basis of observed physical effects upon the divers and the results of initial investigations.

### Nitrogen Balance

Both divers were on fixed diets on entry into the chamber and nitrogen balance was monitored over varying periods, either as predetermined or as their physical condition would allow. This was achieved by Kjeldahl analysis of total oral intake compared with total fecal and urinary output.

### Thyroid Function Tests

Plasma levels of total Thyroxine (T4), Tri-iodothyronine (T3), reverse T3 (rT3), and Thyrotophic Hormone (TSH) were measured at 2-day intervals by radio immunoassay before, during, and upon completion of the trials.

### Liver Function Tests (LFT's)

Because of the limited volumes of plasma available, these tests were confined to assay of enzyme activities commonly used as liver function tests in clinical chemistry. These included aspartate and alanine transaminases (AST and ALT), alkaline phosphate (Alk. Phos.), γ glutamyl transpeptidase (γ GT), and 5′nucleotidase (5′ND).

### Other Plasma Enzyme Studies

In view of the results of the LFT's and the thyroid function tests (to be discussed later), two other plasma enzyme activities were assayed: creatine kinase (CK) and adenylate kinase (AK). In addition, isoenzyme analyses of circulating aldolase, enolase, and creatine kinase were undertaken; starch-gel electrophoretic techniques were used.

### Urine Tests

Erlich's aldehyde test for urobilinogen and Obermayer's indican test were performed upon a limited number of urines collected during the 660-msw dive because of noted discoloration of plasma during the compression phase.

### Other Plasma Constituents Assayed

The plasma myoglobin levels were measured by radio immunoassay in the samples taken during the 660-msw dive.

## RESULTS

### Condition and Well-Being of Divers

Throughout the 540-msw trial both divers remained subjectively and objectively fit; they were fully capable of adhering to the diet and exercise programs exactly as planned.

During the compression phase of the 660-msw dive, nitrogen narcosis symptoms were experienced by both divers on reaching 420 msw (43 bars). Further compression to 540 msw (55 bars) considerably alleviated these symptoms and upon arrival at 660 msw (67 bars) after 3½ days compression both divers were well. After 3 h at bottom, however, both divers developed symptoms and signs of overt debilitating HPNS, which persisted throughout the 36 h at 660 msw and were not greatly relieved until decompression had proceeded to about 580 msw. Therefore, strict diet studies and exercise tests could not be recommended until decompression was well under way.

### Plasma Thyroxine Levels

In both the 540-msw divers the total T4 levels rose steadily from the commencement of compression, reached maximum levels during the middle of the decompression phase, and thereafter tended to fall away again—but never quite back to the predive values. In *Diver Red* the T4's actually rose above the upper limit of normal (160 nmol/L), while in *Diver Blue* the maxima reached were just below the upper limits of normal. A similar sequence of change was observed in the plasma samples taken during the 660-msw dive. In these cases, however, although the changes were clearly evident and significant, they were not as pronounced or consistent as in the shallower dive.

### Nitrogen Balance Studies

Throughout the 540-msw trial both divers remained in good health and were maintained upon their strict diet until the 3rd day of decompression so that strict nitrogen balance studies could be undertaken up to that point (a

period of 5 days predive and 16 days indive). Diver *Red* remained in a state of net negative balance except for a short period (the last 3 days at bottom), when the nitrogen contents of the diets were deliberately raised by 80%; he then fell back into negative balance upon reverting to the original diet. Diver *Blue,* however, remained in net negative balance throughout, irrespective of the increased nitrogen intake. Moreover, on returning to his original diet, he went into an even more pronounced negative balance state.

Even more pronounced states of net negative balance developed during the early stages of the 660-msw dive, but little conclusion can be drawn from this because both divers became so acutely unwell on reaching maximum compression that they were unable to eat properly. Strict balance studies could only be recommenced in *Diver Red* after the 8th day of decompression. He progressed rapidly out of a state of net negative balance to one of neutrality, which was maintained throughout the remaining decompression phase.

## Liver Function Tests

In three of the four series of assays the plasma transaminase activities rose during the compression phase of both trials. The elevations were not gross but nevertheless significant (e.g., 2 × the upper limits of normal). Moreover, intermittent elevations of both enzymes were evident in these three series (both the 540-msw divers and *Diver Red* of the 660-msw trial) throughout all three phases of the dives. It was frequently noted that the ALT levels were above those of AST.

The plasma Alk. Phos. and $\gamma$ GT activities never became abnormal in any of the four series, although some slow drift upwards was apparent in the same three series as manifested in the transaminase disturbances. The 5'ND levels, however, did rise above normal limits—again, in a spiky, intermittent fashion throughout the trial and in the same three series as manifest the AST and ALT disturbances. Only in the samples taken from the 660-msw *Diver Blue* was it possible to say that no significant changes in LFT enzyme activities could be demonstrated.

## Plasma CK and AK Activities

Because the transaminases are found in a wide variety of other tissues as well as the liver (skeletal and heart muscle being two rich sources), the plasma CK and AK activities were measured in all four series of samples. At no time during either of the two dives did the plasma CK levels rise above normal limits. On the contrary, as the trials progressed through compression and decompression, the CK activities fell steadily away to the lower limits of normal in all four series, rising rapidly again at the end of the dives. The levels of AK, however, tended to rise throughout the dives; frequently they were well above the upper limits of normal. The most persistent elevation of AK was evident in the deep 660-msw dive, although it was not possible to

measure AK in as many of the samples as in the 540-msw dive (because there was evidence of hemolysis in the plasma).

### Isoenzyme Studies

Because of the severe HPNS-narcosis symptoms suffered by the divers in the early stages of the 660-msw dive, several analyses of isoenzyme components of circulating enzymes were undertaken on both sets of plasma samples. The enzymes we investigated in this manner were aldolase, enolase, and creatine kinase, to see if any of the fractions of these enzymes normally found in the central nervous system (CNS) had appeared in the circulation where they are not normally demonstrable. The particular fractions being sought were the aldolase *C* fraction, the fast-migrating neurone-specific enolase fractions, and the *BB* isoenzyme of creatine kinase. None of the CNS-located isoenzymes were detectable in any of the plasma samples taken at any stage of the two experiments. A further, though less specific, enzymology test of liver function seemed appropriate here: the assay of total aldolase activities. Only in the plasma samples taken from *Diver Red* (the diver who developed the more severe HPNS) during the 660-msw dive did the total aldolase levels ever rise above normal limits.

### Hemolysis of Blood Samples

Only a few of the plasmas from the 540-msw dive showed any significant trace of hemolysis. Hemolysis was much more commonly seen among the samples taken during the 660-msw dive. Moreover, this was most prominent during the early stages of the dive (compression and at bottom). Apparent also was a strange sequence of pigmentation, which went from simple moderate hemolysis on *Compression Day 1* and became more intense as compression progressed. This sequence was apparent in samples from both divers (though much more pronounced in *Diver Red.* Furthermore, the pigmentation also changed in nature from the simple red of hemolysis to a dirty brown coloration by the time maximum compression had been reached. Thereafter, during the early decompression phase, the pigmentation steadily disappeared, later samples randomly manifesting some degree of simple hemolysis.

Spectroscopic scannings of the plasma taken during this early phase of intensifying red-to-brown discoloration were not conclusive. The absorption spectra showed the general features of hem pigments but did not conform exactly with hemoglobin or methemoglobin. The possibility that some or all of the pigmentation resulted from leakage of myoglobin led to the assay of myoglobin levels (by radio immunoassay). At no time did the circulating myoglobin levels become abnormally high in either diver.

### Urinary Urobilinogen and Indican Levels

The aforementioned sequence of plasma pigmentation raised the question as to whether or not actual in-vivo hemolysis was occurring in these individu-

als. Accordingly, we tested urines collected during this period for excess urobilinogen content, using Erlich's aldehyde test. These tests were positive, becoming increasingly so, seemingly in parallel with the intensifying hemolysis. However, knowledge of the fallibility of Erlich's test and how false positive results may be given by increasing levels of other substance in the urine, particularly tryptophan metabolites, led to submitting the same urines to Obermayer's test for urinary indican. These tests were also positive, an indication of increasing accumulation of indican over exactly the same period. Hence, it was concluded that the positive Erlich's tests were *false* insofar as they were probably not attributable to excess quantities of urobilinogen.

Figures 1, 2, and 3 show the changes in AST and ALT levels, CK and AK activities, total thyroxine levels, and nitrogen balance observed in *Diver Red* during the 540-msw dive.

## DISCUSSION

### Liver Function Tests

The albeit moderate and rather intermittent elevation of transaminases throughout the periods of compression in three of the four series suggest some

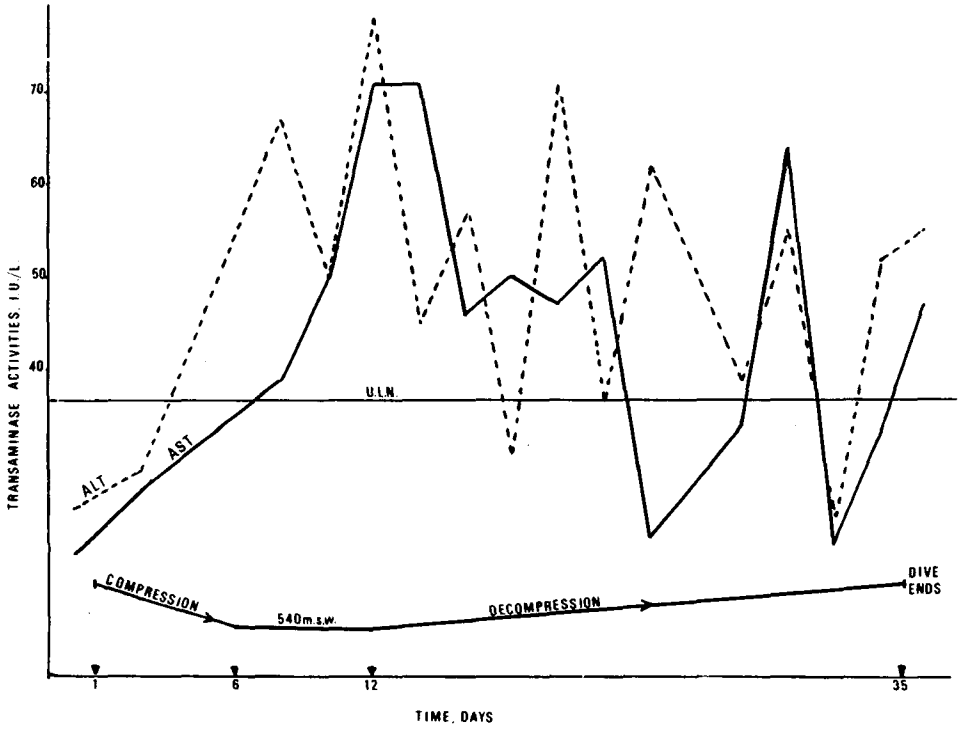



Fig. 1. Plasma aspartate and alanine transaminase (AST and ALT) levels in *Diver Red* during the 540-msw dive.

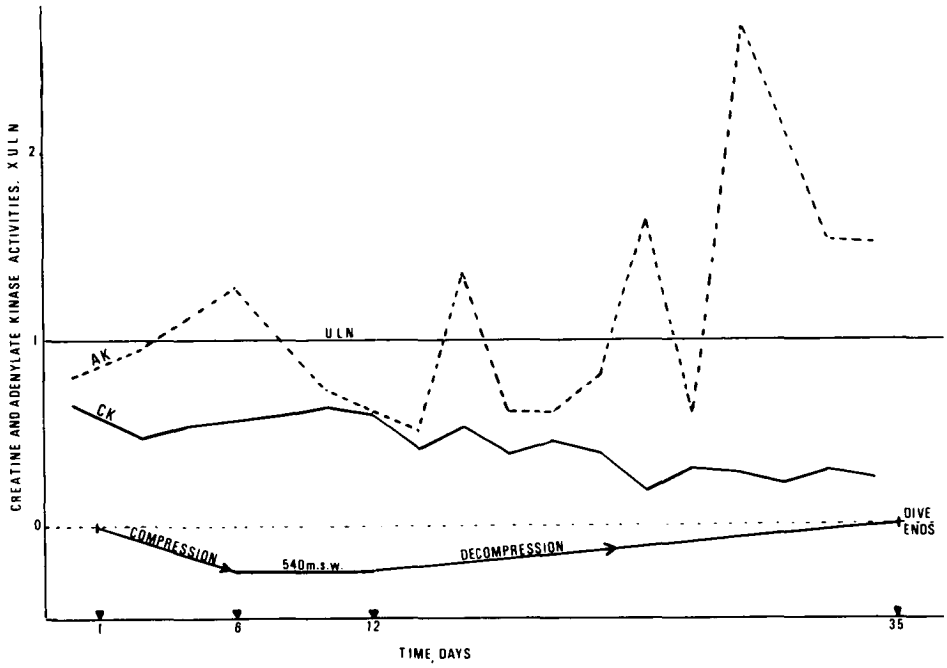



Fig. 2. Plasma creatine kinase (CK) and adenylate kinase (AK) activities in *Diver Red* during the 540-msw dive.

acute hyperbaric hepatocellular "embarrassment," particularly because the ALT's were of the same order as the AST's and, on several occasions, higher.

Interestingly, of the other three commonly assayed "liver" enzymes, Alk. Phos., γ GT, and 5'ND, which are found predominantly in the cells lining the biliary tract, only the 5'ND levels ever rose above normal limits. This enzyme is in no way confined to the liver (although the two richest sources are the liver and the brain) and is indeed found in most tissue cells. It has been used for many years by biochemists undertaking subcellular fractionation of tissues as a marker for the *outer cell membrane* fraction because this is the location of the enzyme within the cell. Its elevation therefore suggests leakage from and damage to the outer cell membrane, membranes of liver cells, and possibly a wide range of other tissues as well.

Unfortunately, there are no suitable isoenzyme techniques available that identify the tissue source(s) of the circulating enzyme; although in relation to its routine clinical usage and behavior in disease, all evidence suggests that the enzyme detected in the plasma is of hepatic origin.

## CK, AK, and Blood Thyroxine Levels

As has been stated, the transaminases are by no means peculiar to the liver—and muscle is another rich potential source—hence, the move to the

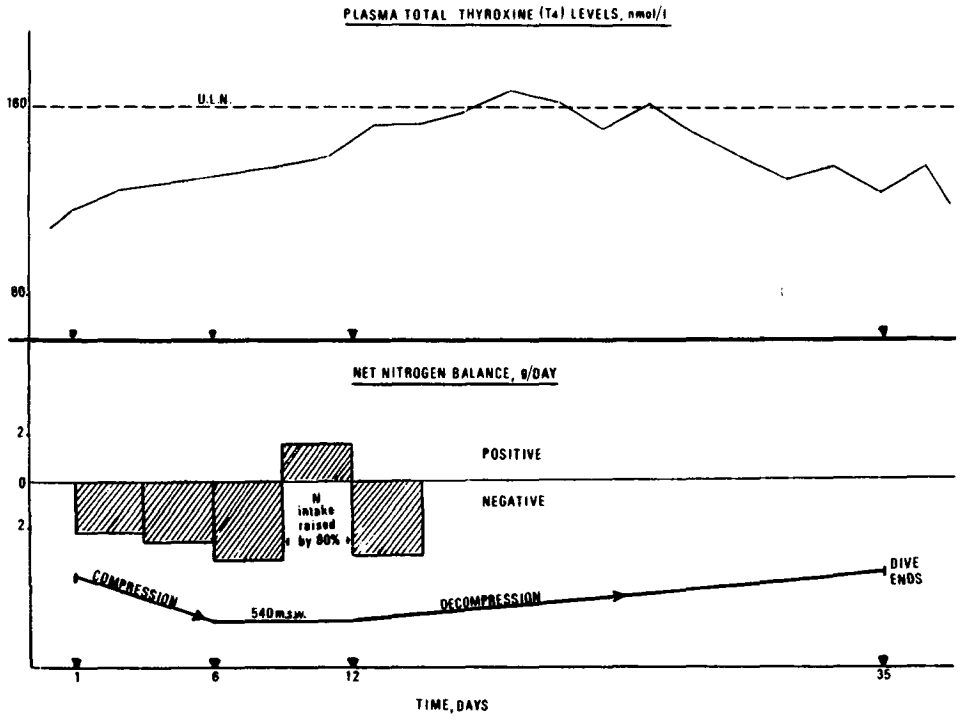



Fig. 3. Changes in total thyroxine (T4) levels and nitrogen balance in *Diver Red* during the 540-msw dive.

study of circulating CK and AK levels. Creatine kinase, although identifiable in many different tissues, has only three really rich sources. Far and away the richest source is skeletal muscle, followed by heart muscle, with brain the only other CK-abundant tissue. Thus, it is that plasma CK activity assays have proved most valuable tests in modern clinical chemistry for detecting muscle or heart damage, or both. Adenylate kinase is far more evenly distributed throughout the body, yet again, if only for reasons of relative tissue bulk, skeletal muscle is by far its richest single source; hence, its old name *myokinase*.

In muscle disease (dystrophies, myopathies, myositis, trauma, and the like), CK elevations are prominent, often massive, and AK changes tend to mimic those of CK but are usually far less pronounced. Yet in all four series of plasma samples taken from our divers, the same trend of change in CK and AK activities was evident. At no time did the CK levels rise above normal limits: the trend was a steady decline of activity to the lower limits of normal. On the other hand, the AK levels tended to rise and were frequently above upper limits of normal—persistently so in the 660-msw dive.

These findings are very interesting when viewed in conjunction with the climb of total thyroxine levels and persistent negative nitrogen balances, which were apparent in all four series.

It is well known that patients suffering from hyperthyroidism have muscle wasting, manifest hyperreflexia, and, almost invariably, a demonstrable muscular weakness and easy fatigability of muscle. In some cases this weakness may be so severe as to constitute a prostrating myopathy. Yet unlike all other forms of myopathy, myositis, or muscular dystrophy, it is never possible to demonstrate elevated plasma CK levels. Indeed, significantly lowered CK activities are the rule in thyrotoxicosis. Furthermore, we have shown the CK and AK no longer behave in similar fashion, i.e., while CK levels are significantly lowered, elevated AK activities are the common feature of thyrotoxicosis (2). Thus, it seems that in these extreme high pressure environments, these divers tended to drift towards a mild thyrotoxic state with rising T4's, falling CK's, and elevated AK's. Such changes cannot in these chamber experiments be attributed to thermogenic responses to cold exposure because chamber temperatures were carefully controlled and the divers were in thermal comfort (no changes in TSH levels were ever detected). This fact leads to the suspicion that the T4 elevation may reflect some impairment of the metabolism and clearance of circulating T4, in which liver plays a major role. The LFT results give some support to this hypothesis, i.e., that the fault could lie in high-pressure-invoked embarrassment of hepatic efficiency. It is well documented that acute liver disease is commonly associated with raised total T4 levels, attributed to a rise in the plasma Thyroxine-Binding Globulin (TBG). A range of other changes have been reported in liver disease according to the severity of the disruption of the hepatic metabolism of thyroxine, e.g., reduced Tri-iodothyronine (T3) with increased reverse T3 and free T4, remarkably analogous to the already noted changes in these and other chamber divers (1,3,4).

## HPNS and Disturbed Liver Function

Although both divers remained in subjectively good health throughout the 540-msw dive, both became acutely unwell during the 660-msw dive on reaching maximum compression.

An interesting question arises in view of the demonstrated disturbances of LFT's. Could it be that the high pressure nervous syndrome is in part a reflection of disturbed liver function? Again, it is well known that varying degrees of liver failure may manifest themselves as CNS signs, i.e., hepatic encephalopathy. Our immediate rejoinder to this hypothesis would be:

> If so, then a) Why did the divers not also become ill during the 540-msw dive despite the evidence of liver embarrassment, and b) Why did *Diver Blue* in the 660-msw dive never manifest LFT abnormalities, even though he became acutely ill?

Nevertheless, this tentative suspicion still remains and investigations are continuing to confirm hyperbaric liver damage inducing clinical chemical anomalies that are known to be associated with hepatic encephalopathy.

### Plasma Pigmentation and Results of Urinary Tests

The bizarre sequence of increasing hemolysis on compression (in the 660-msw dive), with these hem pigments seemingly oxidizing to characteristically brown "met" forms, defies explanation. The failure to demonstrate any increase of urinary urobilinogen upon showing that the initially positive Erlich's tests were probably the result of increase in urinary indican (Obermayer's test) leads to the conclusion that the hemolysis probably did not occur in vivo, and that it was most likely a mechanical handling phenomenon (i.e., decompression of whole blood samples from such depths, or the extraction from the vein). The rise of urinary indican is itself interesting insofar as it could reflect upon hepatic dysfunction. Indican derives from tryptophan, and hepatic failure is known to be associated with elevated free plasma tryptophan levels, and tryptophan toxicity itself has been implicated as one of the etiological factors in hepatic encephalopathy. Yet great caution must be exercised because several other situations may result in elevated circulating tryptophan levels and indicanuria, e.g., gut stasis and starvation.

## SUMMARY OF MAIN FINDINGS

1) Exposure to very high pressure (>50 bars) under strictly controlled chamber conditions;

a) invokes a steady rise in plasma total thyroxine levels and a stimulation of protein catabolism;

b) appears to acutely embarrass the liver as judged by liver function tests so far performed; and

c) is associated with steady decline of plasma creatine kinase activities and increase of adenylate kinase levels, irrespective of the clinical condition and muscular activity of persons so exposed.

2) As is well known, exposure to such pressures may invoke an incapacitating HPNS, which is avoidable to 540-msw ($He\text{-}O_2$) if the compression rate is not too rapid. Nevertheless, the avoidance of clinical manifestations of HPNS does not imply that no harm is being done, i.e., it was still possible to demonstrate the thyroid hormone, nitrogen balance, and liver function disturbances in divers who remained subjectively and objectively well.

## MAJOR CONCLUSIONS AND POSTULATES

The T4 elevations that in turn could arise from hyperbaric embarrassment of hepatic metabolic efficiency could be the major etiological factor underlying the hyperbaric fatigue phenomenon. It is also postulated that some part of the clinical features of HPNS could also be attributable to hyperbaric liver damage.

## References

1. Garrard MP, Carlyle RF, Collis SA. A resume of metabolic balances carried out during the current series of simulated saturation dives to 67 bar. In: Bateman EC, ed. Applied nutrition I. London: John Libbey, 1981:71–73.
2. Doran GR, Wilkinson JH. Serum creatine kinase and adenylate kinase in thyroid disease. Clin Chim Acta 1971;35:115–119.
3. Anderson J, Bennett PB, Carlyle RF, Garrard MP. Thyroid hormone metabolism in men during simulated saturation dives to a maximum depth of 686 metres of seawater (69.6 bar). J Physiol 1982;328:47P–48P.
4. Carlyle RF, Garrard MP, Stock MJ. Observations of some metabolite and hormonal levels in the blood of men during simulated saturation dives to 420 msw (43 bar) in helium-oxygen mixtures. J Physiol 1978;285:115P.

# SUBFERTILITY IN MICE CAUSED BY EXPOSURE TO 50 ATA HELIOX: TOWARDS A MECHANISM

*C. Doré, M. Halsey, S. Monk, S. Rastan, and B. Wardley-Smith*

Previous experiments have demonstrated that exposing male mice to 50 ATA heliox during one complete spermatogenic cycle has a dramatic effect on their functional fertility when they are subsequently mated with untreated females. This effect was seen both as a reduction of 65% in the pregnancy rate and also as a reduction in the live litter size at full term. A subsequent series of experiments demonstrated that this reduction in fertility was not caused by either the high environmental temperature required in 50 ATA heliox (34.5°C) or by the high $P_{O_2}$ (0.5 ATA) (1). These data suggested that the effect was most likely to be caused by exposure to pressure per se. Further investigations of the nature of this subfertility have demonstrated that the two aspects (reduced pregnancy rate and reduced litter size) show different rates of recovery after exposure to pressure, and thus may originate from different events, either in the maturation of sperm, in the process of fertilization, or in early fetal development (2).

Examination of spermatozoa and the testes of mice immediately after exposure to pressure did not provide definitive evidence for the site of the lesion, although the tubules in the testes of the mice exposed to pressure showed a greater degree of disorganization (3). Results from exposing the mice to pressure for only 1 week support the idea that the mature epididymal spermatozoa remain substantially unaffected. This is not unexpected since exposing isolated frog spermatozoa to very high hydrostatic pressure up to 544 atm (4) had no effect on their subsequent fertilizing capacity. Thus, the cause of the functional subfertility is most likely to be a subtle change in some aspect of spermatogenesis.

These data led us to postulate that the mechanism of the subfertility would be associated with a failure in fertilization rather than a subsequent

arrest in development of the zygote. In mice the first cleavage division of the fertilized ovum takes place in the oviduct 24–36 h after mating (5). Failure to cleave may be caused by either a failure of the sperm to penetrate and fertilize the ovum or to various processes affecting development. The latter alternative is revealed by the disintegration and fragmentation of the nonviable cell. These experiments were therefore designed to test first whether the pressure-induced subfertility was revealed in the first 36 h after mating and, secondly, to distinguish between two possible causes of the failure if it existed.

## MATERIALS AND METHODS

### Exposure Schedule

Mice of the inbred strain BALB/c were supplied from the Specified Pathogen Free unit at the Clinical Research Centre. Sexually mature male mice (8–10 weeks old) were randomly allocated to 2 groups of 10. One group (*P*) was exposed to 50 (range 48.5–51) ATA helium-oxygen (heliox: $P_{O_2}$ 0.5, range 0.47–0.53 ATA) in a 20-L steel pressure chamber (6). Chamber temperature was kept at 34.5 ± 1.0°C, which preliminary experiments had shown was required to maintain rectal temperature at 37°C. The second group (*C*) was exposed to air at 1 ATA ($P_{O_2}$ 0.25, range 0.21–0.29 ATA) and chamber temperature was maintained at 26 ± 2°C. The exposure conditions and schedule for *P* and *C* groups were the same as those followed in the 50-ATA and 1-ATA groups, respectively, in previous studies (1,2). Briefly, the pressure group was exposed for 24 h on *Days 1* and *4* of each week for 5 weeks, and was compressed at 1.7 ATA min. They were held at pressure for 22 h, then decompressed on an exponential decompression profile with a duration of 2 h, 40 min. This schedule was found to be free of overt signs both of high pressure neurological syndrome (HPNS) and decompression sickness. Control mice were exposed to 1 ATA air for an equivalent period on *Days 2* and *5* of each week. Food and water were available ad libitum at all times.

### Mating

Three days after the final exposure, male mice were housed individually and given harems of randomly selected female mice of reproductive age (6–8 weeks). Each male was housed with a harem of five females continuously. The females were checked daily for vaginal plugs, and when one was found this was designated *Day 1* of pregnancy.

In these experiments, the ova were examined as follows. Females were killed on the morning of *Day 2* post-coitum, and the oviducts were transferred to 0.8 mL phosphate-buffered saline (PBS) in a petri dish. All subsequent stages involved use of a dissecting microscope. The contents of the oviduct were then flushed out by inserting a flat-tipped 30 G needle into the ostium and gently flushed with 0.2 mL PBS into the petri dish. The criterion of

fertilization in this study was the occurrence of the first cleavage division. The number of ova at the 1-cell or 2-cell stage was counted; in addition, the number that had fragmented within the zonal pellucida was also noted. We analyzed the results for each male, allowing for the non-normal distribution of the data (7).

## RESULTS

In both pressure and control groups the percentage of females mated in each group was 76% (95% confidence limits 57–88%), a finding that indicates no difference in mating frequency. Results for the proportion of 1-cell and 2-cell stages are shown in Fig. 1. Thus, the proportion of ova at the 1-cell stage is significantly higher in the pressure group ($P < 0.01$) and the proportion of ova at the 2-cell stage is significantly lower ($P < 0.03$). There was no significant difference in the number of fragmented or disintegrated cells.

## DISCUSSION

These results demonstrate two aspects of pressure-induced subfertility. Firstly, the phenomena are revealed within 36 h of the subsequent mating with untreated females. This finding confirms and extends the previous observations, which have assessed the effect in terms of either full-term or 15-day fetuses. There now seems to be no possibility that the original observation was a chance effect because it has been repeated in all the other studies (1–3).

Secondly, these results demonstrate that one of the lesions responsible for the pressure-induced subfertility takes place at the fertilization stage. In the females mated with the pressure-treated males at 2 days post-coitum there is a significantly higher proportion of ova at the 1-cell stage with a correspondingly lower proportion at the 2-cell stage compared with controls, an indication that the sperm has failed to fertilize the ovum. It is unlikely that penetration of the ovum has taken place, rather that the first cell-division fails, because fertilized ova that do not divide are not viable, and subsequently disintegrate (5). There was no difference in the proportion of cells that had disintegrated between pressure and control groups.

These results are consistent with the lack of teratogenic effects in the fetuses sired by pressure-treated male mice. If fertilization occurred normally, but subsequently development was impaired, it is possible that many types of developmental abnormality would appear either as an increase in the number of resorptions (when females are killed late in gestation, i.e., *Days 15–20*), or in fetal abnormalities. We have found no increase in the number of resorptions, nor any evidence for either skeletal or histological abnormalities in the fetuses (1).

The cause of the failure of fertilization remains to be elucidated, but one possibility is that the acrosome is affected. The acrosome is a structure found

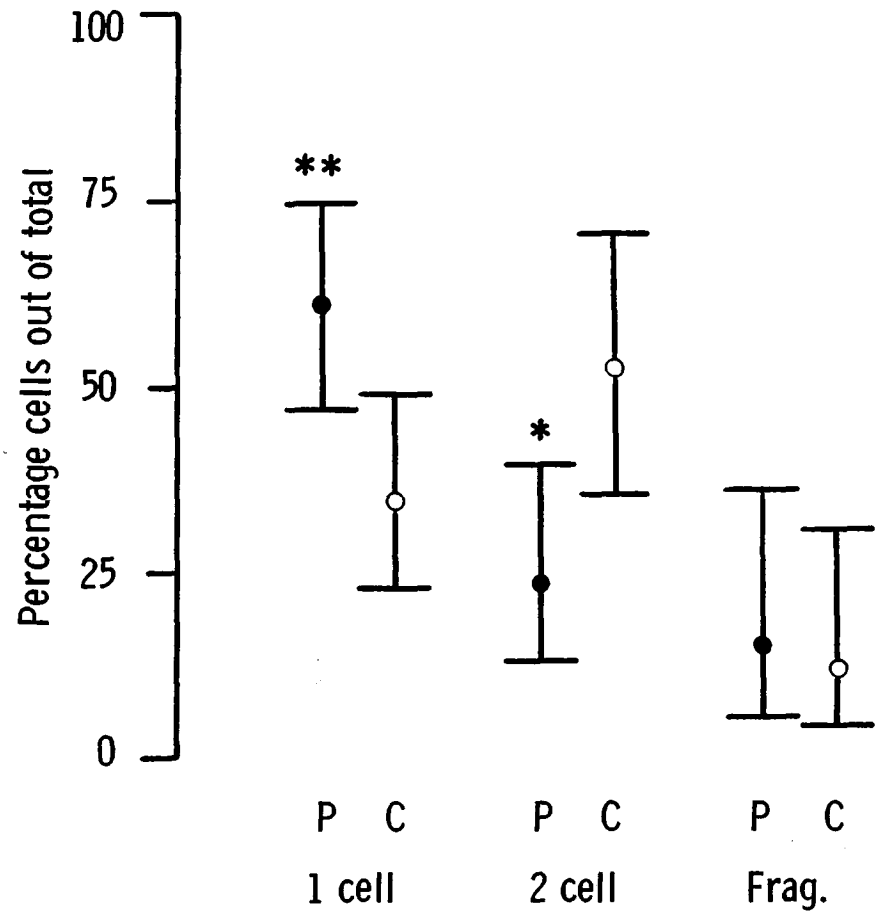



Fig. 1. The percentage of ova at the 1-cell or 2-cell stage and the percentage fragmented within the zonal pellucida. The data for both the pressure (*P*) and control (*C*) groups are presented as the means of 95% confidence limits together with the statistical significances of the differences between the groups (** $P < .01$; * $P < .03$).

on the head region of the spermatozoan, which releases enzymes that aid in penetration of the investments of the ovum. It is likely that an acrosomal lesion would interfere with this process, which leads to fusion of the plasma membranes of the two gametes.

In conclusion, these results indicate that one of the mechanisms of the effect of high pressure on functional fertility of male mice is an impairment of fertilizing capacity of the sperm. Future work should indicate the precise reason for this failure and whether this has any relevance to man.

## References

1. Baden JM, Doré CJ, Green CJ, Monk S, Halsey MJ, Wardley-Smith B. Reduction in fertility of male mice after exposure to high environmental pressure. J Appl Physiol: Respir Environ Exercise Physiol 1982;53:1207–1212.

2. Halsey MJ, Monk S, Wardley-Smith B. Pressure induced infertility in male mice—do they recover after exposure to 50 ATA heliox? Undersea Biomed Res 1982;9 (Suppl):46.
3. Dott HM, Doré CJ, Foster GCA, Halsey MJ, Monk S, Setchell BP, Wardley-Smith B. Effect of exposure to high environmental pressure on spermatogenesis in mice. Gamete Res (in press).
4. Marsland DA, Rugh R. Resistance of sperm of *Rana pipiens* to hydrostatic compression. Proc Soc Exp Biol 1940;43:141–143.
5. Biggers JD, Whitten WK, Whittingham DG. The culture of mouse embryos in vitro. In: Daniel JC, ed. Methods in mammalian embryology. San Francisco: WH Freeman, 1971:86–116.
6. Bailey CP, Green CJ, Halsey MJ, Wardley-Smith B. High pressure and intravenous steroid anaesthesia. J Appl Physiol: Respir Environ Exercise Physiol 1977;43:183–188.
7. Williams DA. Extra-binomial variation in logistic linear models. Appl Stat 1982;31:144–148.

# TIME-DEPENDENT EFFECTS OF PRESSURE AND TEMPERATURE ON AN INTEGRATIVE AXON

*Y. Grossman and J. J. Kendig*

A number of previous studies have been carried out on the effects of pressure on both unmyelinated and myelinated axons. The results of these studies suggest that pressure, like cooling, acts to slow and broaden the conducted action potential (1–3); to depolarize some axons, possibly by decreasing the activity of an electrogenic pump (4); and to cause repetitive impulse activity in a few axons by an unknown mechanism (5). Examination of the ionic currents that underly the action potential reveals that pressure, again like a temperature decrease, slows all the rate constants associated with both sodium and potassium currents (4,6–9). All previous studies on single axons at hyperbaric pressure have employed peripheral axons whose function is faithfully to relay impulse traffic and which therefore have a high safety factor for conduction. The present studies were undertaken to compare the effects of temperature and pressure changes on an axon with a low safety factor, in which selective conduction failure at a branch point at physiological impulse frequencies may serve an integrative role in the animal's behavior.

## METHODS

### Animals

Spiny lobsters used in the study were collected from the Gulf of Eilat (*Panulirus penicillatus*) and from the coast of Southern California (*Panulirus interruptus*). Animals were kept in aquaria with constantly filtered sea water at a temperature of 18°C for *P. penicillatus* and 11°C for *P. interruptus*.

## Preparation

The bifurcating axon used in these studies is from the peripheral nerve supplying the deep abdominal extensor muscles, the anatomical organization of which is given by Parnas and Atwood (10). The nerve was dissected from abdominal segments 2–4 as described in a previous publication (11). The experiments were carried out on the common excitor motor neuron, the main axon (*Ax*) of which divides into two branches: a thick one (*M*) innervating the medial, and a much thinner one (*L*) innervating the lateral abdominal extensor muscles. This axon was functionally isolated from the four others in the nerve by severing the latter with fine scissors.

## Solution

The physiological solution bathing the nerve consisted of NaCl 520 mM, KCl 12 mM, $MgCl_2$ 10 mM, $CaCl_2$ 10 mM, HEPES buffer 5 mM, with pH adjusted to 7.4. In experiments at 1 atmosphere (atm), the preparation was constantly perfused with the solution, whose temperature was regulated by a temperature-controlled circulating bath. In experiments at hyperbaric pressure the preparation was not perfused; temperature was monitored by a microthermistor in the solution 1–2 mm from the axion and controlled by a thermoelectric device beneath the recording chamber.

## Stimulation and Recording

Conventional methods of intracellular and extracellular stimulation and recording were used in an arrangement similar to that described by Grossman et al. (11). Miniaturized suction electrodes were used extracellularly in experiments in which the nerve was exposed to helium pressure in a chamber previously described by Kendig (4). The electrode arrangement is shown diagrammatically in Fig. 1.

## Voltage Clamp

Following enzymatic treatment (10–15 min collagenase 1 mg/mL) the axon was manually stripped of its elaborate sheath. Then it was placed in a modified Vaseline gap voltage-clamp apparatus used in our previous studies (4). The holding chamber was modified to accommodate this large unmyelinated axon by altering gap distances, adding an extra air gap (12), and increasing leakage compensation in the clamp amplifier. The yield of these experiments was very low.

## Pressurization

After the chamber was sealed and control recordings made, helium pressure up to 200 atm was applied in stages. To obtain measurements of transient

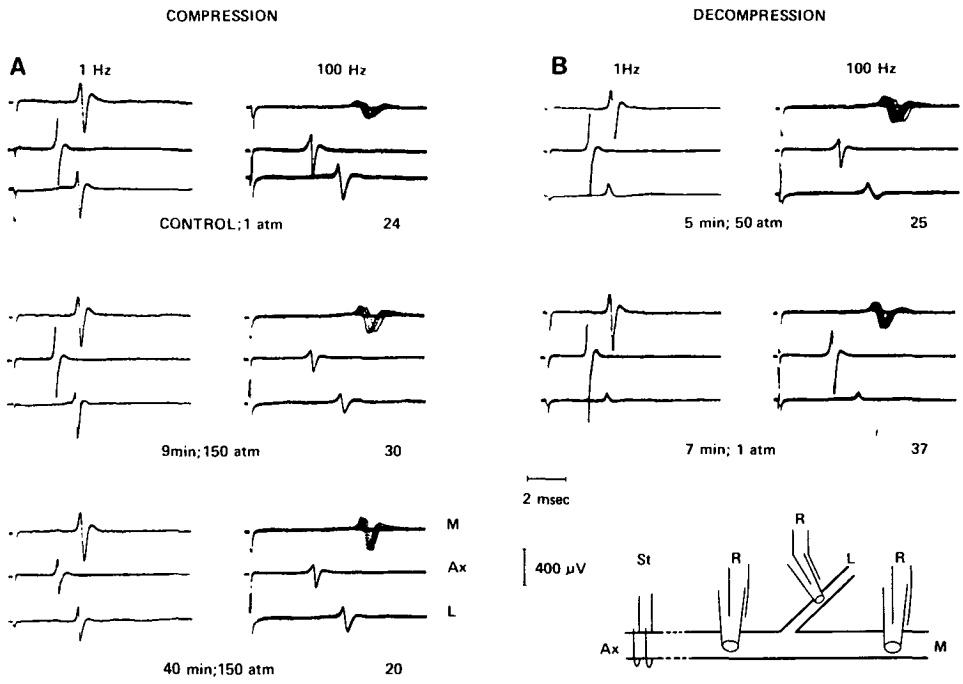



Fig. 1. Pressure effects on conduction of single action potentials (1 Hz) and trains (100 Hz) across the branch point. The configuration of the extracellular stimulating (*St*) and recording (*R*) electrodes on the main axon (*Ax*), the thick branch (*M*), and the thin branch (*L*) is illustrated *in the insert*. The 100-Hz column illustrates the actual moment of conduction block in *M* (*upper traces*). Each photograph is composed of 5–10 superimposed frames. The duration of stimulation required to block the *M* branch is indicated in seconds *below* each set of records. *A:* When the axon was compressed to 150 atm, a transient increase in the ability to carry impulses at 100 Hz is observed at 9 min, which in 40 min decays to a lower steady-state level. *B*: After 5 min at 50 atm on decompression, *M* and *Ax* show increased amplitudes and frequency-following ability (the *L* record was lost as an active response). An even greater transient improvement occurred when the axon was decompressed to 1 atm. Unfortunately, the recording was lost before a steady-state decompression measurement could be made. Temperature 21°C.

responses, we employed relatively high compression and decompression rates of 25–50 atm/min; they were associated with bath temperature increases of 1–4°C, which required a few minutes to resolve before recordings at the new pressure level were made. Because initial findings suggested a temperature hysteresis, recordings were always made on the cooling limb of a temperature adjustment. Recovery on decompression was difficult to obtain; it was fully successful in a minority of cases. Decompression was not attempted in the voltage-clamp experiments.

## RESULTS

### Transient Pressure Effects

The most interesting and unexpected effect of pressure on this axon was a transient increase in excitability on compression to pressures of 50–150 atm.

The excitability increase was manifested as an increase in conduction velocity and in amplitude of the extracellularly recorded action potential in the main axon and both branches; an example is shown in Fig. 1. The increase was observable over the first 3–15 min after a compression step and was followed by a decline to steady-state levels below control (Fig. 1*A*). A similar but even more marked transient increase in amplitude and velocity was seen following a decompression step (Fig. 1*B*). There was also a transient improvement in the ability to conduct high-frequency impulses across the bifurcation. Figure 1 shows an example in which conduction into the *M* branch at 100 Hz was blocked after 24 s of continuous stimulation at 1 atm. Nine minutes after compression to 150 atm, time to block had increased to 30 s, then declined to 20 s after 40 min at 150 atm. Similarly, 5 min after decompression to 50 atm *M* was blocked at 25 s; the interval further increased to 37 s after 7 min at 1 atm. Figure 2 shows a different example in which brief (3-pulse) trains at higher frequencies were used to probe frequency-following ability. This protocol avoids the long recovery time required after prolonged stimulation at lower frequencies. Five to 10 min following compression to 50 and to 100 atm, conduction into the *M* branch was improved as evidenced by a decrease in the minimum interspike interval at which the second or third impulse in a train successfully invaded the branch.

When the cleaned axon was compressed under voltage clamp, there was a large transient increase in the fast inward current observable as soon as

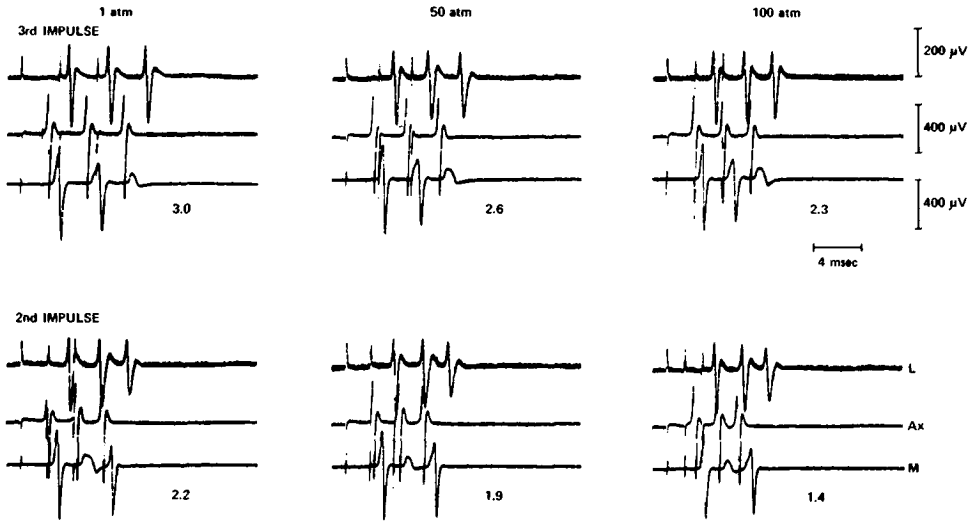



Fig. 2. Transient (5–10 min) effect of pressure on frequency-following ability of the axon. Response of main axon (*Ax*), thick branch (*M*), and thin branch (*L*) to a stimulus train of 3 impulses at pressures of 1, 50, and 100 atm. Increased pressure transiently decreased the interstimulus interval (msec indicated *below* each set of records) at which conduction into *M* of the third (*upper traces*) or the second (*lower traces*) impulse was blocked. Temperature 22°C.

temperature had returned to control levels following a compression step (Fig. 3). The appearance of this enhanced fast inward current and its decline to steady-state levels below control paralleled the corresponding changes in the action potential. The fast inward current is presumably predominantly carried by sodium ions. The effects of pressure on the late outward current, presumably a potassium current, were much less dramatic. Although we did not attempt decompression in the voltage-clamped preparation, the observation of transient increases in inward current could be repeated on successive compression steps, e.g., from 1–50 atm and then 50–100 atm.

## Steady-State Pressure Effects

In contrast to the transient effects observed in the first 15 min, when examined approximately 20 min after a compression step, this axon displayed a response to pressure similar to that observed in other axons. The extracellularly recorded action potential in the *Ax* as well as in both the *M* and *L* branches diminished in amplitude, and conduction velocity was slowed. Figure 1 shows an example at 150 atm. At a uniform temperature of 20–22°C all branches appeared to respond to an increase in pressure in a similar fashion. The percent decrease in action potential amplitude in *Ax* averaged over 10 axons was 1.66 ± 0.53 (SD)/± 10 atm pressure. Similar values were obtained for *M* and *L*. The amplitude of the action potential appeared more sensitive to pressure than the velocity, which showed decreases between 0.55 and 0.58%/10 atm in all branches. These changes were at least partially reversible on decompression.

In the voltage-clamped preparation at steady-state, the effects of pressure on the fast inward current were again consistent with the observations on the extracellularly recorded action potential. There was a decrease to levels below control (Fig. 3). The effects on the late outward current were again less clear-cut than those on the inward current.

The ability of the axon to conduct brief high-frequency impulse trains was also reduced by 20 min exposure to hyperbaric pressure. At pressures above 100 atm, there was a 1.3–4.0-fold increase in the time interval at which the third impulse in a train was just blocked in *Ax*. Sustained exposure to pressures above 100 atm for periods longer than 35 min resulted in failure of even single impulses in *Ax* to cross the bifurcation and successfully invade both branches in 40% of the axons.

## Temperature Effects

The effects of cooling, as in other studies, resembled the steady-state effects of compression: Action potential amplitude and conduction velocity were decreased with $Q_{10}$'s of 1.7 and 2.0, respectively (Fig. 4*A* and *B*), and the ability of the axon to follow high-frequency stimulus trains was diminished (Fig 4*C*). The inward current measured with the Vaseline gap voltage clamp was diminished upon cooling. There was a clear-cut temperature hysteresis;

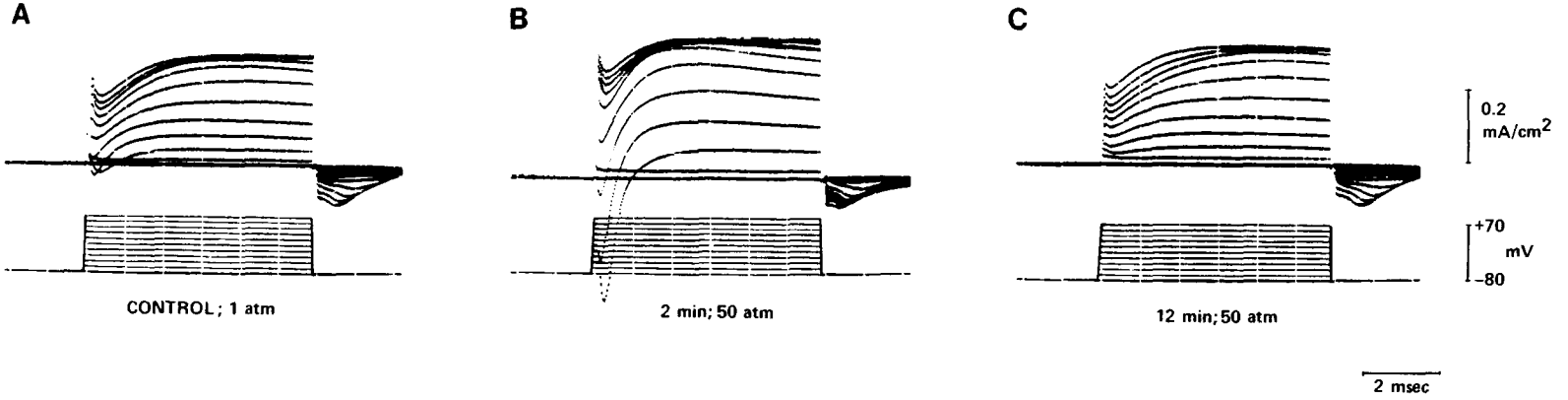



Fig. 3. Transient increase of membrane currents on compression. Membrane currents (*upper traces*) and voltage (*lower traces*) from an isolated axon voltage clamped by Vaseline-air gap technique. The holding potential is −80 mV. Depolarizing voltage steps in 15-mV increments were applied at a temperature of 11.6°C. At control pressure of 1 atm (*A*) the axon had a rather small fast inward current (*downward deflection*) and a comparatively large late outward current. After 2 min at 50 atm (*B*) the fast inward current increased dramatically, the late outward current only slightly. After a further 12 min at 50 atm, the fast inward current declined to nothing without noticeable change in the late outward current.

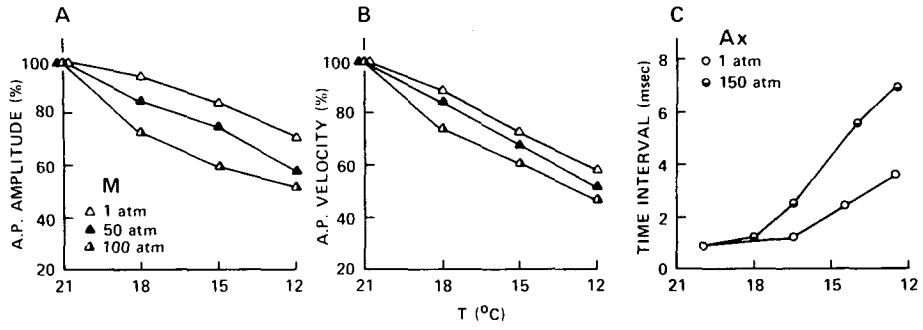



Fig. 4. Steady-state effect of temperature at various pressures on action potential properties. The effect of cooling on amplitude (*A*) and velocity (*B*) of extracellularly recorded action potentials in *M* was enhanced by pressurizing the axon to 100 atm. A similar synergism was observed on the ability of the main axon(*C*) to carry trains of 3 impulses at high frequency. The interstimulus interval at which the third pulse could not be initiated at 12.5°C at 150 atm was twice that obtained at the same temperature at 1 atm. All measurements taken after 20 min of stabilization except that at 18°C, 100 atm. This measurement was made after only 10 min at that pressure, and therefore may be lower than a true steady-state. All data were normalized to the new control at 21°C at each pressure level.

action potential amplitude measured on rapid reheating was greater than the amplitude measured at the same temperature while the preparation was slowly cooled. The same was true of conduction velocity and ability to follow stimuli at high frequency. The magnitude of the temperature hysteresis measured at 1 atm was such that the amplitude and velocity measured on rapid heating corresponded to points measured 3–5°C warmer on the slow cooling curve. A temperature hysteresis determined in this fashion reflects a transient temperature effect. The duration of the temperature transient was at least 10 min, the time required for the responses to return to steady-state following a temperature increase. Thus, in this axon both temperature and pressure evoked transient as well as persistent changes in functional properties.

## Temperature-Pressure Interactions

Under steady-state conditions, compression and cooling interacted synergistically, each enhancing the depressant effect of the other on axonal properties. Compression decreased action potential amplitude, velocity, and ability to conduct at high frequency more markedly at cooler temperatures. Similarly, low temperature effects were more marked at hyperbaric pressures (Fig. 4), pressure increasing $Q_{10}$ for all the axonal properties measured.

In contrast to the synergistic interaction between cooling and compression when both variables were in the steady-state, both variables in the steady-state antagonized each other's transient effects. At hyperbaric pressures the width of the temperature hysteresis was reduced by about 50%. Similarly, low tem-

peratures antagonized the transient increases in excitability associated with both compression and decompression steps; the latter were 2–5-fold greater at 21°C than at 13°C.

## DISCUSSION

Compared to other axons that have been examined for response to hyperbaric conditions, this inhomogeneous branching axon is unique in its putative role as an integrative rather than relay component of the animal's nervous system. Nevertheless, it is not known to possess fundamentally unique properties. The action potential is based on voltage-sensitive sodium and potassium channels, as in other axons. Ion accumulation and active ion transport play important roles in the development of conduction block at the bifurcation (13), but these factors are also common to other axons. The observed transient increases in inward currents, action potential amplitude and velocity, and conducting ability following pressure changes were therefore surprising, inasmuch as they have not been observed in other axons. The possibility of artifact was carefully considered. Temperature increases, which could mimic the transient pressure effects, were excluded by temperature monitoring near the axon; all measurements were made at control temperatures following cooling, to avoid the observed transient effects of warming. Movement artifact is unlikely because changes in conduction velocity and frequency-following ability are independent of electrode contact with the nerve, and because congruent changes were observed with the very different methods of extracellular recording and voltage clamp. Neither squid giant axon (6–9) nor peripheral myelinated nerve (4,14) have revealed similar transient changes with pressure steps. However, some molluscan central neurons, which possess a rich variety of currents different from those in axons, reveal transient responses (15). By contrast, the steady-state depressant effects of pressure on conduction velocity are similar to reports on other axons (1–3), although pressure-related depressant effects appear more pronounced in this axon than in others. Because of the limitations of extracellular recording, and the very preliminary nature of the voltage-clamp studies, it is not yet possible to describe a membrane-level basis for any of the observed effects of pressure in this axon, transient or steady-state.

Although the mechanism of the response to pressure is not yet known, the sensitivity of this integrative axon to pressure is of considerable physiological significance. The observed pressure-related modulation of the axon's ability to conduct trains of impulses across the bifurcation will significantly alter the information coded as a pattern of impulses in the daughter branches.

The relationship between the present observations and the high pressure nervous syndrome (HPNS) is also not clear. In a previous study on peripheral crustacean axons, a small portion of the population was observed to fire repetitively on compression; this behavior was suggested to be related to HPNS (5). The particular axon used in the present studies did not generate

repetitive discharges. Nevertheless, the transient increases in action potential amplitude, and the accompanying improvement in the axon's ability to conduct high-frequency impulse trains, may suggest an additional possible basis for HPNS in axon physiology.

## Acknowledgments

The pressure chamber was adapted to the present experiments by Anesthesia Department Electronics Technician Mr. Lloyd Gano. The technical assistance of Ms. Nancy Erickson is gratefully acknowledged. Supported by Ben-Gurion University research grant to Y. Grossman and by grants NIH NS13108, NSF BNS80-20497, and ONR contract N00014-75-C-1021 to J. J. Kendig.

## References

1. Kendig JJ, Trudell JR, Cohen EN. Effects of pressure and anesthetics on conduction and synaptic transmission. J Pharmacol Exp Ther 1975;195:216.
2. Spyropoulos CS. Responses of single nerve fibers at different hydrostatic pressures. Am J Physiol 1957;189:214–218.
3. Spyropoulos CS. The effects of hydrostatic pressure upon the normal and narcotized nerve fiber. J Gen Physiol 1957;40:849–857.
4. Kendig JJ. Currents in a voltage clamped vertebrate neuron at hyperbaric pressure. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:363–370.
5. Kendig JJ, Schneider TM, Cohen EN. Pressure, temperature, and repetitive impulse generation in crustacean axons. J Appl Physiol: Respir Environ Exercise Physiol 1978;45:742–746.
6. Henderson JV, Gilbert DL. Slowing of ionic currents in the voltage clamped squid axon by helium pressure. Nature 1975;258:351–352.
7. Conti F, Fioravanti R, Segal JR, Stuhmer W. Pressure dependence of the sodium currents of squid giant axons. J Membr Biol 1982;69:23–34.
8. Conti F, Fioravanti R, Segal JR, Stuhmer W. The pressure dependence of the potassium currents of the squid giant axon. J Membr Biol 1982;69:35–40.
9. Shrivastav BB, Parmentier JL, Bennett PB. A quantitative description of pressure-induced alterations in ionic channels of the squid giant axon. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:611–619.
10. Parnas I, Atwood HL. Phasic and tonic neuromuscular systems of the crayfish and rock lobster. Comp Biochem Physiol 1966;18:701–723.
11. Grossman Y, Parnas I, Spira ME. Differential conduction block in branches of a bifurcating axon. J Physiol 1979;295:283–305.
12. Nonner W. A new voltage clamp method for Ranvier nodes. Arch Gen Physiol 1969;309:176–192.
13. Grossman Y, Parnas I, Spira ME. Ionic mechanisms involved in differential conduction of action potentials at high frequency in a branching axon. J Physiol 1979;295:307–322.
14. Kendig JJ. Pressure reversal of inert gas narcosis at node of Ranvier sodium channels. Biophys J 1983;41(2 pt. 2):145a.
15. Wann KT, Macdonald AG, Harper AA, Ashford MLJ. Transient versus steady-state effects of high hydrostatic pressure. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:621–627.

# EFFECT OF HIGH HYDROSTATIC PRESSURE ON SODIUM TRANSPORT ACROSS THE TOAD SKIN

*S. K. Hong, M. E. Duffey, J. M. Goldinger, D. Sambor, and M. Nygogosyan*

A characteristic *hyperbaric diuresis* has been observed in many dry or open-sea saturation diving exeriments carried out in the past (1). Although this diuresis appears to be primarily due to inhibition of the tubular reabsorption of free water (i.e., water diuresis), it is sometimes associated with an increase in the urinary excretion of sodium (Na), especially during the early stage of hyperbaric exposure (2) or during steady-state exposure to a pressure greater than 25–30 ATA (3). The latter phenomenon strongly suggests the possibility that active tubular reabsorption of Na may be affected by high hydrostatic pressure. In fact, Goldinger et al. (4), in our laboratory, reported that the active Na transport (i.e., efflux) in human erythrocytes is markedly inhibited even by a modestly high hydrostatic pressure (e.g., 40% inhibition at 50 ATA). Nevertheless, there is no information regarding the direct effect of pressure on the tubular transport of Na. On the other hand, several investigators have used an amphibian skin preparation (which is considered to be a functional model for the distal segment of the mammalian kidney) to study the effect of pressure on electrical parameters of Na transport, but the results are conflicting and inconclusive (5–9).

The active transport of Na across an epithelium is considerably more complicated than that in a homocellular system, which has a single membrane step. Typically, an epithelium consists of two membrane steps for transport: outer (or apical) and inner (or basolateral) membranes; in addition, there is an intercellular tight junction that is also permeable to Na. Therefore, the effect of pressure on active Na transport across the erythrocyte membrane may not necessarily imply that a similar effect exists for the epithelial Na transport. We undertook the present investigation to study the pressure effects on the baseline

Na transport and on stimulatory actions of vasotocin and cyclic AMP on Na transport across the isolated toad skin.

## METHODS

Toads, *Bufo marinus*, obtained from Mexico, were doubly pithed, and a section of the ventral abdominal skin was mounted as a flat sheet between two Lucite chambers (Ussing-type; 2.5 mL capacity for each), having a cross-sectional area of 0.4 $cm^2$. The composition of the basic medium bathing both outer and inner surface of the skin was (mM): NaCl, 90; KCl, 3; $NaHCO_3$, 5; $CaCl_2$, 1; $MgSO_4$, 0.5; $KH_2PO_4$, 1.2; glucose, 5; and pH, 8.4.

The basic design of the tissue chamber as well as the technique used to apply high hydrostatic pressure to the skin preparation are described in detail elsewhere (10). Briefly, one Lucite chamber exposed to the inner surface of the skin is connected to a closed flow-through perfusion system while the other chamber is fitted with a small magnetic stirrer and a long (4 cm), horizontal, fluid-filled stainless pressure equilibration tube. The tissue chamber system is also equipped with two separate electrode systems: one for the measurement of transepithelial electrical potential difference (PD), and the other for the introduction of external current to determine short-circuit current (Isc), which was used as a measure of the active net Na transport (11). The temperature of the bathing medium was continuously monitored by means of a thermocouple probe fitted into the chamber with the closed flow-through perfusion system. The previously described tissue chamber was placed in a larger hyperbaric pressure vessel (internal volume of 7 L) capable of compression to 400 ATA. The compression of this pressure vessel allowed the transmission of hydrostatic pressure to the skin preparation without permitting access to the gas in the pressure vessel.

Because only one side of the skin was continuously perfused with aerated Ringer solution (5 mL/min) in the present work, the stability and viability of the preparation had to be tested. For this purpose, the electrical parameters of Na transport were continuously measured at 1 ATA for 5 h at room temperature (~20°C). It was found that the Isc was virtually stable for nearly 3 h at a level of 40–50 $\mu A/cm^2$, after which it decayed slowly at a rate of approximately 7 $\mu A/cm^2/h$. The PD fluctuated between 20 and 30 mV, generally following the pattern of changes in Isc. The transepithelial resistance (R), computed from the PD/Isc ratio, slowly increased from 350 $\Omega cm^2$ at the beginning to 750 $\Omega cm^2$ at 5 h.

Based on the above results, subsequent experiments were designed such that all measurements are completed within 4 h after each skin was mounted in the tissue chamber. Typically, the first 1 h was allowed for equilibration of the preparation, following which the pressure vessel was compressed with $N_2$ at a rate of approximately 20 ATA/min to the desired level. At the end of 1 h under high pressure, we added either vasotocin or dibutyryl adenosine 3′:5′-cyclic monophosphate (cAMP) to the inside bathing medium to study its effect

on Na transport for 1–2 h. The pressure vessel was then decompressed and the experiment was terminated.

## RESULTS

Typical experiments illustrating the entire time course are shown in Fig. 1. It can be seen that the baseline Isc increased transiently during the first 10 min after the start of compression and then decreased continuously until it leveled off at approximately 40 min. Although the temperature of the bathing medium increased invariably during the compression phase (by 3–9°C), there was no correlation between the magnitude of transient increase in Isc and temperature. In some experiments, the rate of compression was continuously adjusted to minimize the rise in the temperature, yet a similar transient increase in Isc was still observed. Moreover, the magnitude of this increase in Isc was pressure-independent and 10-15 $\mu A/cm^2$ (~20% of the precompression level) at all pressures (Table I). The transepithelial PD did not change significantly during the first 10 min under pressure.

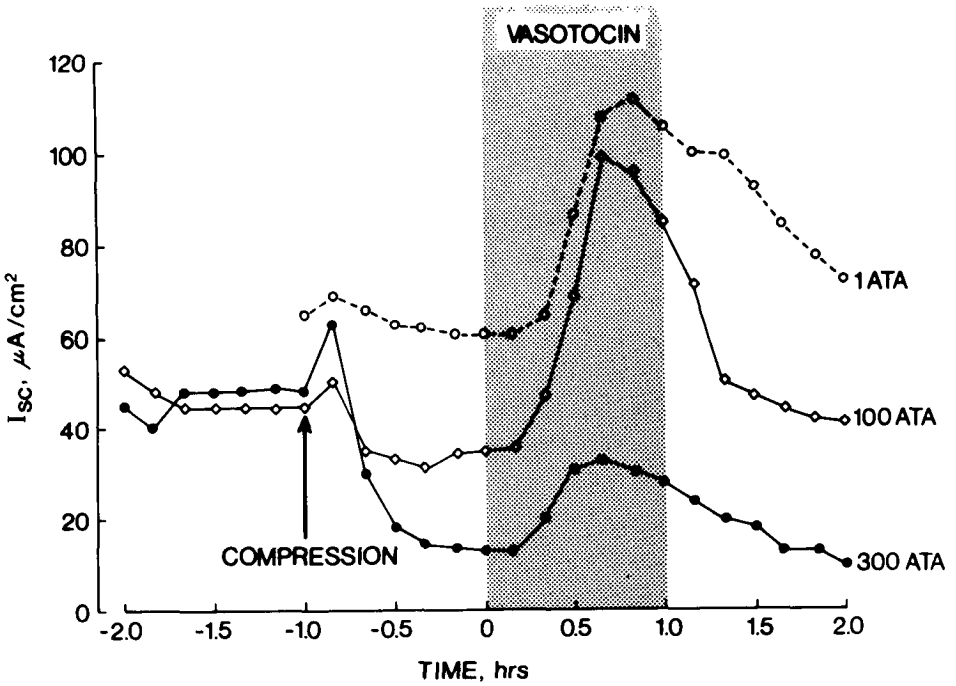



Fig. 1. Typical experiments illustrating the changes of short-circuit current (Isc) under the influence of pressure and vasotocin. Vasotocin was added to the inside bathing medium at a concentration of 100 mU/mL during the 1-h period (*shaded area*). (From Hong et al. [10] with permission from Undersea Medical Society, Inc.)

TABLE I

Short-Circuit Current (Isc, μA/cm²) Responses to Pressure and Vasotocin

| Pressure (ATA) | Isc Before Compression | ISC at Pressure | | |
|---|---|---|---|---|
| | | 10 min | 60 min | + Vasotocin† |
| 1 ($n$ = 4) | 60.7±12.4 | — | — | 55.5± 7.4 |
| 50 ($n$ = 4) | 64.7± 4.8 | +9.2±2.7* | −15.7± 3.2* | 53.7±11.2 |
| 100 ($n$ = 5) | 52.2± 6.2 | +14.4±6.3* | −15.6± 2.3* | 58.6± 7.8 |
| 200 ($n$ = 5) | 47.0± 4.2 | +10.0±2.7* | −25.4± 2.1* | 38.8± 9.5 |
| 300 ($n$ = 5) | 47.8±13.9 | ±9.4±4.2* | −30.8±10.0* | 35.4± 7.5 |

(Mean ±SE). *denotes a significant change ($P<0.05$, paired $t$-test); †represents a peak response to vasotocin added to the inside bathing medium at a concentration of 100 mU/mL.

In contrast, the magnitude of reduction in Isc observed toward the end of 1 h at pressure was pressure-dependent (Table I). As compared to the pre-compression level, the Isc decreased by 25.6±6.5, 29.9±3.1, 55.6±6.3, and 60.3±17.3% at 50, 100, 200, and 300 ATA, respectively. This reduction in Isc was accompanied by increases in the transepithelial R. As shown in Fig. 2, there is a curvilinear relationship between the degree of inhibition of Isc and pressure, with the former leveling off at 200 ATA. Interestingly, the overall pressure-response relationship for the toad skin obtained in the present work is similar to that observed for the active Na transport of human erythrocytes by Goldinger et al. (4) in our laboratory. These results indicate that the active Na transport system is indeed sensitive to high hydrostatic pressure, subject to a 50% inhibition at 200–300 ATA at least in two different types of the cell.

When vasotocin was added to the medium bathing the inside surface of the skin at a concentration of 100 mU/mL (which induces a maximal response at both 1 and 100 ATA), the Isc began to increase within 10 min, reached a peak at 30–40 min, and then decreased slowly even while vasotocin was still present in the medium (Fig. 1). This pattern of Isc response to vasotocin is identical to the pattern of Isc response to vasopressin, reported by Huja and Hong (12). Because the time course of the Isc response to vasotocin varied with the skin, the magnitude of the peak response was compared under various pressures. The peak Isc in the presence of vasotocin was ~55 μA/cm² higher than the prevasotocin level at 1, 50, and 100 ATA (Table I). Yet the magnitude of the peak response decreased to ~35 μA/cm² at 200 and 300 ATA, which was marginally different from the corresponding value at 1 ATA ($0.05<P<0.10$). Vasotocin decreased R at all pressures. These results suggest that a high pressure interferes with the stimulatory action of vasotocin on Na transport.

It is currently believed that there are at least two major steps responsible for the vasotocin action (13). The first step involves the interaction of vasotocin with the specific receptor located on the basolateral membrane, an

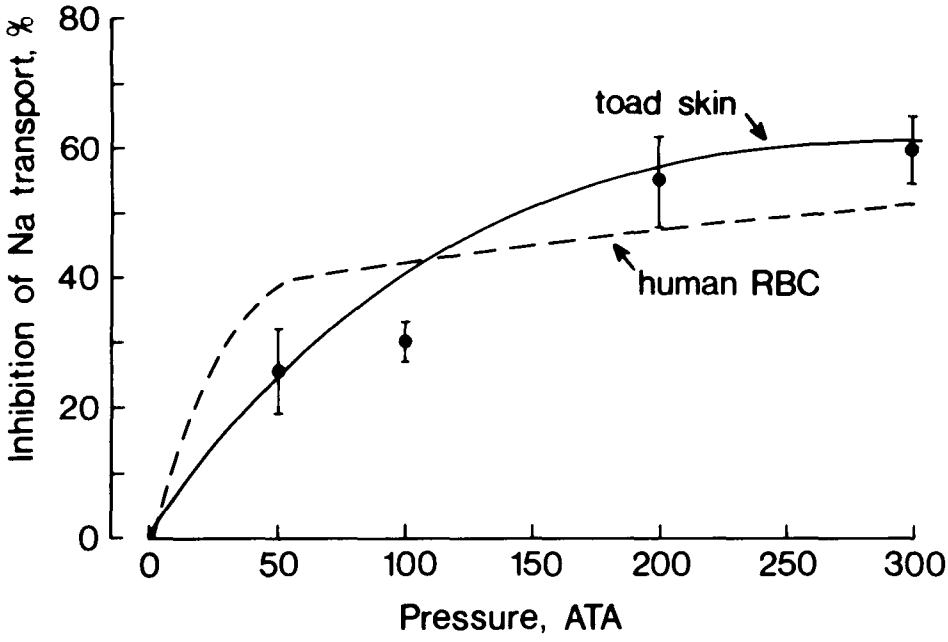



Fig. 2. Percent inhibition of Na transport as a function of hydrostatic pressure in the toad skin and the human erythrocyte. The toad skin data are calculated from the magnitude of changes in short-circuit current observed at 60 min under pressure (*see* Fig. 1), and the human erythrocyte data are from Goldinger et al. (4). (From Hong et al. [10] with permission from Undersea Medical Society, Inc.).

effect leading to the increased production of cAMP through stimulation of adenylate cyclase; the second step represents the cAMP-induced increase in the Na permeability of the apical membrane. It is therefore important to determine the locus of the previously described pressure effect on the vasotocin action. For this purpose, the direct stimulatory action of cAMP on Isc was studied at 300 ATA at which pressure the vasotocin action was inhibited.

As shown in Table II, the addition of cAMP to the inside bathing medium at concentrations of 0.5 and 1.0 mM resulted in increases in Isc in a dose-dependent manner at 1 ATA. More importantly, it can be seen that the magnitude of increase in Isc in the presence of 1 mM cAMP is not different between 1 and 300 ATA despite the significant difference in the level of Isc before cAMP between the two pressures. These results indicate that the direct effect of cAMP on the apical membrane permeability to Na is not affected by high pressure. It thus appears that the main locus of the pressure effect on the vasotocin action is at steps leading to the increased production of cAMP.

## DISCUSSION

It should be stated that the Isc was measured in this investigation as a measure of active net transport of Na across the toad skin. Although this

TABLE II

Effects of Dibutyric cAMP on Electrical Parameters of Na Transport

| Pressure (ATA) | Isc ($\mu A/cm^2$) Before cAMP | ΔIsc-cAMP | PD (mV) Before cAMP | After cAMP | R ($ohm \cdot cm^2$) Before cAMP | After cAMP |
|---|---|---|---|---|---|---|
| 1, 0.5 mM cAMP ($n$ = 3) | 40.7±3.0 | 14.3±4.3 | 25.9±6.9 | 22.4±5.3 | 512±133 | 285±60 |
| 1, 1 mM cAMP ($n$ = 5) | 41.8±4.9 | 25.4±3.2 | 25.7±4.5 | 24.0±3.2 | 529± 90 | 255±62 |
| 300, 1 mM cAMP ($n$ = 3) | 11.7±4.6* | 31.3±0.9* | 5.9±2.0*† | 14.7±3.9 | 427±149 | 182±53 |

(Mean ±SE). cAMP: dibutyryl adenosine 3′:5′-cyclis monophosphate; Isc: short-circuit current; PD: transepithelial electrical potential difference; R: transepithelial resistance. *Significantly different from the corresponding value obtained in the presence of 0.5 mM cAMP at 1 ATA. ($P < 0.05$, unpaired $t$-tests). †Significantly different from the corresponding value obtained in the presence of 1 mM cAMP at 1 ATA. ($P < 0.05$, unpaired $t$-tests).

relationship holds under various experimental conditions at 1 ATA (11), it has never been verified under high pressure. Even though previous investigators also assumed that the above relationship is applicable to the hyperbaric condition, the experimental verification is needed. In the meantime, the results obtained in the present work also will be discussed on the basis of the above assumption.

As soon as the compression of the pressure vessel was begun, the Isc began to increase transiently (Fig. 1). The mechanism for this slight increase is unknown at present, although the accompanying increase in the temperature of the bathing medium may be related to this phenomenon. Brouha et al. (7) also observed a similar change during 3–5 min exposure of frog skins to high hydrostatic pressure; this change was attributed to an increase in the Na permeability of the outer barrier of the skin (8).

A far more important finding in the present work is the pressure-dependent, sustained inhibition of the baseline Isc observed after the initial, transient increase of Isc (Fig. 1). At 200–300 ATA, the Isc decreased by nearly 60%, an effect that may be partly attributable to the reduction in $O_2$ consumption (14). Nevertheless, the Isc increase in response to either vasotocin (Fig. 1) or cAMP (Table II) at these high pressures clearly indicates that the metabolic inhibition is not the primary reason for this pressure-induced inhibition of baseline Isc.

According to the current cellular model for active Na transport across the toad skin, the entry of Na into the epithelial cell across the outer membrane is passive and rate-limiting, while the extrusion of Na out of the cell across the basolateral membrane is active and is mediated by Na-K-ATPase (13). Therefore, the pressure-induced inhibition of the Isc could be explained by the reduction of outer membrane permeability to Na or the inhibition of Na-K exchange pump, or both, in the basolateral membrane. The results obtained so

far in the present work are still insufficient for us to conclude definitively about the exact mechanism. Nevertheless, the results are most consistent with the view that the pressure decreases the outer membrane Na permeability. This view is based on the observations that the Isc suppressed by high hydrostatic pressure could be reversed by either vasotocin or cAMP, which is known to increase the outer membrane permeability (13). In other words, the active Na transport mechanism residing in the basolateral membrane appears to be intact under high pressure and can function adequately provided that the entry of Na across the outer membrane is maintained to insure the supply of substrate (i.e., Na) for the Na-K-ATPase. According to this view, the following sequence may be envisioned to account for the pressure effect: Pressure decreases the outer membrane permeability to Na; this decrease results in a reduction in the intracellular concentration of Na and secondary to the reduction of intracellular Na, a reduction in Na pump activity. Preliminary results from recent studies using current-voltage analyses of the skin under high pressure while the concentration of K in the inside bathing medium is raised to minimize the basolateral membrane resistance indicate that the outer membrane resistance is indeed increased under high pressure. These findings are also consistent with the view that pressure decreases the outer membrane permeability to Na.

The effect of high hydrostatic pressure on the Na-K-ATPase has been investigated by several previous investigators, using either tissue homogenates (15) or erythrocyte ghosts (4). These studies indicated that a modestly high pressure (100–200 ATA) activates the Na-K-ATPase activity in these in vitro systems. Neither the mechanism for, nor the implication of, the above phenomenon is clear at present. Yet the Na-K-ATPase activity is clearly inhibited at extremely high hydrostatic pressure (500 ATA) (16). Clearly, more definitive, systematic studies are needed to clarify the nature of the interaction of Na-K-ATPase with pressure.

As stated in the introduction, there is indirect evidence for inhibition of the tubular reabsorption of Na in human divers exposed to 31 ATA (3). Although the results obtained from the present work using toad skins cannot be directly applied to the human kidney function, they are consistent with the view that high hydrostatic pressure can indeed inhibit active Na reabsorption in the kidney. This view is based on these facts: the pressure effect on active Na transport was comparable between human erythrocytes and toad skins (Fig. 2), and the Na transport system in the toad skin is functionally similar to that in the distal tubule of the mammalian kidney.

## Acknowledgments

The present work was supported by USPHS grant HL-28542. The authors gratefully acknowledge Mrs. Elizabeth Miranda for her valuable assistance in the preparation of this manuscript.

## References

1. Hong SK. Body fluid balance during saturation diving. In: Hong SK. ed. International symposium on man in the sea. Bethesda: Undersea Medical Society, Inc., 1975:127–140.

2. Hong SK, Claybaugh JR, Frattali V, Johnson R, Kurata F, Matsuda M, McDonough A, Paganelli CV, Smith RM, Webb P. *Hana Kai II:* a 17-day dry saturation dive at 18.6 ATA: III: Body fluid balance. Undersea Biomed Res 1977;4:247–266.
3. Nakayama H, Hong SK, Claybaugh JR, Matsui N, Park YS, Ohta Y, Shiraki K, Matsuda M. Energy and body fluid balance during a 14-day dry saturation dive at 31 ATA (SEADRAGON IV). In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh syposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:541–554.
4. Goldinger JM, Kang BS, Choo YE, Paganelli CV, Hong SK. Effect of hydrostatic pressure on ion transport and metabolism in human erythrocytes. J Appl Physiol: Respir Environ Exercise Physiol 1980;49:224–231.
5. Gottlieb SF, Cymerman A. Effects of increased oxygen tension on sodium active transport through frog skin. Aerosp Med 1970;41:661–665.
6. Gottlieb SF, Cymerman A, Metz AV. Effect of xenon, krypton, and nitrous oxide on sodium active transport through frog skin with additional observations of sciatic nerve conduction. Aerosp Med 1968;39:449–453.
7. Brouha A, Pequeux A, Schoffeniels E, Disteche A. The effects of high hydrostatic pressure on the permeability characteristics of the isolated frog skin. Biochem Biophys Acta 1970;219:455–462.
8. Pequeux A. Polarization variations induced by high hydrostatic pressures in the isolated frog skin as related to the effects on passive ionic permeabilities and active Na transport. J Exp Biol 1976;64:587–602.
9. Pequeux A. Effect of pH changes on the frog skin electrical potential difference and on the potential variations induced by high hydrostatic pressure. Comp Biochem Physiol 1976;55:103–108.
10. Hong SK, Duffey ME, Goldinger JM. Effect of high hydrostatic pressure on sodium transport across the toad skin. Undersea Biomed Res 1984;11:37–47.
11. Ussing H, Zerahn K. Active transport of sodium as the source of electrical current in the short-circuited isolated frog skin. Acta Physiol Scand 1951;23:110–127.
12. Huja BS, Hong SK. Characteristics of vasopressin action on Na transport across the isolated toad skin. Comp Biochem Physiol 1976;53:187–191.
13. Macknight ADC, DiBona DR, Leaf A. Sodium transport across toad urinary bladder: a model "tight" epithelium. Physiol Rev 1980;60:615–715.
14. Fenn WO, Boschen V. Oxygen consumption of frog tissues under high hydrostatic pressure. Respir Physiol 1969;7:335–340.
15. Gottleib SF, Koehler GJ, Rhodes LVG. An oxygen- and pressure-sensitive enzyme: Na-K adenosinetriphosphatase. In: Lambertsen CJ, ed. Underwater physiology V. Proceedings of the fifth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1976:431–442.
16. Pfeiler E. Effects of hydrostatic pressure on $(Na^+ + K^+) - ATPase$ and $Mg^{2+} - ATPase$ in gills of marine teleost fish. J Exp Zool 1978;205:393–402.

# EFFECTS OF SODIUM VALPROATE ON HPNS IN RATS: THE PROBABLE ROLE OF GABA

*J. C. Rostain, B. Wardley-Smith, and M. J. Halsey*

The observations made in the baboon *Papio papio* with the breathing mixture He-$N_2$-$0_2$ showed, among other things, that the epileptic seizures that occurred during very rapid compression were localized in the occipital region; however, those which occurred with a breathing mixture of He-$O_2$ were generalized (1–3). These differences showed some resemblance to seizures produced in photosensitive *Papio papio* before and after treatment with drugs that changed the levels of cerebral gamma-aminobutyric acid (GABA) (4,5).

Other experiments using rats and mice have shown that GABAergic substances, or drugs that elevate cerebral GABA levels, increase the pressures at which tremors and convulsions appear (6,7). In these experiments we have tried to determine more precisely the role of GABA in the high pressure neurological syndrome (HPNS) by using substances that elevate the level of GABA. This paper reports results obtained on HPNS with sodium valproate, a drug well known for its antiepileptic action. This drug affects levels of GABA by blocking GABA-transaminase (GABA-T); thus it reduces GABA breakdown (8,9).

## METHODS

Male Sprague-Dawley rats (300–450 g) were used in all experiments (some have been used in several experiments). Under general anesthesia (ketamine/pentobarbitone), the animals were implanted with electroencephalographic (EEG) electrodes and deep electrodes, which were either in the caudate nucleus or in the thalamus (ventral nucleus); the position of these deep electrodes was verified histologically post mortem. The electrodes were at-

tached to a miniconnector, and both electrodes and connector were held in place with dental cement. A minimum of 1 week was allowed for recovery before the animals were exposed to pressure.

The hyperbaric experiments were carried out in a 25-L pressure chamber (maximum pressure 400 bars). During the experiments the rats were placed in a restraining box, which was mounted over a small strain gauge (10). This arrangement permitted accurate determination of the threshold pressures for tremor and myoclonus. The miniconnector was connected via a multicore cable to the electrical plugs in the chamber.

The colonic temperature of the rats was continuously recorded with a thermistor probe. Food and water were available throughout the experiment.

The activities of the EEG and deep structures were monitored by means of an electroencephalograph and recorded on analog magnetic tape via an FM multiplexer adaptor. After interpretation of the EEG traces, we analyzed the recordings by computer (PDP 11/10 and MICRO II) to obtain the power spectra and to calculate the statistical results. The Mann-Whitney U-test (nonparametric) was used for the comparison of the results for the behavioral observations.

Compression was done with a mixture of He-$O_2$ (rate (10 m/min, $P_{O_2}$ = 0.4 bar); the temperature was increased from 29 to 34.5°C to ensure the thermal comfort of the animal. The pressure was increased until either the first convulsion occurred, or until a depth of 1000 m was reached. The stay at pressure was from 30 to 60 min. All decompressions had a linear profile: 1 m/min or 0.6 m/min; $P_{O_2}$ = 0.5 bar (Gardette, personal communication).

The experimental series consisted of control rats without drugs ($n$ = 7); rats with saline injected intraperitoneally (i.p.) ($n$ = 5); and rats treated with sodium valproate in saline (400 mg/kg) injected i.p. 30 min before the compression ($n$ = 7). These rats also received similar injections of saline or sodium valproate in the absence of compression so that we could study any behavioral and electrophysiological changes during a 3-h period (equivalent to the compression and the stay at pressure).

## RESULTS

On the surface, injection of sodium valproate reduced the level of activities of the rats and increased the periods of sleep during the recording time of 3 h. There were modifications of awake EEG activities by an increase in the power spectra of the beta 1 frequencies (14–22 cps), and sometimes by an increase in the delta frequencies (1–4 cps).

Injection of sodium valproate before compression produced a significant attenuation in the HPNS during the dives. The depths at which the symptoms of HPNS appeared were increased:

a) tremor appeared about 300–400 m deeper than those seen in control dives with or without saline (Fig. 1) and was less intense;

b) myoclonia appeared around 100 m deeper;

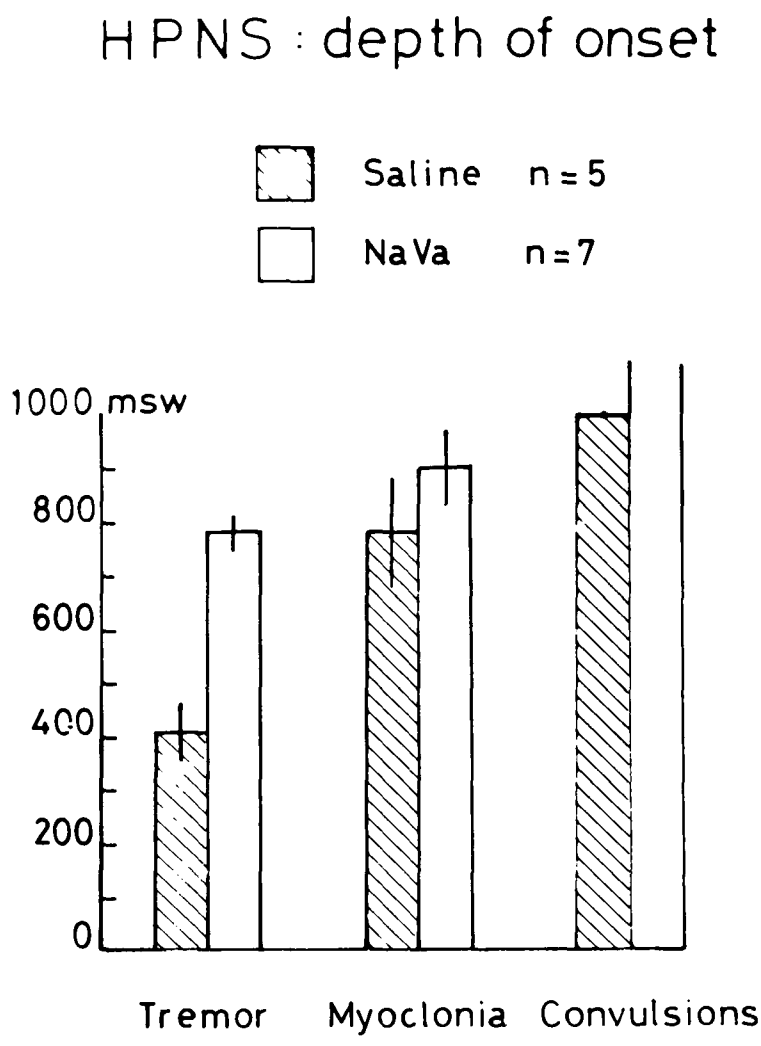



Fig. 1. Depths (mean and SD) of onset of tremor, myoclonia, and convulsions during control experiments with saline ($n$ = 5) and experiments with sodium valproate ($n$ = 7). Tremor: U-test, $P$ = 0.01; myoclonia: U-test, $P$ = 0.05.

c) convulsions were not seen up to the maximum depth (1000 msw) of our experiments with sodium valproate; in control rats, they appeared between 800 and 1000 msw with or without saline.

The EEG modifications were the same as those seen on the surface in the rats treated with sodium valproate. The increase in the slow waves (theta activities 4–7 cps) recorded during compression of the control experiments were not seen during the dives with sodium valproate. We should also note

that the behavioral effects of sodium valproate were shorter during compression than on the surface. The periods of sleep rapidly disappeared and the level of activity began to increase towards 300 msw (about 30 min after the start of compression).

## DISCUSSION

The results obtained with sodium valproate demonstrate that it has a beneficial effect on HPNS. Increasing the level of cerebral GABA will therefore modify and reduce some symptoms of HPNS. The results agree with those obtained for this drug by Bichard and Little (6), as well as for other drugs that elevate cerebral GABA levels. In fact, of all the substances that facilitate the action of GABA which have been used at pressure (6,7; Rostain et al. unpublished data), sodium valproate has given the best results.

It has recently been shown that sodium valproate has not only an inhibitory action on GABA-T, but also facilitates the action of GAD (glutamic acid decarboxylase), an enzyme involved in GABA synthesis (11). In addition, sodium valproate also reduces the level of aspartic acid, an amino acid excitatory neurotransmitter (12).

Thus, the efficacy of sodium valproate may depend on a combination of all these effects. It is possible that the reduction of HPNS symptoms seen with some anesthetics, such as ketamine (13), might result from a depressive effect on excitatory neurotransmitters such as aspartic acid; Anis et al. (14) have shown that ketamine has a depressive effect on aspartic acid.

These considerations have enabled us to propose a hypothesis for the possible role of GABA and excitatory neurotransmitters in the HPNS, which is shown in Fig. 2.

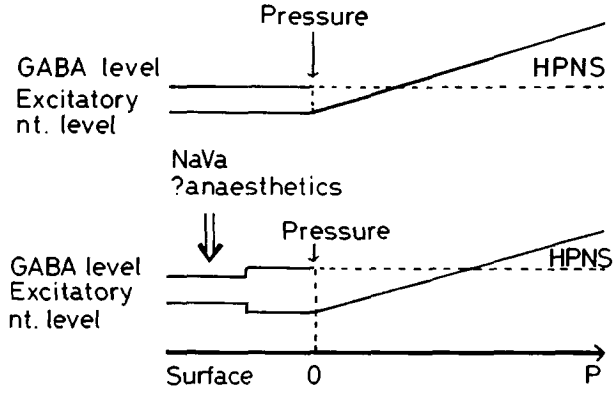



Fig. 2. Diagrams of the hypothesis for the role of GABA and excitatory amino acid neurotransmitters. *Upper graph:* action of pressure alone on GABA level and excitatory neurotransmitter (nt) level. *Lower graph:* actions of sodium valproate (and possibly some anesthetics) and pressure on GABA and excitatory neurotransmitter levels: sodium valproate increases the GABA level and decreases the excitatory neurotransmitter level.

Yet there are many factors that are unknown in this hypothesis which need further study. It is not known whether the synthesis of GABA changes in pressure: it may remain stable, or it may be modified. Excitatory neurotransmitters that may be involved in the HPNS remain to be discovered, but the recent results that we have obtained with a drug that antagonizes the excitation from aspartic acid show that this neurotransmitter has an important role in the appearance and intensity of HPNS (15).

## Acknowledgment

Research supported by Royal Society, M.R.C., D.R.E.T., C.N.R.S.

## References

1. Rostain JC. Le syndrome nerveux des hautes pressions chez l'homme et le singe *Papio papio*. These de doctorat es Sciences Naturelles. Universite d'aix-Marseille I. 1980;1:195;2:130.
2. Rostain JC, Imbert JP, Gardette B, Lemaire C, Dumas JC, Naquet R. Compression methods: II. Study of the effects of profiles and $N_2$ injections on HPNS of the baboon *Papio papio*. Undersea Biomed Res 1978;5:46.
3. Rostain JC, Gardette-Chauffour MC, Doucet J, Gardette B. Problems posed by compression at depths greater than 800 m in the monkey *Papio papio*. Undersea Biomed Res 1981;8 (Suppl):17.
4. Meldrum BS, Balzamo E, Gadea M, Naquet R. Photic and drug induced epilepsy in the baboon (*Papio papio*): The effects of isoniazid, thiosemicarbazide, pyridoxine, and amino-oxyacetic acid. Electroenceph clin Neurophysiol 1970;29:333–347.
5. Meldrum BS, Horton RW. Neuronal inhibition mediated by GABA and patterns of convulsions in baboons with photo-sensitive epilepsy (*Papio papio*). In: Harris P, Mandley C, eds. Edinburgh: The natural history and management of epilepsy. Livingstone 1974:55–64.
6. Bichard AR, Little HJ. Drugs which increase GABA transmission protect against the high pressure neurological syndrome. Br J Pharmacol 1982;76:447–452.
7. Wardley-Smith B, Angel A, Halsey MJ, Mason ST. Interactions of GABA and catecholamine neurotransmitter system in HPNS. Undersea Med Res 1982;9 (Suppl):19.
8. Johnston GAR. Physiologic pharmacology of GABA and its antagonist in the vertebrate nervous system. In: Roberts E, Chase TN, Tower DB, eds. GABA in nervous system function. New York: Raven Press, 1976:395–411.
9. Kupferberg HJ. Antiepileptic drugs. Sodium valproate. In: Glaser GH, Penry JK, Woodbury DM, eds. Antiepileptic drugs: mechanism of action. New York: Raven Press, 1980:643–654.
10. Baker JA, Halsey MJ, Wardley-Smith B, Wloch RT. Assessment of the high pressure neurological syndrome (HPNS): A new method of measuring tremor in animal model. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:415–420.
11. Losher W. Comparative assay of anticonvulsant and toxic potencies of sixteen GABAmimetic drugs. Neuropharmacol 1982;21:803–810.
12. Meldrum BS. Mechanism of action of sodium valproate. Brain Res Bull 1980;5 (Suppl 2):579–584.
13. Green CJ, Halsey MJ, Wardley-Smith B. Possible protection against some of the physiological effects of high pressure. J Physiol (Lond) 1977:267:46–47.
14. Anis NA, Burton NR, Lodge D. Ketamine anaesthesia via N-methyl-aspartate and acetylcholine receptor blockade? Studies on cat and rat Renshaw cells. J Physiol (Lond) 1982;324:74.
15. Meldrum BS, Wardley-Smith B, Halsey MJ, Rostain JC. 2- amino-phosphonoheptanoic acid protects against the high pressure neurological syndrome. European J Pharmacol 1983;87:501–502.

# HYPERBARIC PRESSURE EFFECTS ON THE NICOTINIC CHOLINERGIC RECEPTOR

*R. F. Taylor*

Numerous studies have shown that pressure can exert direct, dose-responsive effects on neural transmission (1–4). Such effects may be responsible for the variety of altered neurological responses observed in humans under hyperbaric conditions. Other studies concerning the action of anesthetics under pressure and pressure effects on macromolecules and model membrane systems indicate that pressure can act directly on cellular membranes (5–8). Such effects may alter the functionality of macromolecules, including neural transmitter receptors, within the membranes. The confirmation of this hypothesis and the definition of the molecular events involved in such pressure effects could advance our understanding and eventual control of neurological diving disorders.

We previously reported the first, direct evidence that pressure can affect the acetylcholine receptor (AChR). Utilizing the isolated nicotinic AChR proteoglycolipid (PGL) from rat gastrocnemius muscle, we showed that increasing pressure caused a decreased binding of cholinergic ligands, including acetylcholine (ACh), to the receptor, apparently due to pressure-induced binding site changes (9). Our observations were subsequently confirmed by other workers using an AChR membrane preparation from the electric tissue of *Torpedo californica* (10). We have continued our studies using the purified ACh isolated as both a protein and PGL from rat gastrocnemius tissue. Our rationale for this approach is that the use of isolated and defined neural receptors, free from other pressure effects (and thus interferences) on whole cells and tissues, will better demonstrate pressure effects at the molecular level. A preliminary report of this work has been presented (11).

## METHODS AND MATERIALS

### Preparation of the AChR

The AChR-PGL was isolated from rat gastrocnemius muscle using chloroform-methanol extraction and purified to homogenity according to our standard method (12). Aqueous preparations of the AChR were prepared in 50 mM $NaHPO_4$ buffer (pH 7.4) containing 50 mM NaCl, 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluroide according to standard methodologies (13,14). After homogenization of the tissue and an initial centrifugation at 1 000 × g for 30 min, the crude membrane fraction was isolated by centrifugation of the 1 000 × g supernatant at 40 000 × g for 60 min. The membrane-associated AChR protein was extracted from the resulting pellet with the original homogenizing buffer containing 2% Triton X-100 for 60 min at 4°C. After centrifugation of this mixture at 30 000 × g for 30 min, >90% of specific cholinergic binding activity was found in the supernatant. This crude membrane receptor preparation ("AChR membrane") was used in initial pressure studies and as the starting material for purification of the AChR protein-utilizing affinity and DEAE-Sephadex chromatography (12,14). The purified AChR protein was shown to be homogenous by polyacrylamide gel electrophoresis and high pressure gel permeation chromatography. Details of these procedures as well as the direct comparisons of the AChR protein and PGL preparations will be published elsewhere.

### Binding Assays

Two different approaches are necessary to assay cholinergic ligand binding to the AChR preparations. For the organic solvent soluble, AChR-PGL, a biphasic (chloroform-aqueous) partition method is used (9,12), in which the (hydrophobic) PGL is dissolved in the lower, organic phase, and the (hydrophilic) ligand is dissolved in the (buffered) upper, aqueous phase. Migration of radioactive ligand from the upper to the lower phase directly correlates to ligand-PGL binding after correction for nonspecific migration with appropriate controls. For the AChR membrane and protein preparations, an ultrafiltration assay utilizing Amicon PM-30 filters is used. Receptor-ligand complex is retained on the filters and the amount of ligand bound is quantitated from free ligand in the filtrate. These two types of assays are also directly applicable to pressure chamber studies to be discussed further.

Using the two assay methods described, we have found that after the amounts of ligands bound are corrected for nonspecific binding (i.e., binding in the presence of 1 μM α-Bungarotoxin (α-BTX), *see* Ref. 12), both the PGL and aqueous AChR preparations have similar binding capabilities in spite of their very different chemical and physical properties. For example, ACh (0.5 μM) binding to the PGL was 12.3 pmol/μg protein (8.2 pmol/μg protein,

specific) as compared to 14.7 pmol/μg protein (13.5 pmol/μg protein, specific) for the AChR protein. The binding of 0.1 μM α-BTX to both preparations was found, as expected, to be totally specific (15.1 and 22.3 pmol μg protein for the PGL and protein, respectively). Thus, both preparations contain the binding site of the nicotinic AChR. Specific ligand binding to the AChR membrane preparations was similar to that observed with the protein, although nonspecific ligand binding approached levels observed with the AChR-PGL.

### Pressure Studies

Our studies of pressure effects on the receptor preparations utilize a temperature-controlled, 1 000 psi, 3 ft × 1.5 ft cylindrical pressure vessel. Our principal diving gas is heliox. For studies such as those reported here, the chamber is outfitted with an apparatus incorporating two mixing vessles for the assay mixtures (*see* Ref. 9 for a detailed description). In studies with the AChR-PGL, both phases of the biphasic partition mixture are stirred by means of overhead and bottom stirring units, and samples for receptor-ligand quantitation are withdrawn directly from the lower phase through in-chamber sample lines connected to exterior stainless steel/Teflon micrometering valves. For studies with the aqueous AChR preparations, the (monophasic) reaction mixture is also sampled through the in-chamber lines but the lines are modified to contain an in-chamber, stainless steel Swinney filter containing a 13-mm Amicon PM-30 filter. After setting up the appropriate apparatus, the chamber is pressurized according to our standard diving protocol (9), i.e., 5 psi/min from 14.7 to 50 psi; 10 psi/min from 50 psi upwards. After each pressure level is reached, the reaction mixture is allowed to equilibrate for 30 min and samples are withdrawn for analysis. We use backflushing of the sample lines with an external helium tank to ensure proper mixing of samples and resuspension of receptor-ligand complex on the (aqueous assay) filters. Decompression to appropriate pressure levels follows the 10 psi/min schedule with, again, a 30-min equilibrium period at each pressure before sampling.

## RESULTS AND DISCUSSION

Table I reports the effects of compression/decompression on the binding of four cholinergic ligands, ACh, α-BTX, bromoacetylcholine (BAC) and d-tubocurarine (dTC), to the AChR protein and PGL. In all cases, pressure causes a decreased binding of ligand to the receptors. This binding is recovered upon decompression, although complete recovery of binding activity was observed only with the AChR protein. With both receptor preparations, ACh binding was most affected by pressure—the result was ≅90% or greater inhibition at 800 psi.

A comparison was also made of ACh binding to the AChR membrane and protein preparations at varying pressure levels. As shown in Fig. 1, the AChR membrane preparation lost ≅55% binding at 800 psi as compared to an ≅80% loss by the purified protein. Thus, of all three AChR preparations, the

TABLE I

Binding of Cholinergic Ligands to the AChR at Normo- and Hyperbaric Pressures*

| Receptor Preparation | Ligand (μM) | pmol Bound/ μg Protein† (14.7 psi; 0 time) | Δ% Bound‡ | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 50 | 100 | 200 | 400 | 600 | 800 | 600 | 400 | 200 | 100 | 14.7 psi |
| Protein | [$^{14}$C]ACh (0.5) | 16.2 | −7 | −32 | −42 | −58 | −75 | −89 | −81 | −60 | −35 | −14 | 2 |
| | [$^{125}$I]α-BTX (0.5) | 24.7 | 20 | 1 | −9 | −11 | −16 | −71 | −59 | −45 | −31 | −18 | −4 |
| | [$^{3}$H]BAC (0.5) | 17.9 | −6 | −27 | −36 | −57 | −73 | −84 | −78 | −58 | −39 | −23 | 1 |
| | [$^{3}$H]dTC (2.0) | 96.2 | −13 | −14 | −20 | −32 | −44 | −75 | −57 | −44 | −20 | −15 | 3 |
| Proteoglyco-lipid | [$^{14}$C]ACh (1.0) | 15.4 | −30 | −51 | −65 | −81 | −90 | −94 | −93 | −80 | −69 | −48 | −14 |
| | [$^{125}$I]α-BTX (0.5) | 18.3 | −20 | −36 | −52 | −67 | −75 | −83 | −79 | −74 | −59 | −44 | −10 |
| | [$^{3}$H]BAC (1.0) | 14.7 | −20 | −43 | −61 | −75 | −82 | −88 | −86 | −77 | −68 | −37 | −12 |
| | [$^{3}$H]dTC (2.0) | 295 | −20 | −36 | −42 | −48 | −51 | −55 | −53 | −49 | −46 | −41 | −6 |

*Protein binding assays were carried out in 5 mL of 5-mM $NaHPO_4$ buffer (pH 7.4) containing 0.1% TX-100, 100 μg/mL of BSA and from 25 to 50 μg of protein. PGL binding assays utilized the biphasic partition method in which a 3-mL chloroform solution of proteoglycolipid containing 30–50 μg protein was partitioned against the appropriate ligand in 3 mL of 50-mM tris-HCl buffer (pH 7.2) containing 100 μg/mL BSA. *See* the text for details. †Each value is the mean of at least two assays at that pressure level with no single value deviating from the mean by more than ±8.3%. ‡The Δ% bound represents the loss (gain) of ligand bound as compared to the amount bound at 14.7 psi (0 time).

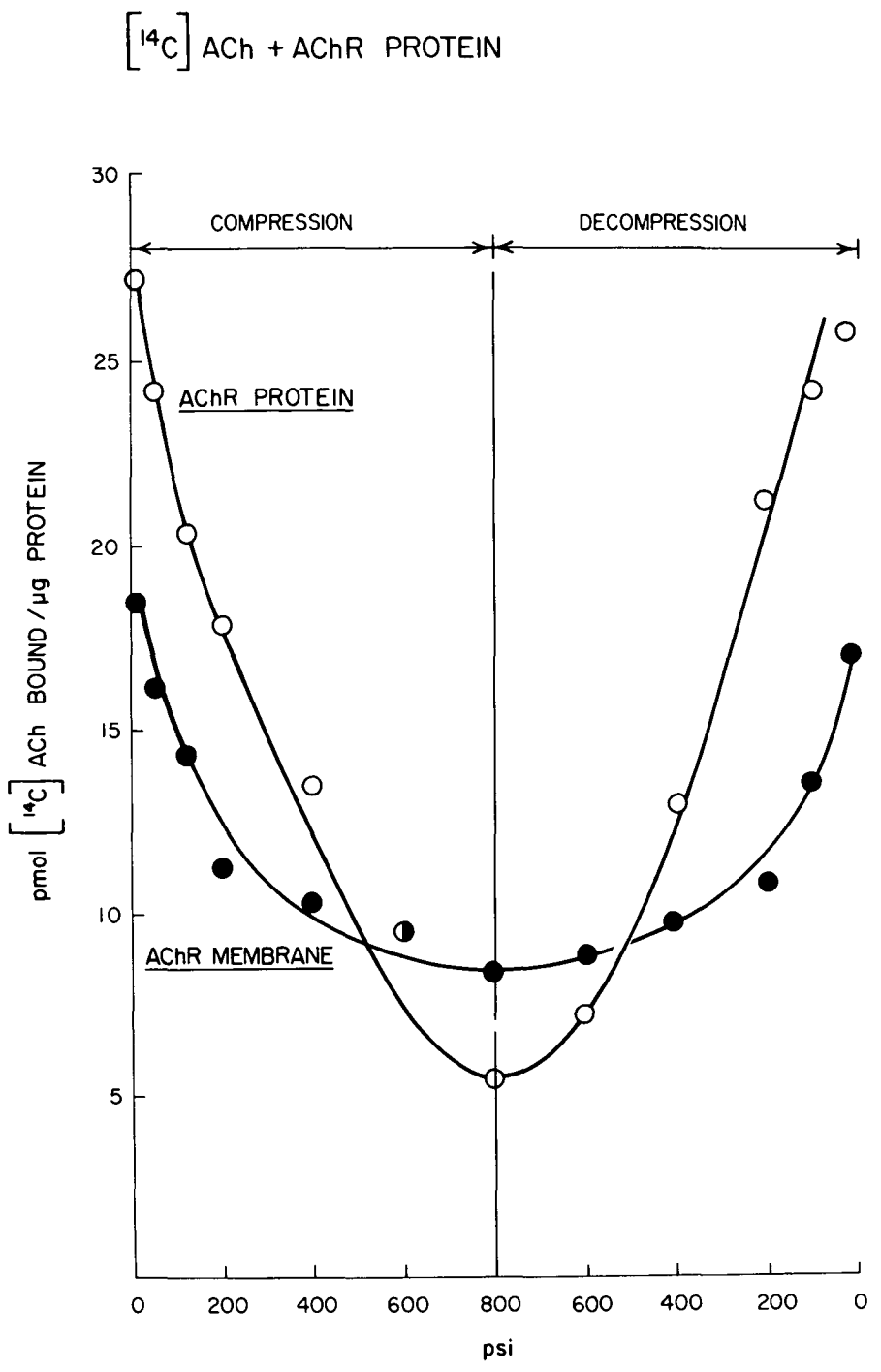



Fig. 1. Effect of pressure on the binding of ACh to the AChR membrane and protein preparations. The reaction mixtures contained ≅50 µg of protein and 1 µM Ach.

AChR membrane preparation was the most resistant to pressure-induced ligand loss. This resistance may be due to stabilization of the AChR toward pressure effects by other membrane components in the (crude) membrane preparation. This, in turn, may reflect the natural stability of the in vivo receptor because if a dramatic loss of binding to the receptor (as occurs with the purified receptor) occurred in divers, total neurological dysfunction would rapidly occur under hyperbaric conditions. Thus, the effects of pressure on neural transmission appear tempered by the natural organization of neural cell membranes.

We have previously reported (9,11) that pressure effects on the AChR can be further potentiated or inhibited by cholinergic drugs/agents. Up to this time,

TABLE II

Effects of Various Drugs and Agents on ACh Binding to the AChR Preparations at Normo- and Hyperbaric Pressures*

| Receptor Preparation | Drug/ Agent† | Δ% pmol [$^{14}$C]ACh Bound/μg Protein‡ | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 14.7 | 100 | 400 | 800 | 400 | 100 | 14.7 psi |
| Protein | None | — | −29 | −46 | −82 | −54 | −15 | −5 |
| | Nicotine | −58 | −77 | −81 | −92 | −86 | −89 | −72 |
| | Muscarine | 7 | −30 | −70 | −68 | −73 | −59 | −13 |
| | d-Tubocurarine | −5 | −27 | −36 | −60 | −33 | −32 | −30 |
| | Decamethonium | −38 | −64 | −83 | −96 | −84 | −82 | −42 |
| | Hexamethonium | 45 | 35 | 36 | −52 | −41 | −36 | 29 |
| | Atropine | 5.4 | −27 | −36 | −60 | −33 | −32 | −30 |
| | DFP | 2.1 | −7 | −8 | −11 | −10 | −5 | −3 |
| | Physostigmine | −53 | −56 | −65 | −70 | −71 | −71 | −62 |
| | Tetrodotoxin | 32 | −14 | −42 | −53 | −44 | −44 | −2 |
| | Veratridine | 12 | −5 | −7 | −21 | −16 | −19 | −7 |
| Proteo-glycolipid | None | — | −39 | −71 | −78 | −74 | −49 | 4 |
| | Nicotine | −15 | −12 | −26 | −54 | −34 | −19 | −16 |
| | Muscarine | −19 | −14 | −29 | −39 | −34 | −23 | −16 |
| | d-Tubocurarine | −27 | −68 | −79 | −90 | −84 | −70 | −12 |
| | Decamethonium | −4 | −63 | −82 | −87 | −81 | −62 | −14 |
| | Hexamethonium | −3 | 10 | −6 | 4 | 3 | 10 | 3 |
| | Atropine | −7 | −19 | −42 | −40 | −34 | −29 | −24 |
| | DFP | −10 | −47 | −49 | −63 | −59 | −53 | −23 |
| | Physostigmine | 4 | −24 | −34 | −52 | −29 | −7 | −6 |
| | Tetrodotoxin | 1 | −20 | −33 | −66 | −44 | −3 | −2 |
| | Veratridine | −4 | −5 | −18 | −29 | −21 | −11 | −9 |

*Assays were carried out using the ultrafiltration or biphasic partition method described in Table I except that the aqueous buffer in both assays was 5 mm $NaHPO_4$ (pH 7.4). In each reaction mixture, 45 μg (AChR protein) or 12 μg (AChR-PGL) of protein was reacted with drug/agent for 15 min before addition of [$^{14}$C]ACh. Final ACh concentrations were 1.0 μM (AChR protein) or 2.5 μM (AChR-PGL). Under these conditions, AChR protein bound 18.5 pmol ACh/μg protein and the AChR-PGL bound 33.6 pmol ACh/μg protein. †For the AChR protein, all agents and drugs, except DFP, were at a final concentration of 5 μM in the reaction mixtures. DFP was at a final concentration of 10 μM. For the AChR-PGL, all agents and drugs were at a final concentration of 15 μM. ‡The Δ% binding represents the loss (gain) of [$^{14}$C]ACh bound as compared to the amount bound at 14.7 psi in the absence of any drug/agent.

as shown in Table II, we have tested a variety of drugs/agents for their effect on pressure-induced inhibition of ACh binding to its receptor. For example, Fig. 2 illustrates that decamethonium (DMet; a neuromuscular, nicotinic, cholinergic antagonist) further inhibits, as expected, ACh binding to the AChR-PGL and thus potentiates the pressure effect; hexamethonium (HMet; a ganglionic, nicotinic, cholinergic antagonist) exhibits the surprising ability to inhibit pressure-induced loss of ACh binding. In the case of the AChR protein (Fig. 3), DMet again potentiates binding while HMet inhibits loss of binding up to $\cong$400 psi. This action of HMet is intriguing: it suggests that the drug may be binding to another (non-ACh) binding site on the AChR, with a resulting stabilization of the receptor toward pressure or activation of the receptor (or both) to a more avidly (ACh) binding form. This hypothesis is supported by other studies, which have shown that agents, including HMet, may interact directly with the AChR and result in the conversion of the receptor to a higher affinity form in vitro able to bind more AChR. This high affinity, in vitro state parallels the in vivo state of receptor desensitization (16,17). Thus, our observations may reflect a balance between pressure-induced loss of ACh binding with drug-induced potentiation of ACh binding until, as in the case of the AChR protein (Fig. 3), the drug effects are overcome by pressure effects. It would be interesting to see if the in vitro observations on HMet action at various pressure levels correlate with in vivo studies using the drug in animals under hyperbaric conditions.

Diisopropylfluorophosphate (DFP), a potent anticholinesterase, is also known to bind to a non-ACh binding site on the AChR and can cause desensitization of the in vivo AChR (17,18). Thus, we studied the effect of DFP on the pressure-induced loss of ACh binding to the AChR. In the case of the AChR-PGL, DFP offered only moderate protection against the pressure effect. In the case of the AChR protein, however, DFP significantly protected the receptor from pressure effects at all levels tested (Fig. 4). Because DFP is a reversible binding agent at the AChR (18), we also monitored [$^3$H]DFP at each pressure level. As shown in Fig. 4, significant amounts of DFP were displaced from the receptor with increasing pressure. This displacement suggests that although DFP can protect the AChR from pressure effects, at least a portion of the agent binds to pressure-sensitive regions of the receptor. These studies with DFP further support the hypothesis that agents which can interact with the AChR at non-ACh binding sites and cause receptor desensitization may provide the means to protect the AChR against pressure effects.

It is also interesting that veratridine, an alkaloid toxin which specifically interacts with the regulatory component of the AChR sodium action potential ionophore (19,20) antagonizes pressure-induced loss of ACh binding; while tetrodotoxin, which specifically interacts with the ion-transport components of the ionophore provides significantly less protection (Table II). Also, atropine, a muscarinic cholinergic agonist, appears to afford some protection against pressure effects on the nicotinic AChR. These results suggest that other classes of neural-active drugs besides cholinergic desensitizing agents may provide protection against hyperbaric disruption of the AChR.

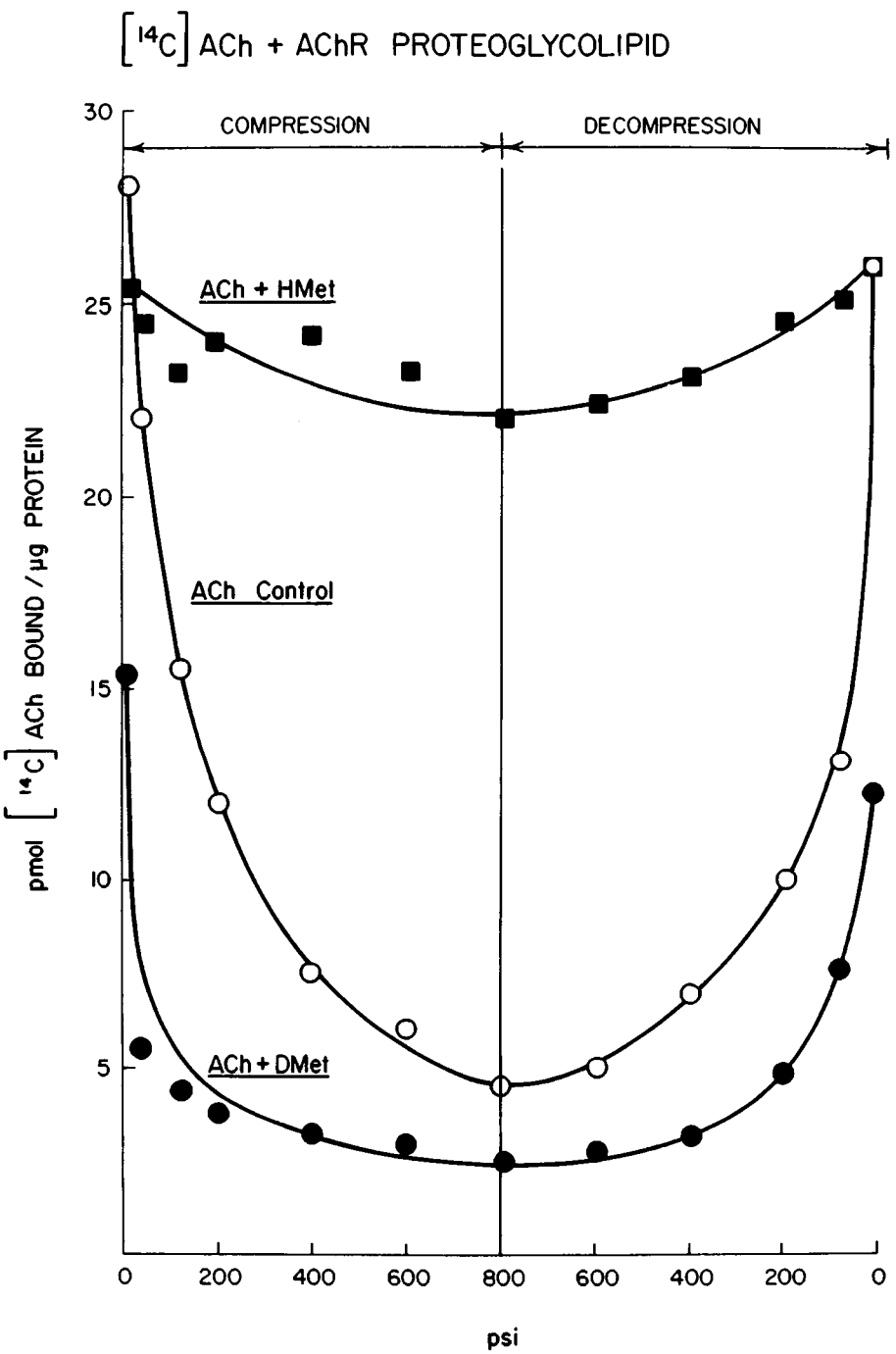



Fig. 2. Effect of pressure on the binding of ACh to the AChR-PGL per se and in the presence of decamethonium bromide (DMet) and hexamethonium bromide (HMet). Each reaction mixture contained ≅25 µg of PGL protein and 2.5 µM ACh.

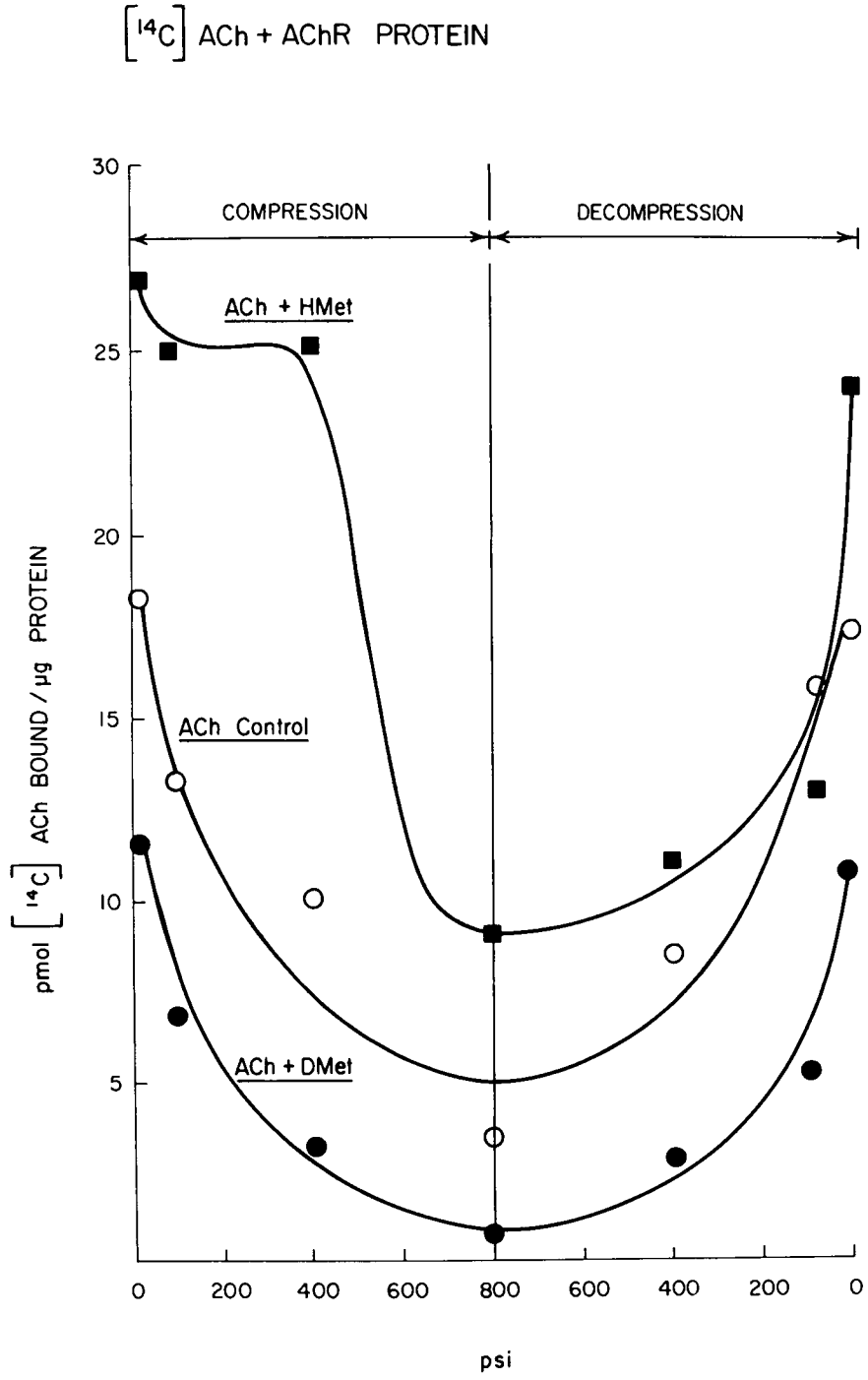



Fig. 3. Effect of pressure on the binding of ACh to the AChR protein per se and in the presence of DMet and HMet. Each reaction mixture contained ≅50 µg of protein and 1 µM ACh.

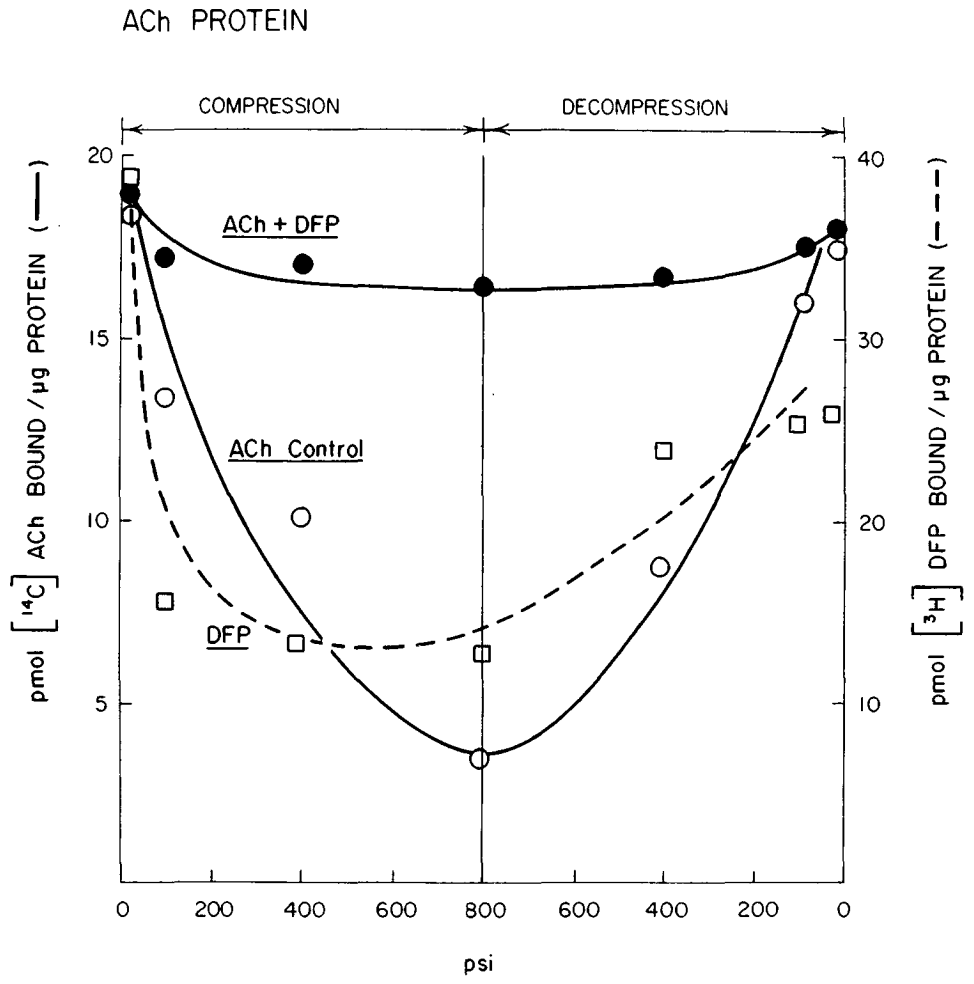



Fig. 4. Effect of pressure on the binding of ACh to the AChR protein per se and in the presence of Diisopropylfluorophosphate (DFP). Each reaction mixture contained ≅50 µg of protein, 1 µM [$^{14}$C]ACh and 10µM [$^{3}$H]DFP.

We have carried out further studies to define the binding kinetics of ACh to the AChR. It is known (12,21) that the AChR has two saturable binding sites for ACh termed *high* and *low* based on their binding affinity for ACh. Previously we had shown, using the AChR-PGL, that the AChR-ACh dissociation constant ($K_d$) for the high affinity binding site only was affected by pressure, an effect resulting in an apparent increased affinity for ACh even though total ACh binding to the receptor decreased. We have repeated these experiments with the AChR protein and observed similar results (Table III), although the total change in the $K_{dl}$ for the protein from 14.7 to 400 psi was

TABLE III

ACh Binding Kinetics to the AChR at Normo- and Hyperbaric Pressures

| | AChR Protein* | | | AChR Proteoglycolipid† | | |
|---|---|---|---|---|---|---|
| psi | ACh Bound† | $K_{d1}$(μM) | $K_{d2}$(μM) | ACh Bound | $K_{d1}$(μM) | $K_{d2}$(μM) |
| 14.7 | 26.3 | 0.052 | 1.7 | 21.4 | 0.16 | 9.2 |
| 50 | 18.2 | 0.049 | 1.7 | 16.1 | 0.76 | 9.2 |
| 100 | 15.6 | 0.046 | 1.7 | 15.7 | 0.039 | 9.2 |
| 200 | 10.4 | 0.034 | 1.7 | 15.0 | 0.033 | 9.2 |
| 400 | 9.1 | 0.024 | 1.7 | 9.3 | 0.030 | 9.2 |
| 600 | 8.1 | 0.023 | 1.7 | —§ | — | — |

*Each saturation experiment utilized 6 assays (*see* Table I for description of method), each containing ≅50 μg of protein and increasing amounts of [$^{14}$C]ACh from 0.01 to 2.0 μM. The amount of ACh bound at each concentration and pressure was determined and used to construct double reciprocal plots. The amounts of ACH bound at saturation and the apparent $K_d$ values were taken from the plots. †Values from Ref. 9. ‡pmol [$^{14}$C]ACh bound/μg protein at saturation. §Not determined.

less than for the PGL, −54 vs. −81%, respectively. Thus, it appears that compression directly alters the suprastructure of the AChR and results in changes not only in its binding capacity for ACh, but also in its (high) binding site affinity for ACh.

Based on our studies to this time, it is proposed that pressure affects the isolated, in vitro AChR as depicted in Fig. 5. At normobaric pressure, ACh binds at both high (partially buried) and low (surface) receptor sites (*H* and *L*, respectively). At elevated pressure, deformation of the receptor causes decreased total ACh binding and presumably affects binding to the *L* site, because this site binds the majority of ACh under normal conditions. At the same time, compression causes an increase in ACh affinity at the *H* site, presumably because of increased accessibility of the ligand to the site. These pressure effects are reversible up to at least 800 psi and may be inhibited by agents such as HMet and DFP. The protection of the AChR in its natural membrane environment precludes such dramatic pressure effects in vivo. Our studies indicate, however, that the neurological disorders observed in hyperbaric environments may be caused by subtle, yet significant, effects of pressure on neural receptor molecules.

## CONCLUSION

Our studies have shown that a) the nicotinic AChR from rat gastrocnemius tissue, isolated as either a protein or proteoglycolipid, is altered in its binding ability for cholinergic agents by pressure; b) the pressure effect is reversible upon decompression; c) the decreased binding of a ligand to the

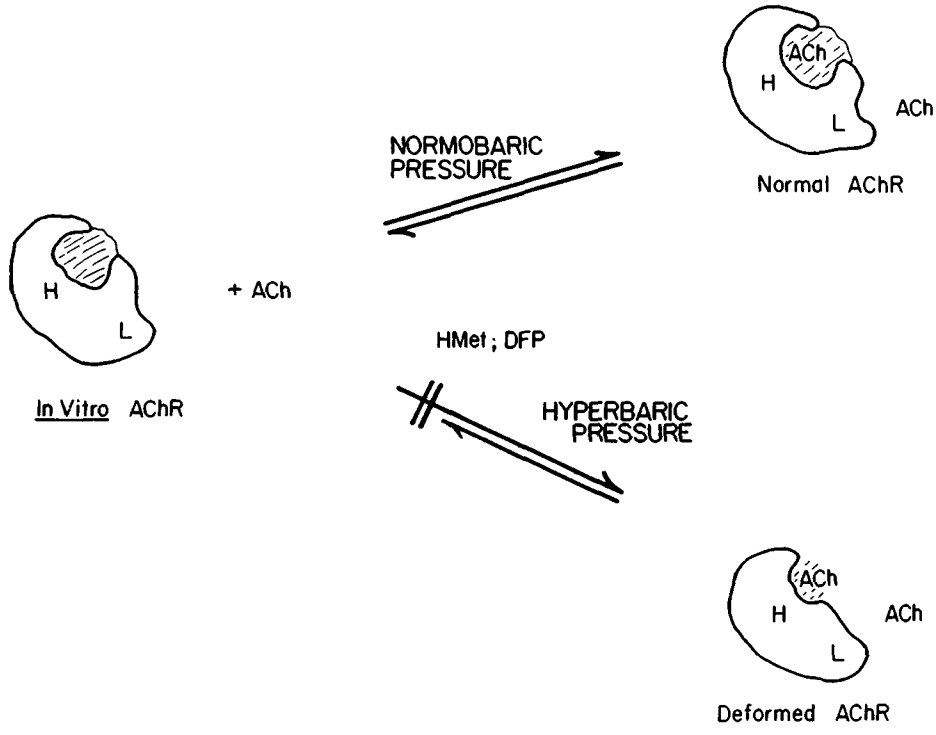



Fig. 5. Hypothetical representation of ACh interaction with the isolated AChR at normo- and hyperbaric pressure. *See* the text for details.

receptor during compression may, itself, be antagonized by certain drugs that appear to stabilize the receptor to pressure effects; and d) the effect of pressure on the AChR appears to be caused by conformational changes at the ACh binding sites.

## Acknowledgments

The technical expertize of Ms. Ingrid Marenchic for receptor preparation is gratefully acknowledged. This work was supported by ONR Contract N0014-80-C-0707.

## References

1. Ebbecke U, Schäeffer H. Effect of high pressure on the action potential in muscle and nerve. Pfluegers Arch 1935;236:679–692.
2. Kendig JJ, Cohen EN. Neuromuscular function at hyperbaric pressures: Pressure-anesthetic interactions. Am J Physiol 1976;230:1244–1249.

3. Henderson JV, Lowenhaupt MT, Gilbert DL. Helium pressure alteration of function in squid giant synapse. Undersea Biomed Res 1977;4:19–26.
4. Kaufmann PG, Bennett PB, Farmer JC Jr. Differential effects of pressure on the mammalian central nervous system. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:371–379.
5. Featherstone RM, Hegeman S, Settle W. Effects of inert gas pressures on protein structure and function. In: Lambertsen CJ, ed. Underwater physiology. Proceedings of the fourth symposium on underwater physiology. New York: Academic Press, 1971:95–100.
6. Akers TK, MacCarter DK. Effects of high pressure on alpha and beta adrenergic receptors of the rabbit duodenum. Aerosp Med 1973;44:60–62.
7. Miller KW. Inert gas narcosis, the high pressure neurological syndrome, and the critical volume hypothesis. Science 1974;185:867–869.
8. Galla HJ, Trudell JR. Pressure-induced changes in the molecular organization of a lipid-peptide complex. Biochim Biophys Acta 1980;602:522–530.
9. Taylor RF, Robertson RF. Effects of normo- and hyperbaric pressure on an acetylcholine-binding proteoglycolipid from rat gastrocnemius tissue. J Neurochem 1980;34:1166–1174.
10. Sauter JF, Braswell L, Wankowicz P, Miller KW. The effects of high pressure of inert gases on cholinergic receptor binding and function. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:629–637.
11. Taylor RF. Effects of hyperbaric pressure on isolated molecular components of the nicotinic acetylcholine receptor. Undersea Biomed Res 1982;9(Suppl):32.
12. Taylor RF. Isolation and purification of cholinergic receptor proteolipids from rat gastrocnemius tissue. J Neurochem 1978;31:1183–1198.
13. Dolly OJ, Barnard EA, Shorr RG. Characterization of acetylcholine receptor protein from skeletal muscle. Biochem Soc Trans 1977;5:168–170.
14. Kemp G, Morley B, Dwyer D, Bradley RJ. Purification and characterization of nicotinic acetylcholine receptors from muscle. Member Biochem 1980;3:229–257.
15. Blackman JG, Gauldie RW, Milne RJ. Interaction of competitive antagonists: The anti-curare action of hexamethonium and other antagonists at the skeletal neuromuscular junction. Br J Pharmacol 1975;54:91–100.
16. Lee T, Witzemann V, Schimerlik M, Raftery MA. Cholinergic ligand-induced affinity changes in *Torpedo californica* acetylcholine receptor. Arch Biochem Biophys 1977;183:57–63.
17. Kuba K, Albuquerque EX, Daly J, Barnard EA. A study of the irreversible cholinesterase inhibitor, diisopropylfluorophosphate, on time course of end-plate currents in frog sartorius muscle. J Pharmacol Exp Ther 1974;189:499–512.
18. Bullock JO, Farquharson DA, Hoskin FCG. Soman and receptor-ligand interaction in *Electrophorus* electroplaques. Biochem Pharmacol 1977;26:337–343.
19. Catterall WA. Cooperative activation of action potential $NA^+$ ionophore by neurotoxins. Proc Nat Acad Sci USA. 1975;72:1782–1786.
20. Taylor RF. Isolation and purification of a veratridine binding proteolipid from rat gastrocnemius tissue. J Neurochem 1978;31:1199–1207.
21. Almon RR, Appel SH. Cholinergic sites in skeletal muscle. I. Denervation effects. Biochemistry 1976;15:3662–3671.

# NEUROCHEMICAL BASIS FOR THE HIGH PRESSURE NEUROLOGICAL SYNDROME: ARE CHOLINERGIC MECHANISMS INVOLVED?

*B. Wardley-Smith, A. Angel, M. J. Halsey, and J. C. Rostain*

Numerous reports have described the signs and symptoms of the high pressure neurological syndrome (HPNS) in both man and animals, together with the alterations in physical conditions (e.g., breathing mixture, compression rate) that will influence the onset of these effects (for review, *see* Ref. 1). However, the neurochemical basis for the genesis of the HPNS is still unknown, as are the precise regions of the central nervous system (CNS) where these events take place. Recent work has suggested that gamma-aminobutyric acid (GABA) may be involved. Bichard and Little (2) have shown that drugs which facilitate GABA transmission raised the threshold at which pressure-induced tremors and convulsions occur in mice, although the drugs they found most effective (sodium valproate, flurazepam) affect other neurotransmitter systems as well. The involvement of catecholamine neurotransmitter systems was first suggested in 1978 (3) when reserpine, which depletes monoamine stores, was shown to lower the compression rate dependence of the convulsion threshold in mice. The effect of reserpine on the HPNS could be partially reversed by drugs whose actions antagonize specific reserpine effects, such as amphetamine, tranylcypromine, and L-tryptophan.

More recently, drugs that selectively deplete different monoamine neurotransmitters were administered to mice and any resulting changes in the behavioral aspects of HPNS were noted (4). No single monoamine was found to be important in the appearance of HPNS, and the authors concluded that a balance between different neurotransmitter systems might be more important than absolute neurotransmitter levels. This concept was further supported when it was demonstrated that chronic depletion in rats of brain noradrenaline with the neurotoxin 6-hydroxydopamine had no effect on the behavioral HPNS

thresholds. However, administration of muscimol, a GABA agonist, to the noradrenaline-depleted rats significantly raised the onset pressure for the appearance of tremors and convulsions (5,6).

To elucidate complex interactions of neurotransmitters, one must first study the effects of changes in single systems, although because of the complexities of brain neurochemistry it is virtually impossible to change a single neurotransmitter without concurrent changes in other systems. This paper reports our experiments using drugs affecting acetylcholine (ACh). We have treated rats with atropine, a muscarinic receptor blocker that crosses the blood-brain barrier; methylatropine, a muscarinic receptor blocker that does not cross the blood-brain barrier; and mecamylamine, a nicotinic receptor blocker active centrally but not at the neuromuscular junction. After treatment we observed any changes in the onset pressures for initial tremor as well as continuous tremor and convulsions as convenient behavioral endpoints for assessing the HPNS. In addition, the frequency of the tremor was measured.

## METHODS

Male Sprague-Dawley rats were used in all experiments. The drugs were dissolved in saline and injected intraperitoneally 30 min before the start of compression. The following doses were used: Atropine sulphate (Sigma Ltd.) 5 mg/kg; Atropine methyl nitrate (Sigma Ltd.) 5 mg/kg; mecamylamine hydrochloride (Sigma Ltd.) 5 mg/kg; saline alone was used for control animals. The rats were individually housed in a small cage mounted over a strain gauge (Fig. 1), designed to accurately determine the onset pressure for tremor (7); the cage was placed in a 25-L, 400-ATA pressure chamber. The output from the strain gauge was recorded for subsequent analysis of tremor frequency and, in addition, the rat was observed continuously. Oxygen was added to bring the partial pressure to 0.5 ATA; subsequent compression was with helium at a rate of 3 ATA/min. Three endpoints were used for assessing the HPNS: the onset of initial tremor, continuous tremor, and the appearance of the first convulsions. All observations were rated blind, and coded solutions were used for all injections. As soon as the first convulsion occurred, the rats were humanely subjected to euthanasia with an overdose of nitrous oxide before they had fully recovered from the seizure.

Results were analyzed in terms of the mean and standard error of the mean for each endpoint used. We used the Mann-Whitney U-test to test for significance.

## RESULTS

Results are summarized in Fig. 2 and presented as onset pressure in ATA (SEM) for the 3 endpoints used. Five rats were used in each group.

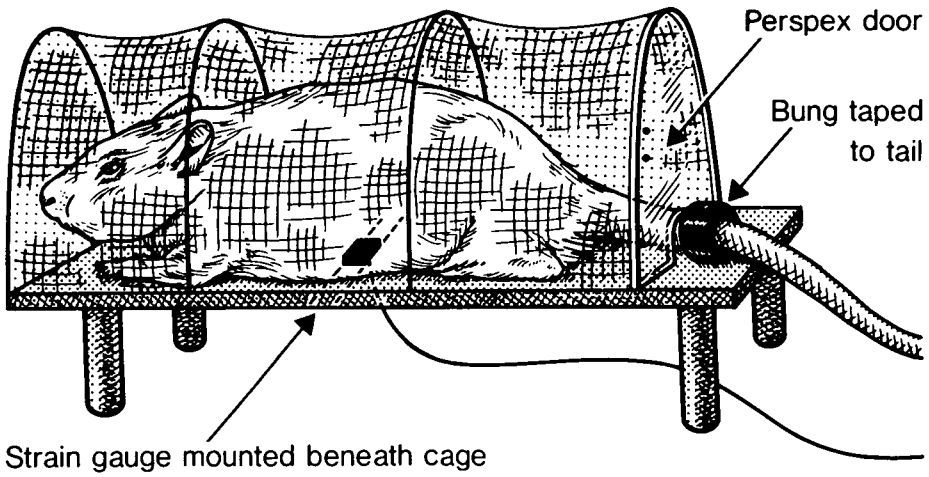



Fig. 1. Apparatus used to assess tremor in a rat at pressure. The cage has a solid rubber base and nylon net sides, which eliminate problems of resonance. The rat was restrained only by taping a bung to the tail, which kept the orientation with respect to the strain gauge approximately constant. This procedure caused no distress to the rat.

## Atropine

There was no significant difference for the onset of tremors and convulsions in the rats treated with atropine. Yet the characteristics of the tremor were slightly altered in these rats. We have previously shown that the frequency of pressure-induced tremor seen in rats is 12–14 Hz. In these experiments the tremor seen in control animals was 13.7 (SD 1.0) Hz; in the rats given atropine the mean frequency of the tremor was slightly increased to 15.4 (SD 0.6) Hz ($P<0.05$ Mann Whitney U-test).

## Methylatropine

There was no significant difference in the onset pressures for the behavioral signs of the HPNS in the rats treated with methylatropine. In addition, the frequency characteristics of the tremor were not altered: they had a mean frequency of 13.8 (SD 1.1) Hz compared with that of the saline controls, which had a frequency of 13.7 (SD 1.0) Hz.

## Mecamylamine

In the rats treated with mecamylamine, the threshold for the initial tremor was reduced by 27% from 35.7 (SD 2.6) ATA to 25.9 (SD 1.6) ATA ($P < 0.01$). Neither continuous tremor nor convulsions occurred at a significantly

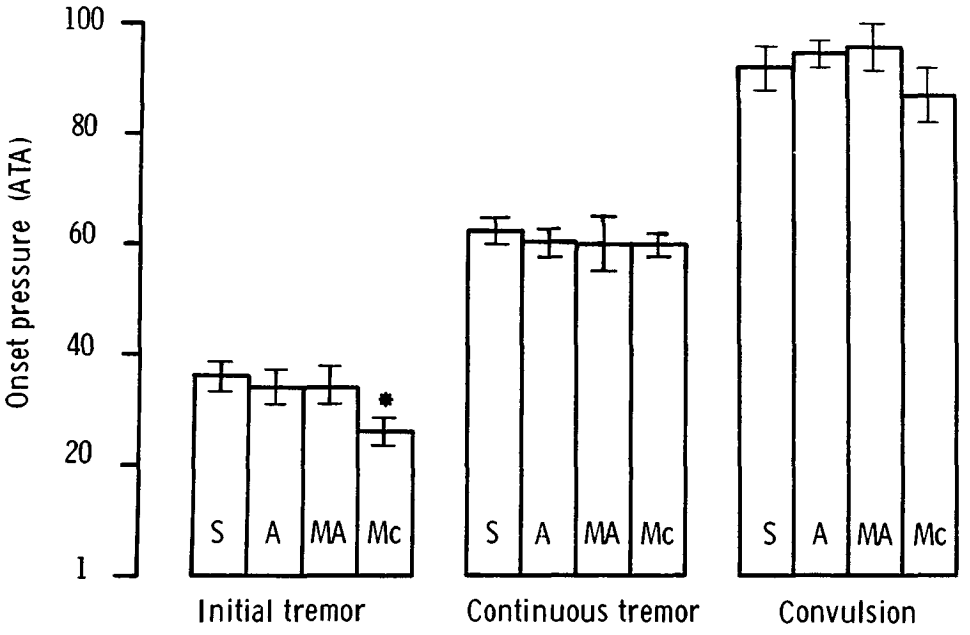



Fig. 2. Histogram showing the results of the different drugs on the endpoints used to assess the HPNS: initial tremor, continuous tremor, and the first convulsion. Vertical axis: onset pressure in ATA. S: Saline; A: Atropine; MA: Methylatropine; Mc: Mecamylamine. Error bars ± 1 SEM; $*P < 0.01$ (Mann-Whitney U-test).

lower pressure than in the saline-treated controls. The characteristics of the tremor were also unchanged, with a frequency of 13.9 (SD 0.6) Hz.

## DISCUSSION

These results appear to us to demonstrate that acetylcholine does not play a major role in the neurochemical development of the HPNS. In these experiments we deliberately used relatively high doses of both mecamylamine and atropine to ensure the maximum obtainable receptor blockade that was not accompanied by systemic toxicity. Interestingly, mecamylamine had a significant effect on only one endpoint, the initial tremor threshold, reducing it by 27%. It is possible, therefore, that nicotinic mechanisms are involved in the initial onset of the HPNS tremor, and this would provide further support for the view that the various aspects of the HPNS have both different mechanisms and sites of origin. It is, however, difficult to be certain that the effect of mecamylamine is primarily caused by its blockade of nicotinic acetylcholine receptors per se; the effect of this blockade on postganglionic noradrenergic

terminals may be of equal importance in the modification of the initial tremor threshold by mecamylamine.

Because of the various roles proposed for cholinergic mechanisms in both arousal and seizures, the lack of dramatic results is surprising. In one model of epilepsy, in which catechol is used to produce tremor, myoclonus, and convulsions, cholinergic mechanisms are thought to play a major role (8). But, in those experiments seizures were blocked by both atropine and mecamylamine, and thus a similar mechanism is unlikely to apply to the HPNS, especially because mecamylamine reduced the onset pressure of the HPNS tremor. This effect also emphasizes the difficulties in selecting a suitable *model seizure* for studies of the HPNS; currently, there is no type of experimental convulsion that closely resembles the seizures seen in animals as a result of exposure to high pressure.

It is interesting that treatment with atropine apparently changed the underlying frequency of the pressure-induced tremor. This change in frequency was not seen after treatment with methylatropine, and thus the alteration must be central in origin. One possibility is that this effect is mediated via Renshaw inhibitions in the spinal cord. It is known that treatment with atropine suppresses the late Renshaw cell activities, which are inhibitory (9). The initial latency of the response is also reduced after treatment with atropine. Therefore, in these experiments, atropine could decrease the activities of Renshaw cells, which in turn could account for the increase in frequency of the HPNS tremor. Investigators have noted (10) that the effect of atropine on the Hoffman response is similar to that of pressure. Yet the lack of any effect of atropine on the thresholds for the HPNS suggests that there is no major antimuscarinic effect of pressure in the brain.

In conclusion, results of both central and peripheral blockade of cholinergic systems produce no dramatic changes in the HPNS, and thus cholinergic mechanisms are unlikely to be primarily involved. Nevertheless, a secondary role for these mechanisms, including control of tremor frequency, is possible.

## References

1. Halsey MJ. Effects of high pressure on the central nervous system. Physiol Rev 1982;62;1342–1377.
2. Bichard AR, Little HJ. Drugs that increase GABA transmission protect against the HPNS. Br J Pharmacol 1982;76:447–452.
3. Brauer RW, Beaver RW, Sheehan ME. Role of monoamine neurotransmitters in the compression rate dependence of HPNS convulsions. In: Shilling CW, Beckett MW, eds. Underwater physiology VI. Proceedings of the sixth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1978:49–60.
4. Koblin DD, Little HJ, Green AR, Daniels S, Smith EB, Paton WDM, Brain monoamines and HPNS. Neuropharmacology 1980;19:1031–1038.
5. Wardley-Smith B, Angel A, Halsey MJ, Mason ST. Interactions of GABA and catecholamine neurotransmitter systems in HPNS. Undersea Biomed Res 1982;9(Suppl):19–20.

6. Angel A, Halsey MJ, Wardley-Smith B. Interactions of GABA and NA in the high pressure neurological syndrome. Br J Pharmacol 1983;19:725–729.
7. Baker JA, Halsey MJ, Wardley-Smith B, Wloch RT. Assessment of the high pressure neurological syndrome (HPNS): A new method of measuring tremor in an animal model. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:415–420.
8. Angel A, Clarke KA, Dewhurst DG. A pharmacological study of the spontaneous convulsive activity induced by 1,2 dihydroxybenzene (catechol) in the anaesthetized mouse. Br J Pharmacol 1977;61:433–439.
9. Krnjević K. Chemical nature of synaptic transmission in vertebrates. Physiol Rev 1974;54:418–540.
10. Hugon M, Fagni L, Rostain JC, Seki K. Somatic evoked potentials and reflexes in monkey diving saturation dives in dry chamber. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:381–390.

# INVITED REVIEW: MOLECULAR AND CELLULAR EFFECTS OF PRESSURE: THE CURRENT EMPHASIS

*M. J. Halsey*

This review is intended:

1) To summarize a selection of the recent contributions to the overall investigations of the molecular and cellular effects of pressure. The continuing series of *Underwater Physiology Proceedings* has approached the subject from different aspects and the concept was to complement the previous publications rather than simply update them.

2) To put the presentations allocated to this Section into context and perspective. Their diversity indicated the expansion of the field and it is becoming increasingly difficult to retain the necessary background knowledge.

3) To assess where the molecular and cellular studies may interrelate with other major areas of investigations into underwater physiology. Such studies are unlikely to have immediate applications, but I believe that it is important that any of them, however fundamental, can be demonstrated to be relevant to the potential problems of deep sea diving.

This review concentrates on the first objective.

## MOLECULAR RESPONSES TO PRESSURE

The molecular and cellular effects now being studied at pressure are very complex, but it is important to continue to remember that the fundamental mechanisms involved are relatively simple. For example, the effect of a variable such as pressure on the position of a chemical or physical equilibrium was summarized by LeChatelier as long ago as 1888:

> Any change in one of the variables that determines the state of a system in equilibrium causes a shift in the position of equilibrium in a direction that tends to counteract the change in variable under consideration.

This effect can be expressed in terms of classical thermodynamics:

$$\left(\frac{\delta \ln K}{\delta P}\right)_T = \frac{-\Delta V}{RT} \tag{1}$$

where $K$, P, V, R, and T are the equilibrium constant, pressure, volume, gas constant, and temperature, respectively.

Similarly, if the event being studied is a rate of reaction then:

$$k_p = k_{oe}^{-P\Delta V^{\ddagger}/RT} \tag{2}$$

where $k_p$ and $k_o$ are the rate constants at high pressure and at atmospheric pressure, respectively; $\Delta V^{\ddagger}$ is the volume of activation of the reaction.

Such textbook equations provide a quantitative description of the effects of high pressure in simple homogeneous systems, but the responses become much more complex in biological systems. Thus, the effects of pressure on lipid monolayers are relatively straightforward but the effect on protein stability is much more difficult to understand. For example, application of pressure to metmyoglobin (at low temperature) unfolds the protein but if, at ultra high pressures (>1000 ATA) the temperature is increased, then the protein begins to fold again spontaneously (1). Similar effects have been observed with the denatured/native ratios of chymotrypsinogen (2).

This behavior reflects the complexity of the hydrophobic effects and it should be noted that *pressure reversal* in molecular studies of proteins refers initially to $\Delta V > o$ in the unfolding, but at high pressures (or temperatures) refers to $\Delta V < o$. I am unaware of any similar reversal in sign being observed in lipid studies, i.e.,

$$(\delta V/\delta P)_T < O. \tag{3}$$

Lipids also differ from proteins in the magnitude of their response to pressure. Classical descriptions of globular proteins refer to the *oil drop* model (3), although it is noted that the packing of residues is nonuniform (4). Studies of the effects of pressure on ribonuclease (5) led Brandts to comment on the extremely low coefficient of compressibility of the protein ($< 5 \times 10^{-6}$ $atm^{-1}$), comparable to that of solid tin or rock salt rather than a liquid oil drop. It is possible to estimate the fraction of filled space within a protein. Surprisingly, for proteins of very different molecular weights the average value is 75 ± 1%, which when compared with water (36%) and hydrophobic solvents suggests that the protein interior is quite densely packed (6). Other complexities of molecular interreaction at pressure have recently been reviewed by Macdonald (7), and it is clear that even in simple biological systems there is still much to understand.

The concept that biochemical reactions involving large overall volume changes will be the most affected by pressure, according to LeChatelier's Principle, has directed attention to certain particular reactions. This means that enzyme-substrate reactions involving a decreased volume change will be stim-

ulated by pressure and reactions involving a large increased volume will be inhibited (8). For example, the aggregation of several enzymes with their substrates, which involve increased molecular volume, has been shown to be inhibited by high pressure (9). The spontaneous relaxation of muscle fibers involves a reaction with an unusually large volume change of 350 mL/mol and inhibition of this could explain high pressure paralysis (10).

It is important to note that disruption of enzymic processes generally occurs at very high pressures (i.e., above 200 ATA). One important exception is the activity of adenosinetriphosphatase (ATPase) enzymes, the reactions of which involve large volume changes. A 20% inhibition of intestinal $Na^+/K^+$-ATPase and cardiac $Na^+/K^+/Mg^{2+}$ ATPase is produced by only 20 atm (11). Another study showed a steeper decline in $Na^+/K^+$-ATPase activity from a surface-dwelling teleost than from a deep-sea teleost at pressure; a demonstration of adaption to deep-sea conditions (12). Hyperbaric inhibition of this enzyme could lead to an accumulation of intracellular $Na^+$ ions in axons (13).

The effects of high pressure on passive ion movements across inexcitable model membranes can also provide information about membrane structure and function at pressure. Pressure of 100 atm increased the passive permeability of isolated frog skin to sodium ions (14). In contrast, helium pressure has been shown to decrease $Na^+$, $K^+$ and glucose passive permeability in liposomes (15,16). Such results are consistent with mechanisms involving decreased membrane fluidity (and an associated decrease in overall volume) caused by pressure. Decreased fluidity of membrane components has been measured in artificial and natural membrame systems (17,18) at high pressure and low temperature. Decreased fluidity has been suggested to explain effects such as decreased transmitter release and decreased binding and postsynaptic responses at pressure, but are not helpful when increased transmitter release can also be shown at high pressure (*see* section on NEUROTRANSMITTER RELEASE AND RECEPTOR BINDING). The complexities of the inter-relationships between molecular processes and the cellular consequences are only now beginning to be studied seriously.

## NEUROTRANSMITTER STUDIES IN THE INTACT ANIMAL

Definitive advances have proved difficult to make in this area because of problems inherent in such investigations and technical difficulties in applying appropriate techniques at high pressure. This section starts with an introduction for the uninitiated before outlining the existing hyperbaric data.

The metabolism of the brain differs from that of other organs in having a disproportionately high energy and oxygen consumption. Glucose is the main energy-generating substrate and there is almost no glycogen reserve. However, relatively large pools of certain free amino acids linked to the tricarboxylic acid cycle (glutamate, aspartate, and γ-aminobutyric acid [GABA]) can be used for energy metabolism as well as for their more specific roles as neurotransmitters. It is difficult to apply to the brain the classical biochemical

techniques that have been useful in the studies of other organs. For example, the liver is made up from cells with similar properties and the response of the whole organ can be predicted from the responses of the individual components. But the brain has a far greater diversity of cell types and, more importantly, their individual functions are crucially dependent on the metabolic and structural interrelationships between cells. Thus, functional changes may be associated with negligible biochemical changes when the brain is studied as a whole. The rate of utilization (turnover) of transmitters in general reflects the rate of impulses transmitted. But, the absolute level of neurotransmitters in the brain depends on their synthesis, release, re-uptake, and metabolism rates. The direct study of turnover is not simple. It is usually not possible simply to measure excretion products in the blood or urine because many of the substances are also produced by other organs. A more difficult problem to overcome is that such neurochemical determinations have a time scale of minutes to hours, whereas the actual neuronal events occur in milliseconds or seconds.

The neurotransmitters include acetylcholine; amino acids with actions that are either inhibitory (GABA glycine) or excitatory (glutamate, aspartate); and amines (adrenaline, noradrenaline, seratonin, and dopamine). It is now known that some neurones may contain more than one transmitter, but the general principle is that a given neurone releases the same transmitter at every one of its synaptic terminals.

The effect of high pressure on neurotransmitter storage, release, and turnover (an obvious area for future study) has been only partially explored, primarily because of the technical difficulties of working at pressure. In 1975 Brauer and coworkers (19) found that reserpine lowered the convulsion threshold at slow but not at rapid compression rates. This abolished the convulsion rate dependence and suggested that the monoaminergic pathways might be important. Pretreatment with the monoamine oxidase (MAO) inhibitor tranylcypromine, an agent known to build up local stores of monoamines, blocked the reserpine effects. Similarly, amphetamine given after reserpine enhanced the effect of any monoamines still available and partially reversed the effect. The tryptophan-reserpine interaction also has been studied (20). In summary, the effect of reserpine on high pressure neurological syndrome (HPNS) convulsions is probably mediated through its known action on monoamine storage rather than by some other mechanism. Additional experiments with small doses of reserpine injected directly into the lateral ventricles indicated that the phenomenon is a central effect on central release of monoamines (21).

More recent work (22) has emphasized the technical difficulties and complexities of interpretation of the neurotransmitter studies at high pressure. Pressure alone elevated total brain 5-hydroxyindoleacetic acid but had little or no effect on the concentrations of serotonin, dopamine, or norepinephrine. It is difficult to interpret this finding, however, without the details of metabolite concentrations. Inhibition of synthesis of either norepinephrine and dopamine (with γ-methyl-p-tyrosine) or of serotonin (with parachlorophenylalanine) did

not affect HPNS (23), however. It is probably the balance of the different neurotransmitter systems that is important, but much more definitive work will be required before the issue is resolved.

The role of GABA in controlling central nervous system (CNS) excitability suggests that HPNS may be associated with specific effects on this neurotransmitter. This possibility has been tested by pretreatment with drugs that facilitate GABAergic transmission; sodium valproate and amino-oxyacetic acid (which inhibit GABA metabolism), and diaminobutyric acid (which inhibits GABA uptake) (24). These drugs elevated the threshold pressures for HPNS convulsions. The effects on pressure correlated with those of the pretreatment drugs on the threshold concentrations of bicuculline (a specific GABA receptor-blocking agent), causing convulsions.

The underlying neurotransmitter perturbation at pressure is unlikely to be as simple as a sole effect on GABA. For example, experiments with muscimol, a GABA agonist, indicate that this drug has little effect on HPNS in rodents, whether injected systemically or intraventricularly. Similarly, pretreatment from birth with 6-hydroxydopamine—a neurotoxin that permanently depletes the catecholamine content of some regions of the brain (including the cortex and cerebellum)—does not affect the rat's susceptibility to HPNS. But rats treated with both drugs had significantly higher onset pressures for both HPNS tremors and convulsions (25). These data indicated that manipulation of two neurotransmitter systems protects against the HPNS. It would be unjustified to draw any conclusions as to the regions of the CNS involved, especially because such pharmacological intervention may be merely treating the symptoms of the HPNS rather than influencing its genesis.

Finally, it should be noted that it is extremely difficult, if not impossible, to find a drug which categorically affects only one neurotransmitter system. A good example is sodium valproate, which has now been studied in mice, rats, and baboons. This drug is a clinical antiepileptic agent that is known to produce a number of biochemical changes including increases in GABA levels, primarily by inhibition of the GABA-transaminase reaction. It should be noted that it is not only the neurotransmitter which is affected and, for example, one additional hypothesis is that excitatory transmission, especially that mediated by aspartate, is reduced by valproate (26). The mechanisms of the anticonvulsant action of valproate still await clarification by a variety of biochemical and neurophysiological techniques (27).

A hopeful advance in neurotransmitter research is the wide range of compounds that has recently been evaluated as antagonists of excitatory amino acids (28). One of these 2-amino-7-phosphonoheptanoic acid (2-APH) has been shown to have a dramatic protective effect against HPNS (29). This compound (2-APH) is a selective antagonist of excitation produced by N-methyl-D-aspartic acid (NMDA) but with minimal activity against excitation produced by kainic acid or quisqualic acid (30). It had already been demonstrated to have anticonvulsant activity when tested against audiogenic and chemically induced seizures (31,32). In the pressure experiments (29) the most dramatic effect was on tremor (increasing the threshold pressure by a factor of

2.2). The tremor that did occur at the higher pressures was much less severe in the 2-APH group compared with their saline controls; it remained both mild and intermittent up to the convulsion onset pressure. The percentage changes in tremor onset pressures seen with 2-APH were greater than has previously been described with the most effective nonanesthetic agents such as sodium valproate and flurazepam (24). Although highly significant, the effect of 2-APH on both myoclonus and convulsions was less pronounced than the effect on tremor. These data support the view that the different phases of the HPNS have different sites of origin.

## NEUROTRANSMITTER RELEASE AND RECEPTOR BINDING

The effects of pressure on synaptic transmission may be caused by one or more mechanisms. Presynaptically, the mechanism could be transmitter synthesis, storage, release, or re-uptake. The postsynaptic alternatives are transmitter-receptor binding, metabolism, uptake or receptor sensitivity. There are also possible effects on secondary mechanisms such as those involving cyclic AMP.

There have been a number of in vitro studies on the effects of pressure on the release and receptor binding of neurotransmitters. Acetylcholine release from the guinea pig ileum has a mechanism similar to that in the CNS. Helium pressures up to 136 ATA increased the spontaneous release of acetylcholine (by 21%) but had no significant effect on the electrically stimulated release (33). Acetylcholine-receptor binding can be studied in the membranes isolated from the electroplaque of the stingray *Torpedo californica*. Helium pressures up to 300 ATA decreased both tritiated acetylcholine and tritiated (+)-tubocurarine binding at equilibrium (34). This effect could be caused by a decrease in the number of receptor sites available or, more likely, a decrease in the affinity for acetylcholine (i.e., an increase in the dissociation constant).

Lower pressures (up to 17 ATA) also decreased acetylcholine binding to proteoglycolipid extracted from rat gastrocnemius tissue (35). It was postulated that pressure affected cholinergic ligand-binding sites (i.e., those that could be specifically saturated). The effects were much larger than expected (50% decrease at 7 ATA), and it is particularly interesting that this work has now been extended to a membrane preparation of the receptor. The crude preparation is more resistant to the effects of pressure, which is consistent with the natural stability of the system in vivo (36).

Finally, Fish et al. (37) demonstrated that pressures up to 100 ATA decrease both the basal and dopamine-stimulated levels of cyclic AMP in rat caudate nuclei homogenates. At the moment, the in vitro studies have not been extensive enough to form any overall picture of the possible perturbations of pressure.

## NEURONAL STUDIES AT PRESSURE

The effects of pressure have been studied in rat, squid, toad, and snail neurons, starting with the work of Ebbecke (38), Grundfest (39), and their

associates, in the 1930's. There was then a long period during which, with very few exceptions (e.g., Spyropoulos [40]), there was no interest in the subject. In the last 5 years, however, at least four different groups of workers have begun to investigate the subject more thoroughly.

The consensus of reports on axonal pressure effects is that there is a slight slowing of conduction velocity, a pronounced prolongation of the action potential, and an increased propensity for repetitive firing (41).

The conduction velocity has been measured in rat superior cervical ganglion (42), in squid axon (43), and in frog nerve (40,44) and the effect of pressure is small. The most consistent effect of pressure is a prolongation of the action potential. Comparisons of four different studies indicate that at 100 ATA the increase varies between 20 and 70%, a finding suggesting that pressure perturbs the kinetics of the excitation processes. Experiments with the voltage-clamped squid axon demonstrate that helium pressure reversibly prolongs the time course of both early and late ionic currents; there is little or no change, however, in the maximum sodium or potassium currents. It has been postulated that the number of ionic channels that open during excitation does not change but that pressure primarily affects the gating processes. Similar experiments with snail (*Helix aspersa*) neurons (47,48) are consistent with the squid axon data; perhaps pressure primarily affects the inward calcium conductance. At the moment, however, underlying mechanisms are speculative.

The early observations of repetitive activity in both the frog sciatic nerve (49) and the squid axon (40) were made only at high pressure (above 300 ATA); interestingly, Grundfest and Cattell (49) noted that the refractory period was also prolonged. Initially, these phenomena were regarded as complicating factors at high pressure—they were hard to quantitate and relatively unimportant. Kendig and her colleagues (50), however, have become particularly interested in the role of repetitive impulse generation in HPNS. They studied crayfish claw nerves and observed that a single stimulus generated repetitive impulses at pressures as low as 30 ATA. As the pressure increased (up to 200 ATA), the train of repetitive impulses became longer, and, eventually, the neurons began to generate impulses spontaneously in the absence of a stimulus (51). At present, only indirect evidence relates repetitive activity to HPNS.

The effect of pressure on synaptic transmission appears to be generally depressant, but most studies have been made of excitatory, cholinergic transmission. In 1975 Kendig and her colleagues (42) performed synaptic-transmission experiments that used the rat superior cervical sympathetic ganglion, which Larrabee and Posternak (52) had also used as a model system for their classic anesthetic studies. From all previous data both in that model system and from intact-animal studies, researchers anticipated that pressure would produce some form of increased excitatory response. Surprisingly, pressure depressed the postganglionic response. Both fast and slow excitatory transmissions were depressed in the unanesthetized ganglion, and the degree of inhibition was pressure dependent. These effects were observed at pressures as low as 35 ATA.

The sympathetic ganglion is a complex structure; the postganglionic response is an ill-defined indication of the processes occurring within it. These processes include inhibitory transmission and the poorly understood, slow excitatory transmission in addition to the fast nicotinic cholinergic pathways studied in the experiment described previously. To test the possibility that results on the sympathetic ganglion were not truly indicative of pressure effects on an excitatory synapse, investigators (53–55) have studied a simpler model of excitatory transmission, i.e., the neuromuscular junction. Initial experiments with the rat phrenic nerve-diaphragm preparation indicated that pressures up to 200 ATA appeared to exert little effect on either the pre- or postjunctional response (54). However, at this neuromuscular junction there is a high safety factor for transmission; this factor permits depression of the postsynaptic response to low levels before failure appears as depression of the muscle compound action potential. When the safety factor was decreased, either by partial curarization or by exposure to a low calcium concentration, pressure inhibited transmission.

## CONCLUSION

The present review has concentrated on those aspects of the molecular and cellular studies that are particularly relevant to the majority of papers in this section of the symposium. It is clear that there are still many questions to be answered but, in this case, the long-term goal is a satisfactory understanding of the effects of high pressure on the CNS. This is, of course, important in itself as well as being directly related to drug-pressure interactions. The practical pharmacological implications are not confined to the amelioration of the HPNS but extend into the treatment of accidents at pressure and even into the potential problems of drug abuse in divers.

Pressure effects on the CNS may also have more indirect and subtle consequences. For example, the unexpected finding of hyperbaric-induced subfertility (56) may well be related to endocrine changes mediated via effects on the nervous system. It is likely that the future developments of the cellular and molecular studies will include more investigations into the long-term consequences of diving as well as assessing acclimation at pressure both in model systems and in the intact animal.

## References

1. Zipp A, Kauzmann W. Pressure denaturation of metmyoglobin. Biochemistry 1973;12:4217–4221.
2. Hawley SA. Reversible pressure-temperature denaturation of chymotrypsinogen. Biochemistry 1971;10:2436–2442.
3. Kauzmann W. Some factors in the interpretation of protein denaturation. Adv Protein Chem 1959;14:1–63.
4. Lee B, Richards FM. The interpretation of protein structures: estimation of static accessibility. J Mol Biol 1971;55:379–400.

5. Brandts JF, Oliveira RJ, Westort C. Thermodynamics of protein denaturation. Effect of pressure on denaturation of ribonuclease A. Biochemistry 1970;9:1038–1047.
6. Klapper MH. On the nature of protein interior. Biochem Biophys Acta 1971;229:557–566.
7. Macdonald AG. Hydrostatic pressure physiology. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving. 3rd ed. London: Baillière Tindall, 1982:157–188.
8. Landau JV. Hydrostatic pressure on the biosynthesis of macromolecules. In: Zimmerman AM, ed. High pressure effects on cellular processes. New York: Academic Press, 1970:45–70.
9 Zobell CE, Kim J. Effects of deep sea pressures on microbial enzyme systems. Symp Soc Exp Biol 1972;26:125–146.
10. Brown DES, Goodall MC. Reversal of relaxing mechanism in muscle fibre system by hydrostatic pressure. Nature 1956;178;1470–1471.
11. Gottlieb SF, Koehler GJ, Rhodes LVG. An oxygen- and pressure-sensitive enzyme: Na-K adenosinetriphosphatase. In: Lambertsen CJ, ed. Underwater physiology V. Proceedings of the fifth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1976:431–442.
12. Moon TW. Effects of hydrostatic pressure on gill Na-K-ATPase in an abyssal and a surface-dwelling teleost. Comp Biochem Physiol 1975;52B:59–65.
13. Gershfield NL, Shanes AM. The influence of high hydrostatic pressure on cocaine and veratrine action in a vertebrate nerve. J Gen Physiol 1959;42:647–653.
14. Brouha A, Pequeux A, Schoffeniels E, Disteche A. The effects of high hydrostatic pressure on the permeability characteristics of the isolated frog skin. Biochim Biophys Acta 1970;219:455–462.
15. Johnson SM, Miller KW, Bangham AD. The opposing effects of pressure and general anaesthetics on the cation permeability of liposomes of varying composition. Biochim Biophys Acta 1973;307:42–57.
16. Johnson SM, Miller KW. The effect of pressure and the volume of activation of the monovalent cation and glucose permeabilities of liposomes of varying composition. Biochim Biophys Acta 1975;375:286–291.
17. Gause EM, Mendez VM, Rowlands JR. A spin label study of the effects of hydrostatic pressure and temperature on cellular lipids. Spectroscopic Lett 1974;7:477–490
18. Chin JH, Trudell JR, Cohen EN. The compression ordering and solubility disordering effects of high pressure gases on phospholipid bilayers. Life Sci 1976;18:489–498.
19. Brauer RW, Mansfield WM Jr, Beaver RW, Gillen, HW. Stages in development of high pressure neurological syndrome in the mouse. J Appl Physiol: Respir Environ Exercise Physiol 1979;46:756–765.
20. Brauer RW, Beaver RW, Sheehan ME. Role of monoamine neurotransmitters in the compression rate dependence of HPNS convulsions. In: Shilling CW, Beckett MW, eds, Underwater physiology VI. Proceedings of the sixth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1978:49–59.
21. Brauer RW. High pressure neurological syndrome. In: Halsey MJ, Settle W, Smith EB, eds. The strategy for future diving to depths greater than 1,000 feet. The eighth Undersea Medical Society Workshop. Bethesda: Undersea Medical Society, Inc., 1975:58–61.
22. Daniels S, Green AR, Koblin DD, Lister RG, Little HJ, Paton WDM, Smith EB. Pharmacological investigation of the high pressure neurological syndrome: brain monoamine concentrations. In: Bachrach AJ, Matzen MM, eds. Underwater Physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:329–336.
23. Koblin DD, Little HJ, Green AR, Daniels S, Smith EB, Paton WDM. Brain monoamines and the high pressure neurological syndrome. Neuropharmacology 1980;19:1031–1038.
24. Bichard AR, Little HJ. Drugs that increase GABA transmission protect against the HPNS. Br J Pharmacol 1982;76;447–452.
25. Angel A, Halsey MJ, Wardley-Smith B. Interactions of γ -amino butyric acid and noradrenaline in the high pressure neurological syndrome. Br J Pharmacol 1983;19:725–729.
26. Meldrum BS. Mechanism of action of sodium valproate. Brain Res Bull 1980;5:579–584.
27. Chapman A, Keane PE, Meldrum BS, Simiand J, Vernieres JC. Mechanism of anticonvulsant action of valproate. Prog Neurobiol (Oxf) 1982;19:315–359.
28. Watkins JC, Evans RH. Excitatory amino acid transmitters. Ann Rev Pharmacol Toxicol 1981;21:165–204.

29. Meldrum B, Wardley-Smith B, Halsey M, Rostain JC. 2-amino-phosphonoheptanoic acid protects against the high pressure neurological syndrome. Eur J Pharmacol 1983;87:501–502.
30. Evans RH, Francis AA, Jones AW, Smith DAS, Watkins JC. The effects of a series of ω-phosphonic δ-carboxylic amino acids on electrically evoked and excitant amino acid-induced responses in isolated spinal cord preparations. Br J Pharmacol, 1982;75:65–75.
31. Croucher MJ, Collins JF, Meldrum BS. Anticonvulsant action of excitatory amino acid antagonists. Science 1982;216:899–901.
32. Czuczwar SJ, Meldrum BS. Protection against chemically induced seizures by 2-amino-7-phosphonoheptanoic acid. Eur J Pharmacol 1982;83:335–338.
33. Little HJ, Paton WDM. The effects of high pressure helium and nitrogen on the release of acetylcholine from the guinea-pig ileum. Br J Pharmacol 1979;67:221–228.
34. Sauter JF, Braswell LM, Miller KW. Action of anesthetics and high pressure on cholinergic membranes. Prog Anesthesiol 1980;2:199–207.
35. Taylor RF, Robertson RF. Effects of normo- and hyperbaric pressure on an acetylcholine-binding proteoglycolipid from rat gastrocnemius tissue. J Neurochem 1980;34:1166–1174.
36 Taylor RF. Hyperbaric pressure effects on the nicotinic cholinergic receptor. In: Bachrach AJ, Matzen MM, eds. Underwater Physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:607–619.
37. Fish E, Shankaran S, Hsia JC. High pressure neurological syndrome: Antagonistic effects of helium pressure and inhalation anesthetics on the dopamine-sensitive cyclic AMP response. Undersea Biomed Res 1979;6:189–196.
38. Ebbecke U. Wirkung allseitigen krompression auf den frockmuskel. Pfluegers Archiv Gesamte Physiol Menschen Tiere 1914;157:75–116.
39. Grundfest H. Effects of hydrostatic pressures upon the excitability, the recovery, and the potential sequence of frog nerve. Cold Spring Harbor Symp Quant Biol 1936;4:179–187.
40. Spyropoulos CS. The effects of hydrostatic pressure upon the normal and narcotized nerve fiber. J Gen Physiol 1957;40:849–857.
41. Wann KT, Macdonald AG. The effects of pressure on excitable cells. Comp Biochem Physiol A 1980;66:1–12.
42. Kendig JJ, Trudell JR, Cohen EN. Effects of pressure and anesthetics on conduction and synaptic transmission. J Pharmacol Exp Ther 1975;195:216–224.
43. Henderson JV, Gilbert DL. Slowing of ionic currents in the voltage-clamped squid axon by helium pressure. Nature (Lond) 1975;258:351–352.
44. Ritchie DM, DiPalma JR, McMichael RF. Helium effect on isolated nerve activity. Arch Int Pharmacodyn Ther 1978;231:57–62.
45. Shrivastav BB, Parmentier JL, Bennett PB. A quantitative description of pressure-induced alterations in inonic channels of the squid giant axon. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981;611–619.
46. Spyropoulos CS. Response of single nerve fibers at different hydrostatic pressures. Am J Physiol 1957;189:214–218.
47. Harper AA, Macdonald AG, Wann KT. The action of high hydrostatic pressure on voltage-clamped *Helix* neurones. J Physiol (Lond) 1977;273:70P–71P.
48. Harper AA, Macdonald AG, Wann, KT. The action of high hydrostatic pressure on the membrane currents of *Helix* neurones. J Physiol (Lond) 1981;311:325–339.
49. Grundfest H, Cattell McK. Some effects of hydrostatic pressure on nerve action potentials. Am J Physiol 1935;113:56–57.
50. Kendig JJ, Schneider TM, Cohen EN. Repetitive impulses in nerves: a possible basis for HPNS. Undersea Biomed Res 1978;5(Suppl):49.
51. Kendig JJ, Schneider TM, Cohen EN. Pressure, temperature and repetitive impulse generation in crustacean axons. J Appl Physiol: Respir Environ Exercise Physiol 1978;45:742–746.
52. Larrabee MG, Posternak JM. Selective action of anesthetics on synapses and axons in mammalian sympathetic ganglia. J Neurophysiol 1952;15:91–114.
53. Friess SL, Durant RC, Hudak WV, Boyer RD. Effects of moderate pressure He-$O_2$ saturation and response modifiers on neuromuscular function. Undersea Biomed Res 1975;2:35–41.

54. Kendig JJ, Cohen EN. Neuromuscular function at hyperbaric pressures: pressure-anesthetic interactions. Am J Physiol 1976;230:1244–1249.
55. Yeandle S. Neuromuscular transmission in the frog at 31 ATA helium pressure. Undersea Biomed Res 1977;4:159–168.
56. Baden JM, Dore CJ, Green CJ, Monk S, Halsey MJ, Wardley-Smith B. Reduction in fertility of male mice after exposure to high environmental pressure. J Appl Physiol: Respir Environ Exercise Physiol 1982;53:1207–1212.

# Part VIII

## BEHAVIORAL AND NEUROLOGICAL EFFECTS OF HYPERBARIC CONDITIONS

# EFFECTS OF DIFFERENT He-$N_2$-$O_2$ BREATHING MIXTURES ON HPNS AT 45 BARS

*J. C. Rostain and C. Lemaire*

Studies using an He-$N_2$-$O_2$ breathing mixture under hyperbaric pressure have shown that the differing effects of nitrogen depend on the amount used and the moment at which it is injected into the beathing mixture (1–6). To investigate more precisely the role of nitrogen at pressure, we have studied the effects on the high pressure neurological syndrome (HPNS) of breathing gas mixtures of different compositions during the course of a stay at 45 bars.

## METHODS

Four professional divers were compressed according to the method determined in the monkey (*Papio papio*) and extrapolated to man (7,8). The compression from 0 to 45 bars lasted 38 h and had stages of 150 min every 100 m. Nitrogen was injected before each stage and the level of nitrogen at 450 m was 4.8% ($N_2$ = 2.2 bars; $PI_{O_2}$ = 0.4 bar; T = 32° ± 1°C).

During the stay of 12 days at 45 bars, the subjects inhaled these breathing mixtures from a mask: a) 6th day, He-$N_2$-$O_2$: 7.5% nitrogen (3.45 bars); b) 7th day, He-$N_2$-$O_2$: 10% nitrogen (4.60 bars); c) 9th day, He-$O_2$ only.
Each mixture was inhaled for 2 h.

The subjects had electroencephalographic (EEG) electrodes (platinum wire) mounted in the scalp in the frontal, central, mid-temporal, and occipital region of the right hemisphere. The EEG activities were recorded before, during, and after each inhalation period (−30, +30, +60, +100, and +180 min). The duration of each recording period was 15–20 min. Tremor was measured with an accelerometer at the same periods of time.

Two psychometric tests were carried out before and between 75 and 100 min of breathing the mixtures: one, a vigilance test consisting of visual choice reaction time; the other, an intellectual test consisting of the recognition of letters.

## RESULTS

### EEG Modifications

Taking the group of divers as a whole, we found there were no consistent changes in the theta frequencies after breathing any of the mixtures. Yet each of the subjects had individual responses to a change of mixture (Fig. 1).

### Tremor

Breathing 7.5 and 10% $N_2$ in the He-$N_2$-$O_2$ mixtures produced a significant reduction in tremor in 3 out of 4 subjects ($P < 0.05\%$ to $P < 0.001$). The He-$O_2$ mixture produced a reduction in tremor in the same three subjects ($P < 0.05\%$ to $P < 0.001$). The fourth subject (*P3*) showed an increase in tremor (Fig. 2).

### Psychometric Performances

During the first period of breathing 7.5% $N_2$ in the He-$N_2$-$O_2$ mixture, the reaction time was reduced in two subjects (*P1*: −4%; *P2*: −7%) and increased in the other two subjects (*P3*: +9%; *P4*: +8%) (Fig. 3).

The number of letters recognized varied in the same way that the reaction time varied. Two subjects, *P1* and *P2*, were more vigilant and crossed out more letters at the end of the inhalation period than at the beginning; *Subjects P3* and *P4* recognized fewer letters (*P4*: −25%) (Fig. 4).

For the second inhalation period ($N_2$ = 10%), the variations in reaction times were reversed. In this period, *Subjects P1* and *P2* showed a reduction in vigilance not seen in the letter-recognition test. They improved their performance in the reaction-time test, but showed a deterioration in the letter-recognition test: (*P3*: −42%, *P4*: −23%) (Figs. 3 and 4).

Finally, during the third inhalation period with the He-$O_2$ mixture, the reaction times were stable in all four subjects (variation ± 0.01 s). The performance in the letter-recognition test was worse in all subjects (respective variations of −24, −2, −36, and −18%) (Figs. 3 and 4).

## COMMENTS

The results show that once everything is stable at 45 bars pressure, the divers will have varied reactions to breathing the different mixtures. With four

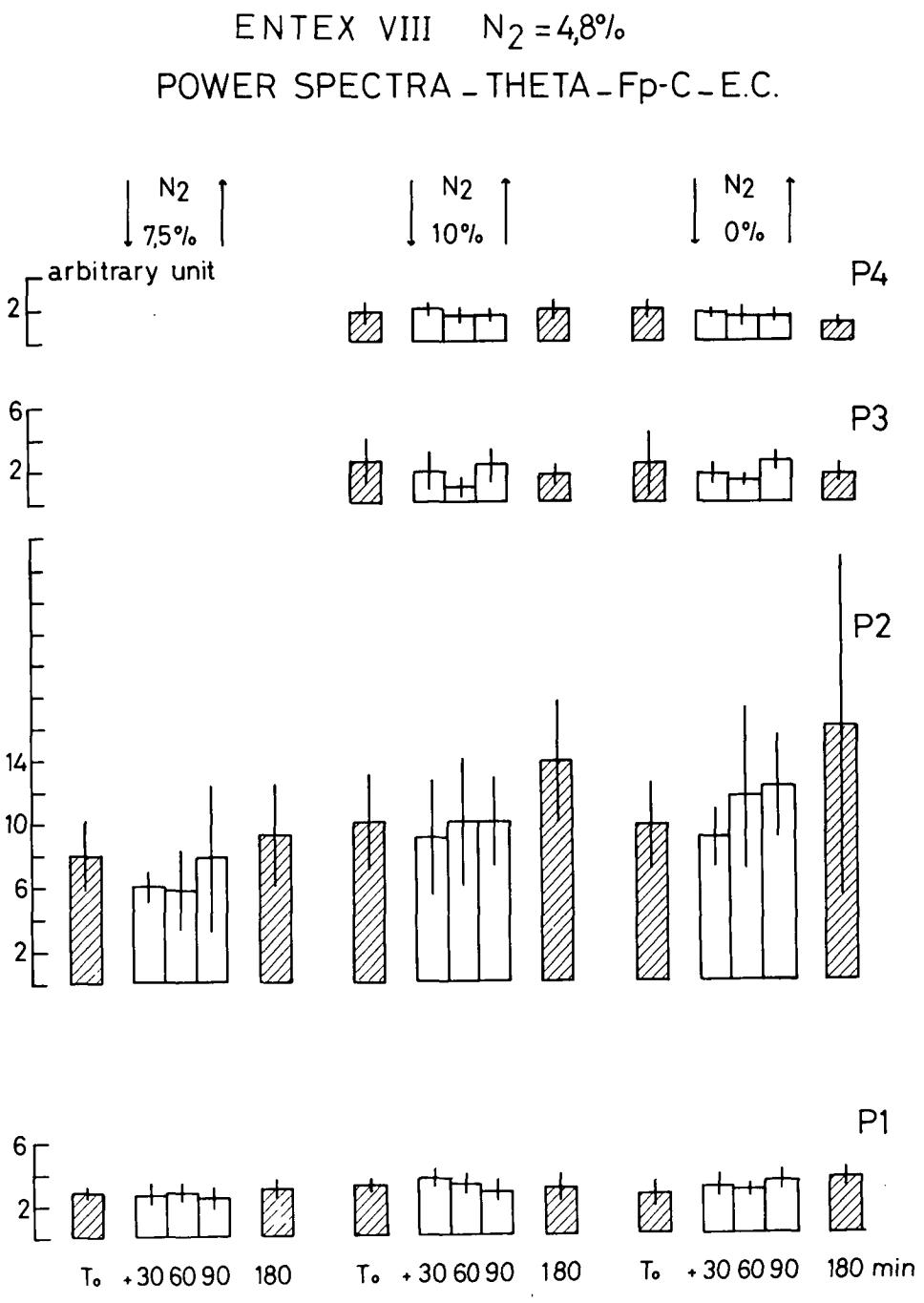



Fig. 1. Evolution of the power of EEG theta band during respiration of different breathing mixtures. Four subjects are represented from *top* to *bottom*. For each inhalation, the diagram represents: the value of the power in arbitrary unit in He-$N_2$-$O_2$ breathing mixture with 4.8% $N_2$, *before* the inhalation from the mask *(To)* (*hatched bars*); the values *during* inhalation of the mixtures with different nitrogen levels; the value 90 min *after* the end of inhalation in He-$N_2$-$O_2$ at 4.8%.

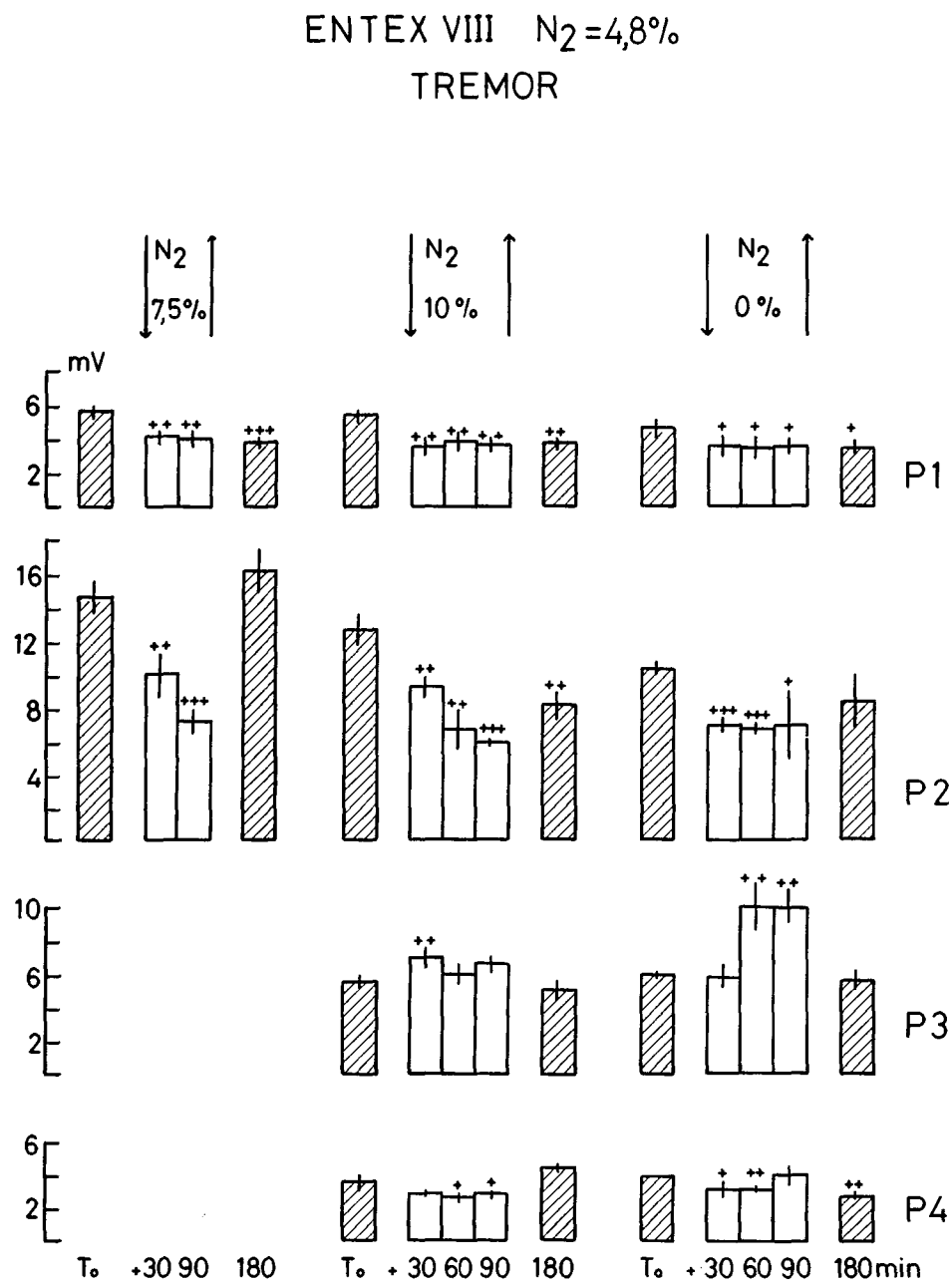



Fig. 2. Evolution of tremor during inhalation of different breathing mixtures. Four subjects are represented from *top* to *bottom*. For each inhalation, the diagram represents the intensity of tremor at different times. *Hatched bars:* the value of the power in arbitrary units in He-$N_2$-$O_2$ breathing mixture with 4.8% $N_2$ *before* the inhalation from the mask (*To*). Shown also are the values *during* inhalation of the mixtures with different nitrogen levels and the values 90 min *after* the end of inhalation in He-$N_2$-$O_2$ at 4.8%.

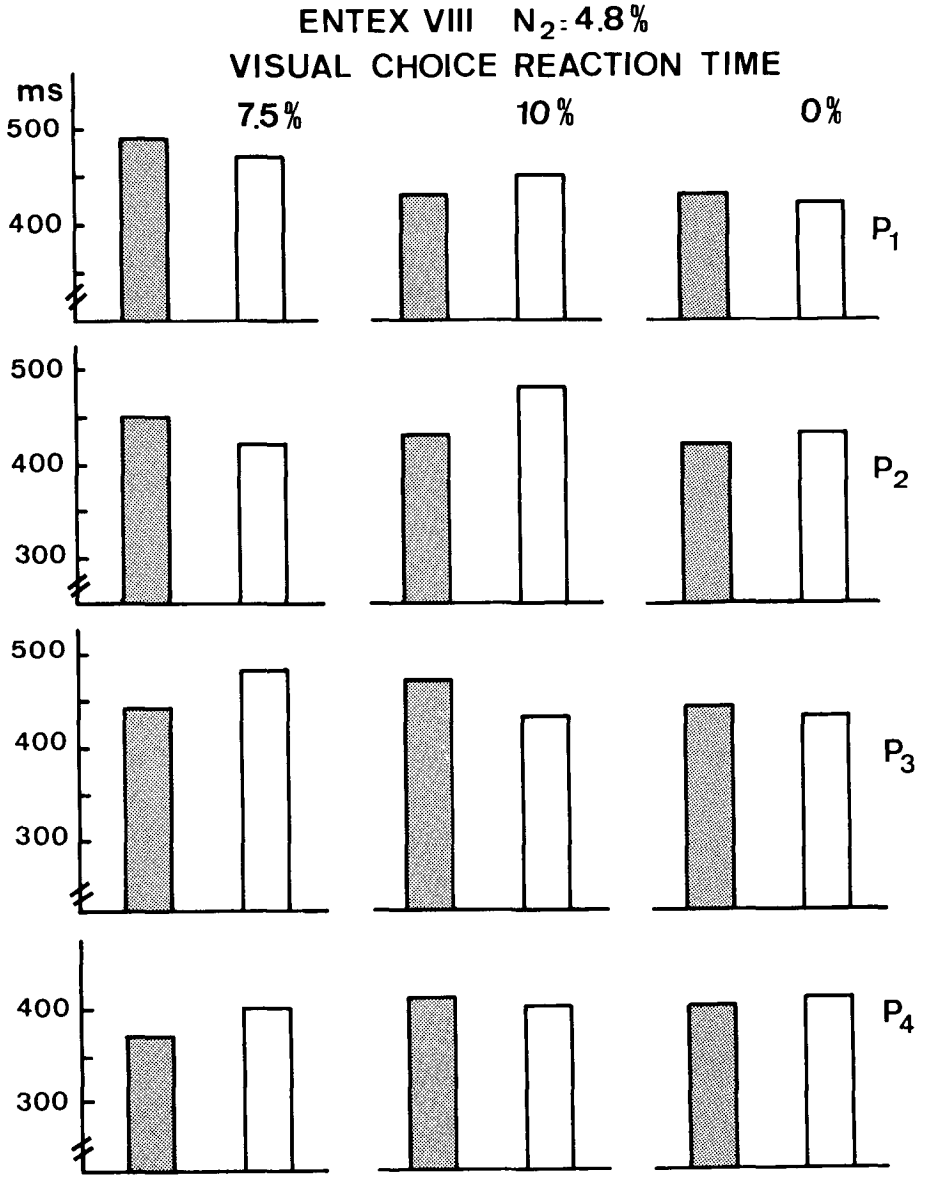



Fig. 3. Values of visual-choice reaction time before inhalation of breathing mixtures with different nitrogen levels (*gray bars*) and 60 min after the beginning of inhalation (*white bars*). *See* the differences between *Subjects P1, P2* and *P3, P4* for the mixtures with 7.5 and 10% $N_2$.

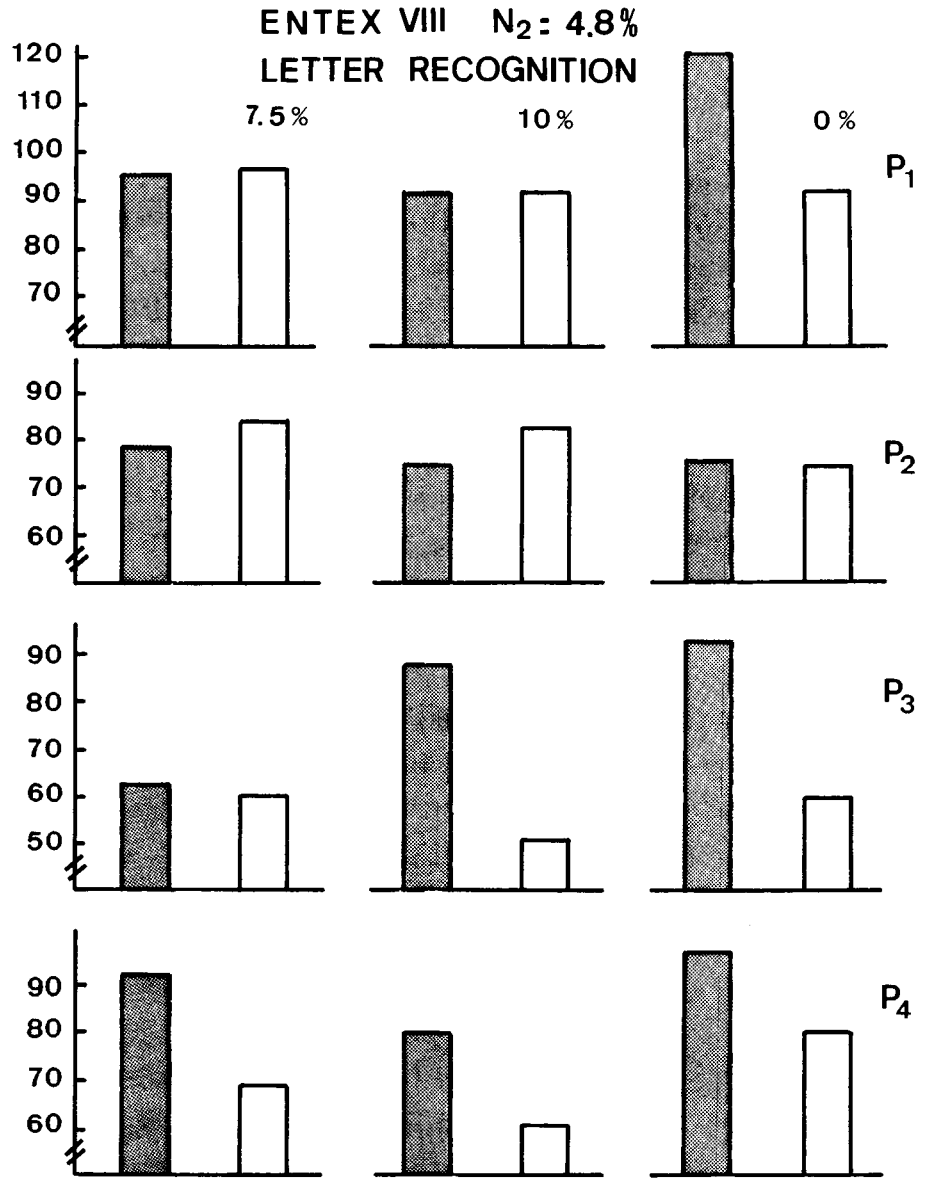



Fig. 4. Number of letters recognized *before* and *during* inhalation of breathing mixtures with different nitrogen levels (*gray bars*) and 60 min *after* the beginning of inhalation (*white bars*). Number of letters recognized in 4 min are shown in *abscissae*. Subjects *P3, P4* seem to be more impaired than *P1, P2* with every mixture.

subjects for only one session for each mixture, there was more variability between individuals than there was produced by the change in inspired mixture.

As is usual with the mixture He-$N_2$-$O_2$, the clinical symptoms of the HPNS were most affected and showed consistent changes in all subjects; tremor was reduced in three out of four subjects. But, this variation in tremor appears to be linked to the change in the mixture rather than to its composition. This is an important fact, which still needs to be explained.

The performances of the subjects were more or less worse than those found during dives at 180 m in an He-$N_2$-$O_2$ or He-$O_2$ mixture, where the different reactions seen depended on the subject (9). We are not certain whether or not the subjects were hypersensitive to other factors (e.g., pressure, fatigue) during these tests. In summary, two subjects were more vigilant with 7.5% $N_2$ while the other two had optimum concentration with 10% $N_2$.

Although these experiments are only an initial study and should be extended, they show that changing the inspired mixture for 2 h is possible during a prolonged stay at constant pressure without producing serious difficulties. This finding is important because it enables us to envisage changing the mixture of He-$N_2$-$O_2$ to one with less nitrogen and thus with a lower density; this change would make possible a working dive with a consequent reduction in respiratory problems.

## Acknowledgment

Grant: DRET: Technical support: GISMER, CEPISMER (French Navy, Toulon).

## References

1. Bennett PB, Blenkarn GD, Roby J, Youngblood D. Suppression of the high pressure nervous syndrome in human deep dives by He-$N_2$-$O_2$. Undersea Biomed Res 1974;1:221–237.
2. Bennett PB, Coggin R, Roby J. Control of HPNS in humans during rapid compression with trimix to 650 m (2132 ft). Undersea Biomed Res 1981;8:85–100.
3. Bennett PB, Coggin R, McLeod M. Effect of compression rate on use of trimix to ameliorate HPNS in man to 686 m (2250 ft) Undersea Biomed Res 1982;9:335–351.
4. Rostain JC, Naquet R, Fructus X. Study of the effects of "Trimix" and "Heliox" mixtures during rapid compression. Undersea Biomed. Res 1976;3:A13–A14.
5. Rostain JC, Imbert JP, Gardette B, Lemaire C, Dumas JC, Naquet R. Compression methods: II. Study of the effects of profiles and $N_2$ injections on HPNS of the baboon *Papio papio*. Undersea Biomed Res 1978;5(Suppl):46.
6. Rostain JC, Gardette-Chauffour MC, Naquet R. HPNS during rapid compressions of men breathing He-$O_2$ and He-$N_2$-$O_2$ at 300 m and 180 m. Undersea Biomed Res 1980;7:77–94.
7. Rostain JC, Gardette B, Gardette MC, Dumas JC, HPNS of baboon *Papio papio* during slow exponential compression with $N_2$ injection to 1000 msw and beyond. Undersea Biomed Res 1979;6 (Suppl):45.
8. Gardette B, Rostain JC. Compression technique in deep sea dives, for man and animals in He-$N_2$-$O_2$ mixture. Undersea Biomed Res 1981;8(Suppl):15–16.
9. Lemaire C. Capacité de travail psychosensoriel en ambiance hyperbare. Le travail humain 1979; 42:13–28.

# CONSIDERATIONS REGARDING THE NEED FOR, AND APPROACHES TO, THE STUDY OF RESIDUAL EFFECTS OF EXPOSURE TO DEEP DIVING ENVIRONMENTS

*R. W. Brauer and W. M. Hinson*

Motto: Read the instructions before and not after spoiling this instrument by faulty use.
Carl Zeiss, *Note on Their Instruments*, ca. 1930.

In the history of environmental physiology, studies concerning the effects of each hitherto unexplored stressful situation have tended to be focused initially, and for a long time thereafter, upon the relatively dramatic and easily detected acute effects of exposure; experimental investigations of the more subtle residual effects were initiated only much later, frequently at a time when the first indications that such effects might occur in human subjects were already being suspected (1). The physiology of deep diving appears to be no exception to this pattern. Sufficient knowledge has now been gained concerning the acute problems of exposure to high-pressure gaseous environments to warrant a look at the possibility that deep-diving subjects might run the risk of delayed effects.

## NATURE OF EFFECTIVE STRESSES

If we exclude the hazards of decompression, the stress factors peculiar to the deep-diving environment include confinement, exposure to elevated oxygen partial pressures, thermal stress, respiration in atmospheres of high density, the effects of elevated hydrostatic pressure, and the pharmacologic effects of expo-

sure to high concentrations of the nonoxygen constituents of deep-diving atmospheres. The first three of these stress factors are familiar from other settings and enough information appears to be available to justify the assumption that under properly controlled diving conditions their residual effects can be neglected. The three last components all are associated with the exposure of individuals to high pressure environments and, hence, represent the stress factors setting the deep-diving environment apart from all others. Among these, in turn, the respiration effects of high gas density alone appear to be smaller than was at one time suspected and, at least at the depths to which man or even experimental animals are currently exposed, are largely manifested in the form of changes in respiratory mechanics, which appear to be fully and readily reversible (2). (A peculiar form of respiratory distress encountered in very deep dives appears to represent a special component of the effects on the central nervous system (CNS) of high pressure exposures and, hence, properly falls under that heading [for example, *see* Ref.3].)

The effects of pressure, as such, and the pharmacologic effects of the several conceivable constituents of deep-diving atmospheres, are a different matter. The results of a recent analysis of the literature attempting to separate effects due to high pressure as such from these pharmacologic effects are summarized in Tables I and II (4). A number of effects associated with the functioning of excitable tissues and the properties of presumptive models of bilayer membranes were found to yield a coherent picture (Table I). The several metabolically inert gases explored invariably antagonize the effects of hydrostatic pressure, and the relative potencies with which they exert this effect follow a common pattern, both from the point of view of rank order and relative magnitude. By contrast, a second group of biological responses to high-pressure exposures fail to fit the *excitable tissue* pattern in one or more respects (Table II); however, the manner of divergence differs sufficiently between the several reactions of

TABLE I

Summary of Relative Intrinsic Potencies $N^2_{2R_i}$ for Inert Gas-High Pressure Interactions Characterizing Various Group A Responses

| | $N^2_{aR_i}$ Anesthesia and HP Reversal | $N^2_{cR_i}$ HP Convulsions | $N^2_{bR_i}$ HP Bradycardia | $N^2_{fR_i}$ Fluidity of Liposomes | $N^2_{TR_i}$ Phase TR of Liposomes |
|---|---|---|---|---|---|
| HP | −0.2 | −0.6 | −0.7 | −0.70 | −1.6 |
| He | 0.07 | 0.00 | 0.17 | 0.12 | 0.2 |
| Ne | 0.18 | — | 0.33 | — | — |
| $H_2$ | 0.39 | 0.34 | — | 0.25 | — |
| $N_2$ | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| $N_2O$ | 22.6 | 42 | 22 | 26 | 38 |

>Marks "neutral" gas for each response. (From Ref. 9, with permission of *Comparative Biochemistry and Physiology*)

TABLE II

Qualitative and Semi-Quantitative Characteristics of the Effect of Helium, Hydrogen, Nitrogen, and Hydrostatic Pressure on Group Non-A Biological Responses, and Comparison with Group A

| Responding Organism, Response, and No. for Response | Relative to Hydrostatic Pressure | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Pressure | | Helium | | | Nitrogen | | |
| | No Effect | Effect | No Effect | Antag. | Potentiate | <He | =He | >He |
| *Spirostomum Ambiguum* | | | | | | | | |
| 1. Swimming speed | | X | | X | | X | | |
| 2. Reversal dur. | X | | | | X | | X | |
| 3. Toxicity | X | | | | X | | | X |
| *Echinosphaerium Nucleofilium* | | | | | | | | |
| 4. Axopod length | | X | | | X | X (2 phase) | | |
| 5. Toxicity | X | | | | X | | | X |
| Echo Virus | | | | | | | | |
| 6. Growth | | (±) | X | | | | X | |
| Herpes Simplex Virus | | | | | | | | |
| 7. Replication | | (±) | X | | | | X | |
| 8. Pathol. | X | | | | X | | | X |
| Marine Bacterium EP-4 | | | | | | | | |
| 9. Growth | | X | | X | | — | — | — |
| *Acholeplasma laidlawii* | | | | | | | | |
| 10. Growth | | X | | X | | — | — | — |
| *Tetrahymena pyriformis* | | | | | | | | |
| 11. Multiplication | | X | | X | | | | X (but $<H_2$) |
| *Saccharomyces cerev.* | | | | | | | | |
| 12. Growth | | X | | Over Compens. | | — | — | — |
| All Group A Responses | | X | | X | | | | X |

this group to preclude their being gathered into only one or two coherent additional categories.

From the point of view of residual effects of high-pressure exposures, these observations raise several problems:

1) The relative effectiveness of pressure as such—unlike the relative effectiveness of each of the several gases included in Table I—varies widely from

one response to another (*Row 1, Table I*). This variation implies that any manipulations of the compression atmosphere will exert substantially different net effects upon different components of the high pressure syndrome, so that the clinical picture may vary widely from one gas mixture to the next. Thus, it seems quite possible that a gas mix suppressing a particular pressure effect may allow compression to proceed to the point where serious degrees of change, perhaps unperceived, may be imposed upon other excitable-tissue-type functions;

2) The problem would be compounded if the antagonistic effects of the pharmacologically active atmosphere component would prove to be caused by a dampening or blocking of manifestations by effects exerted at a site different from that primarily affected by pressure (e.g., blocking of convulsion onset by anesthesia) rather than to a true antagonism (i.e., reversal of the pressure-induced changes in the CNS at the identical sites as those giving rise to convulsions). In the former case, the inert gas anesthetic, by masking visible clinical effects of high pressure, might seriously enhance the chances that under cover of this effect pathological change unaffected by the anesthetic might progress to the point of more or less slowly reversible injury (*cf.* analysis of problem of the nature of inert gas/high pressure antagonism in Ref.4);

3) Finally, the existence of the type of effect listed in Table II further compounds the problem by raising the possibility that changes in cell motility, in cell replication, or in cell survival might accompany high-pressure exposure and prove either little or not at all affected by inert gas anesthetics, or actually potentiated by them.

Quite apart from these effects, all of which are associated with problems raised by manipulation of the compression gas composition to minimize some particular manifestations of high pressure effects, there is the more general question of the heterogeneity of the high pressure neurological syndrome (HPNS). This is already implicit in the data of Table I, which show very different balance of pressure and inert gas potencies for the different effects explored. No fewer than five components of the HPNS were found to be distinguishable, in the terminology of the critical volume hypothesis, on the basis of different combinations of compressibility and solubility characteristics of the several target sites (5). Other observations, focused on the two components of the convulsion stage of the murine HPNS, have shown differences in response to injectable anesthetics, in genetic determinacy, in age dependence, and in dependence upon compression rate (6). Thus, it would appear that any manipulation yet suggested to minimize the severity of specific components of the HPNS is liable to encounter potential difficulties because of the heterogeneity of the properties of the several HPNS components, and to entail risk of producing residual injury in the same manner just described for the inert gas anesthetics.

## TIME FACTORS AFFECTING PRESSURE RESPONSES

As a next step, attention must be turned to the dependence of the effects of exposure to high pressure upon the time characteristics of such exposure.

As will be seen presently, these can be recognized at three different levels: multi-generation exposures leading to adaptation; prolonged exposure of particular individuals to high-pressure environments leading to pressure acclimation; and short-term accommodation changes occurring during the course of a single pressure exposure of duration comparable with those encountered in deep-diving practice. To begin these considerations, one must note that all of the stress factors discussed so far are associated with sufficiently large changes in pressure, and their magnitude is correlated with the magnitude of this pressure change. There is reason to surmise that these effects reflect a fundamental property of excitable tissues: If one confines one's attention to terrestrial or to shallow-water aquatic forms, all species tested down to the level of flatworms—vertebrate and invertebrate alike—show dramatic changes in behavior at threshold pressures from 50–150 ATA (7). Therefore the existence of a rich deep water fauna at greater depths, down to regions where pressures exceed 1000 ATA, implies that the response to the pressure stress must have an additional qualifier, time of sojourn.

The nature of this time dependence is indicated by the results of recent studies on cottoid fishes of Lake Baikal, the world's deepest lake. Because of its geologic history and geographic location near the middle of the world's greatest landmass, this lake has been a major evolutionary laboratory. Among other taxa, the order of cottoid swimbladderless fishes has developed in this lake an extraordinary wealth of closely related species, including those forming the shallow-water-preferring family *Cottidae* and the deep-water-preferring family *Abyssocottidae* (8). For a number of typical members of these two cottoid families, a well-defined relation was found to exist between the depth of water they inhabit and the hydrostatic pressure at which they undergo high-pressure convulsions (Fig. 1). As one progresses from shallow to increasingly deeper water animals, on the average the convulsion threshold pressures increase by an amount that is only a little larger than the corresponding increase in habitat pressure (1.3 ATA/10 m) (9). To a reasonable degree of approximation, one can describe these data by stating that all of these fish undergo high-pressure convulsions when hydrostatic pressures exceed habitat pressures by just over 100 ATA. Although in principle this could reflect either acclimation or adaptation, the Baikalian fauna has kindly provided *cross-over species*, i.e., a few members of the shallow-water cottids that have come to adopt a deep-water mode of existence, and, conversely, one or two members of the abyssocottids that live in shallow waters. These are relatively rare species, and only limited information on the first of these groups is available (*open circles* in Fig. 1), namely, on the deep-water cottid *Batrachocottus nikolskii*. Unlike the remaining cottoids, convulsion threshold pressures for this species were a mere 40 to 45 atm greater than their habitat pressure, a finding strongly suggesting, at least in this case, that the animals may have taken the genetic make-up of their shallow-water forebears into the depths and increased their limited inherited pressure tolerance only by acclimation. The contrast between this pressure tolerance and the much higher pressure tolerance of the abyssocottids, then, would be a reflection of the importance of genetic, presumably adaptational, factors

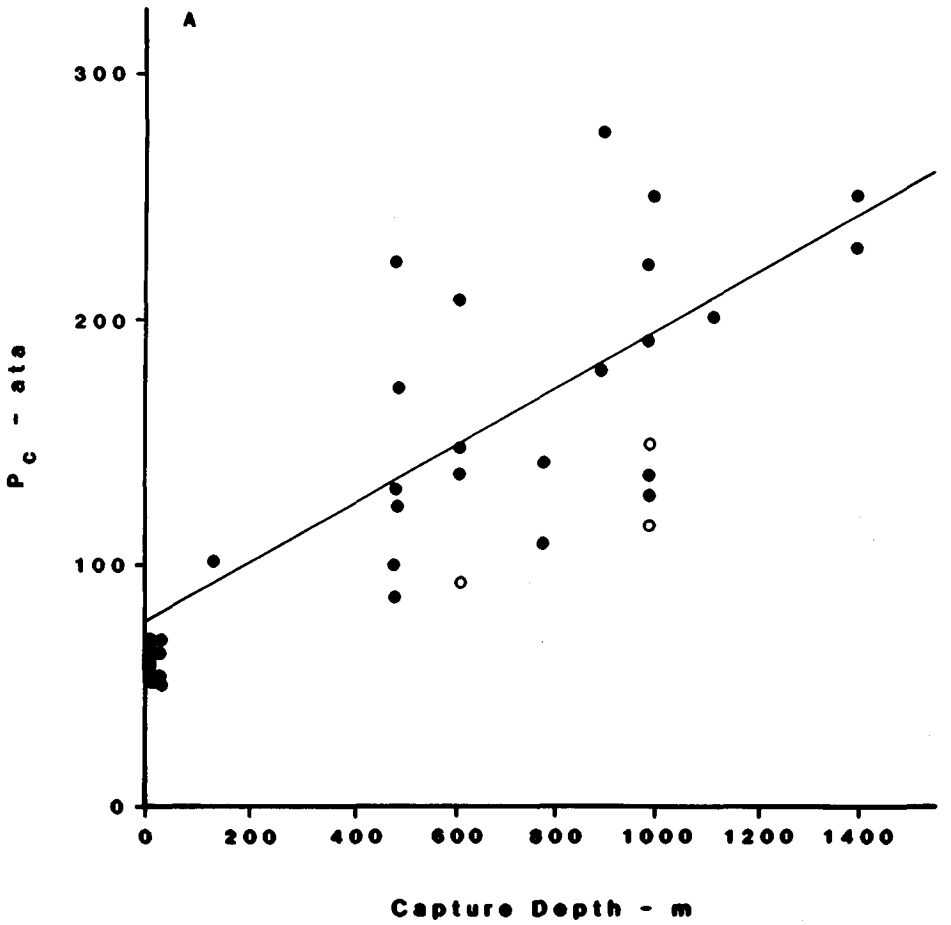



Fig. 1. Pressure tolerance of Baikalian cottoid fishes. Correlation diagram showing convulsion threshold pressure ($P_c$: *ordinate*) as a function of capture depth (*abscissa*). *Solid line*: at least-square regression of $P_c$ on capture depth. Cluster on and near *ordinate* represents shallow-water cottids. Remaining *solid points* represent *Abyssocottids*. *Open circles* indicate *Batrachocottus nikolskii*. (From Ref. 9 with permission of *Comparative Biochemistry and Physiology*)

in providing for the overall pressure tolerance that enables animals to survive in deep waters.

The same basic mechanisms are present as well in terrestrial animals and, in particular, in mammals. Thus, using susceptibility to one of the components of the HPNS as the index of pressure tolerance, investigators have shown that among mice there is a considerable degree of genetic control over this factor, accounting for at least 20 atm difference in convulsion-threshold pressures without any specific selection for pressure tolerance (10).

Acclimation of individual mice to high-pressure environments has also been demonstrated (Fig. 2) (Hinson and Brauer, unpublished data): Acclimation of

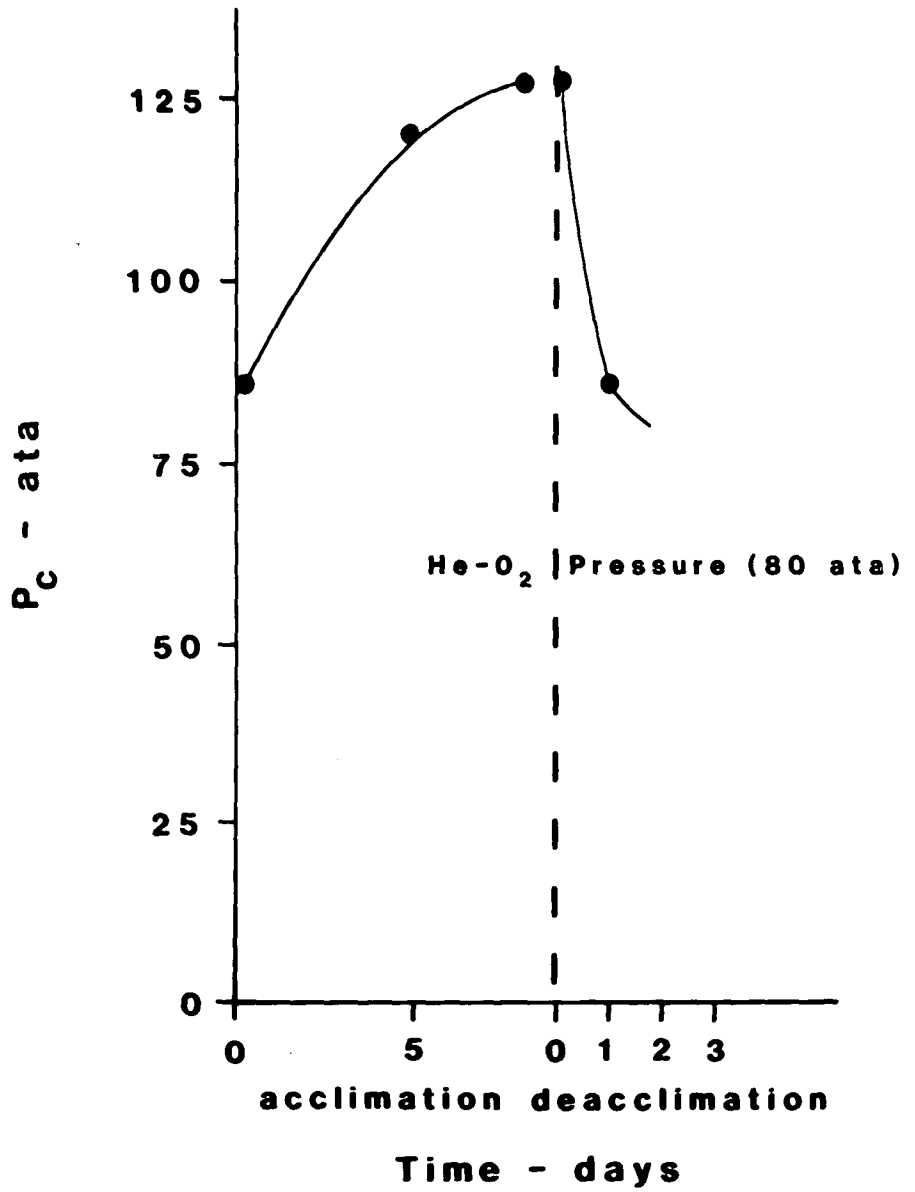



Fig. 2. Change in convulsion threshold pressure of CD-1 mice ($P_c$) as a result of exposure for up to 2 weeks to 80 ATA in He-$O_2$ (*left panel*). Subsequent duration of sojourn in air at 1 ATA (*right panel*). (From Hinson and Brauer, unpublished data)

CD-1 mice to 80 ATA resulted in an increase by 35 atm in convulsion-threshold pressure, a ratio of 0.44 atm/ATA, which is almost the same as the ratio 0.40 atm/ATA for the 35-atm increase in mean convulsion-threshold pressure shown by *Batrachocottus nikolskii* from 870 m mean habitat depth relative to the rest of the cottids from 0 to 75 m depth.

The time course of development of pressure acclimation in the mice would appear quite compatible with the assumption that it reflects substantial changes in tissue composition, for instance, the lipid changes accompanying temperature acclimation in poikilotherm vertebrates (*cf.* Ref. 11). On the other hand, deacclimation is nearly complete within 24 h, but not compatible with changes in tissue pH or ionic composition alone, which proceeds much more rapidly; it may reflect the participation in the overall acclimation processes of more volatile neurochemical events: for instance, changes in neurotransmitter release inculpated in accommodation changes (to be discussed presently), or changes in the distribution of ions between intercellular and extracellular spaces. Data currently available do not permit more specific hypotheses regarding these mechanisms.

Taken together, these data imply two time-dependent mechanisms modifying the effects of pressure on animals: a) multi-generation, genetically determined adaptation to pressure, and b) acclimation involving time spans of the order of weeks in an individual animal's life. The original identification of the effective stress factor thus needs to be amended to imply not pressure, as such, but pressure increase above the level to which a species is adapted, or to which an individual has become acclimated. Furthermore, the data suggest that although under appropriate selection conditions adaptation can raise the tolerated pressure by the actual value of the adaptation pressure, acclimation is less than half as effective in this respect. Furthermore, acclimation appears to be fully reversible with a brief time course; as yet no corresponding data for the effect of sojourn at lower pressures upon pressure tolerance of deep-water organisms are available.

More rapidly acting accommodations to pressure stress in the higher vertebrates are indicated by observations during acute diving procedures and in studies of the effects of varying compression rate. It is a common experience that many of the manifestations of HPNS in deep human dives and in animal experiments are most severe shortly after arrival on the bottom and tend to regress, if not disappear, with time spent at constant pressure (12).

Again, in many, but not all, vertebrates the critical pressure at which a given symptom is elicited during compression decreases with increasing compression rate (13). Of the several possible explanations for this behavior, the one we judge most likely is that the accommodation reflects the interaction of two sets of effects: rate-independent effects attributable to the hydrostatic pressure change, as such, and time-dependent reversal of, or compensation for, these pressure-induced effects. Lessening of symptom severity with time as well as rate dependence of the overall effects produced would be accounted for on this hypothesis by assuming that the compensatory reactions are relatively slow, and that they are triggered by the primary pressure-induced changes (14). It

has proved possible in mice to separate the effects due to compression rate, as such, from those due to total time spent at pressure by manipulation of the compression profile. For this purpose, we have used compression profiles involving an initial compression at 1000 atm/h to some intermediate pressure, followed by a sojourn at that pressure lasting from a few minutes to 24 h, and finally a second compression step, again at 1000 atm/h, to determine the resultant convulsion-threshold pressure (15). Such interrupted compression profiles yield convulsion-threshold pressures close to those for compression experiments in which the same critical pressure was attained after the same total time, but at a single, continuous compression rate, which, of necessity, is very much smaller than the 1000 atm/h used in the step procedure. Thus, to a first approximation, total duration of compression rather than the rate at which the critical pressure is actually approached just before the seizure appears to be the factor determining convulsion-threshold pressure.

More detailed analysis of such experiments showed that the hypothetical recovery process is nonlinear and that its initial rate is directly proportional to the pressure at which the animal finds itself (15) (Fig. 3). This finding led us to suggest that the optimal compression profile to reach the greatest depth in a minimum of time with freedom from serious HPNS symptoms should be one that proceeds as rapidly as possible to the greatest depth attainable without severe HPNS symptoms and then follows a compression profile adapted to the

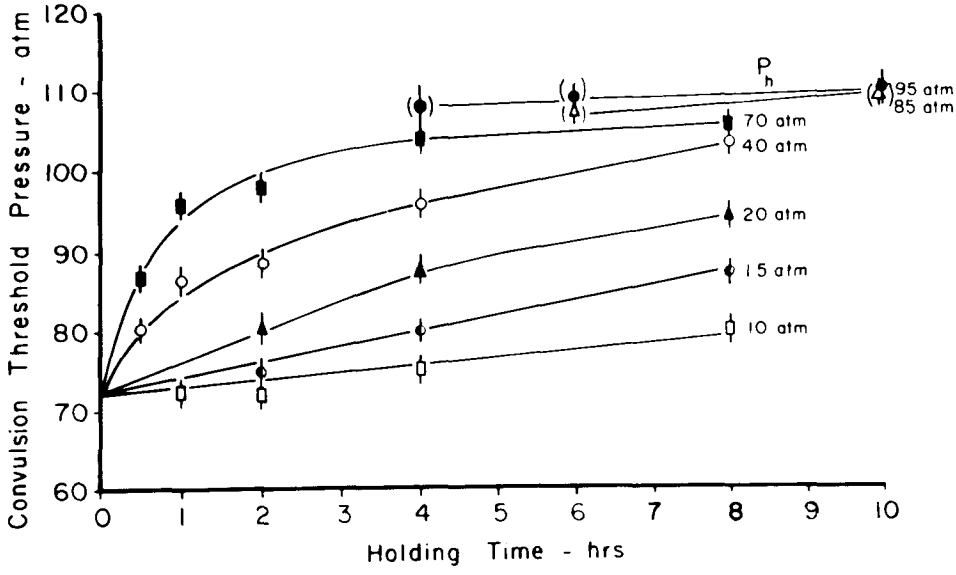



Fig. 3. Change in final convulsion threshold pressure of CD-1 mice in interrupted compression experiments as a function of duration of the interruptions (i.e., the *holding time*) and of the pressure at which the compression was interrupted (the *holding pressure*: $P_h$). Rate of initial and final compression steps: 1000 atm/h. (Data from Ref. 15)

recovery rate so that depth is increased at a rate determined by the instantaneous rate of the recovery reaction. This procedure yields a pseudo-exponential profile very similar to profiles that since that time have come to be widely adopted by those seeking to attain great depths in human dives.

The rates at which such recovery occurs for different HPNS symptoms may vary substantially; thus, compression-rate dependence varies widely for different components of the HPNS as illustrated by changes in the threshold pressures of two different components of the convulsion stage of the HPNS with changing compression (16) (Fig. 4). With regard to the nature of the hypothesized recovery processes, little is known at the present time. In at least one species, recovery processes can be blocked effectively by agents that interfere with storage or release of two or more of the three principal monoamine neurotransmitters (14).

## SCALING OF TIME PARAMETERS

To round out these considerations concerning the time parameter in the development of CNS effects of pressure displacements, one must consider ways

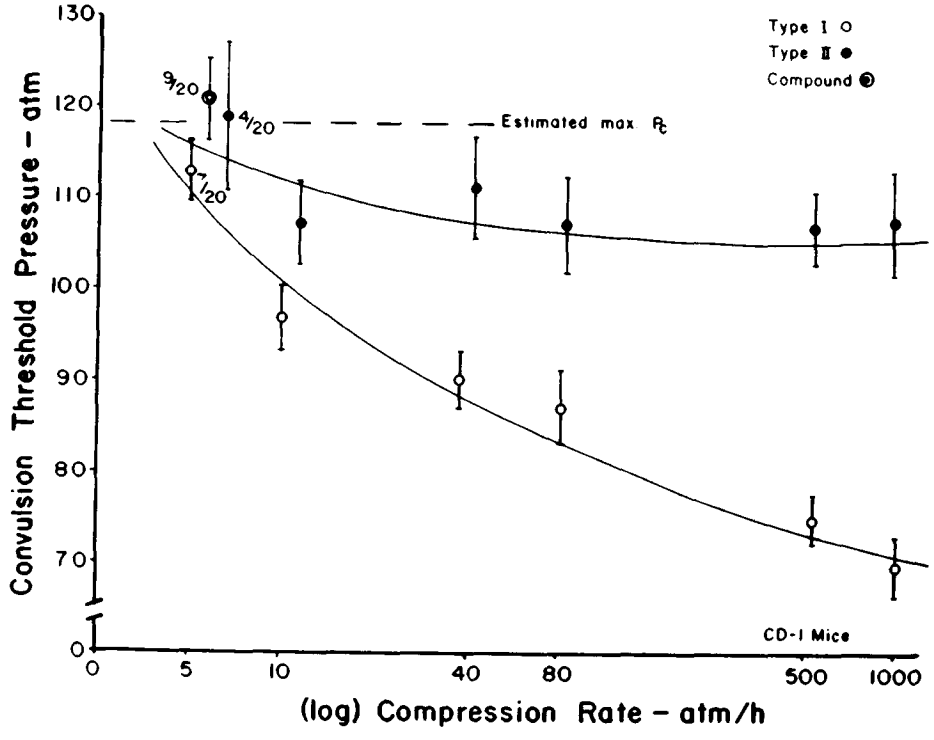



Fig. 4. Variation of convulsion threshold pressures as functions of the logarithm of the compression rate. ○ Type I HPNS seizures; ● Type II HPNS seizures. (Data from Ref. 16)

of translating the results of animal experiments to a time scale applicable to man.

One might consider the possibility of using the magnitude of the compression-rate effect as the basis for such scaling. Unfortunately, the compression-rate dependence of a typical HPNS effect varies approximately with the logarithm of the compression rate (15–17), so that its absolute magnitude cannot be used for scaling purposes; on the other hand, the coefficient by which the compression rate must be multiplied to establish numerical values for the compression-rate effects varies in an apparently random fashion from one species to the next (13), with some suggestion that it will prove far smaller for man and perhaps baboon than for mouse or squirrel monkey. This randomness might be expected in terms of the previous assumption, which assumes that the recovery rate determining the magnitude of the compression-rate effect is the result of complex neurochemical changes; to a high degree, these changes appear to be species-specific and thus probably would not lend themselves to scaling for our present purposes, in which attention is focused upon residual rather than acute effects of pressure exposure. A more satisfactory basis for such interspecies scaling might be sought either in relations governing metabolic rate and functions coupled to it, such as tissue perfusion or respiratory capacity (18), or in terms of the reciprocal of life span and functions like mitotic rate, which are coupled to it (19). Taking actual known values for the metabolic rate and the life spans of the species, one arrives at scaling factors for translation of data from the mouse to man of approximately 22 for both criteria—this calculation implies that a reasonable scaling factor for these two species would be in the neighborhood of 22. For the squirrel monkey, corresponding relations lead to a scaling factor in the neighborhood of 3.5. Thus, in comparisons of compression profiles, the data would imply that a compression rate of 60 atm/h—typical for many mouse experiments—would correspond to an average compression rate for man of 2.7 atm/h. Studies of compression-rate effects in the mouse have involved mean compression rates ranging from 1 to 1000 atm/h and would thus correspond to mean compression rates in human studies ranging from 0.05 to 45 atm/h, rates that bracket the range of compression rates pertinent to operational diving.

Some discussion has revolved around the effects of specific shapes of compression profiles. In general, the profiles proposed for manned deep dives conform to an approximately exponential relation between time required to reach a given pressure and that pressure itself. As pointed out above, such an exponential relation follows directly from the differential equations deduced from the animal experiments if one subjects them to an optimization procedure seeking to minimize total time required to attain a given depth. Considering the uncertainties in the human experimental material inevitably associated with the difficulties of conducting such experiments, and the evident reluctance of investigators to replicate such manned studies under conditions in which only a single parameter is modified at a time, it would appear that for the moment there is no basis for assuming that any qualitative difference in response should result from such manipulation of the compression profile. Therefore, for pres-

ent purposes simple multiplication of compression rates by the scaling factors deduced previously would appear to provide an appropriate and rational method of translating the results of animal experiments to a form applicable to human diving experience, regardless of the shape of the compression profile used.

## MECHANISMS POTENTIALLY CAPABLE OF CAUSING RESIDUAL CHANGE

We have sought to define the peculiar stress factors that need to be taken into consideration in relation to deep diving and have defined, as far as now possible, the temporal constraints that govern the manifestation of acute effects associated with these stresses. Returning to the problem of residual effects from such exposures, one should scan the list of mechanisms by which exposures to environmental stress might give rise to changes that endure beyond the end of the exposure period (*cf.* Ref. 1) and seek out those that might be activated in deep diving exposures. The resulting list includes:

1) Possible slowly reversible components of the changes giving rise to the primary acute effects, including changes in the excitable cells themselves and in the microenvironment surrounding these cells;

2) Actual cell injury associated with the primary stress in highly sensitive components overexposed because of reliance on other components of the heterogeneous substrate as indicators of stress;

3) Secondary effects of such pressure-induced changes in CNS function as convulsions or changes in thermal perception;

4) Slow reversal of compensatory mechanisms activated by the pressure exposure;

5) Changes in brain composition or neurotransmitter activity with pressure acclimation during prolonged exposures; and

6) Changes in tissues (other than the CNS) that reflect pressure effects on cell integrity or cell replication.

Analogous instances of all of these mechanisms have been shown to be involved in producing residual effects from various types of environmental stress (1). Up to this time one cannot exclude the possibility that any one of these mechanisms might actually be activated in deep-diving exposures. The analysis of the stress factors presented in the preceding pages suggests that the hazards are likely to be least in acute exposures to heliox, and because of the heterogeneity of the substrate discussed in Part I of this *Proceedings*, to increase substantially as steps are taken to attain greater pressures by modified compression procedures, as well as during more prolonged pressure exposures.

## EXPERIMENTAL EVIDENCE FOR RESIDUAL EFFECTS OF HIGH PRESSURE EFFECTS

Experimental evidence to test these inferences has been notably meager. Quantitative studies of changes in reflex function in men exposed to HPNS-eliciting pressures have shown that although these functions do not revert to

normal at once, they appear to have done so well before the completion of the inevitable long decompression period (20). Similarly, hemopoietic changes, especially in the platelet count, appear to revert to normal by the end of the decompression period (21). Behavioral changes associated with simulated deep dives have been too vague to permit any valid inference pro or con from the available test data. Animal experiments have shown significant reduction in male fecundity after a rather strenuous sequence of 10 compression and decompression exposures to 50 ATA in heliox (22); more recent data from the same laboratory suggest that the magnitude of this effect decreases with time after the last exposure and may become undetectable after about 4 weeks. Probably pertinent to the present discussion are the observations in baboons carried to maximal pressure by manipulation of compression schedule and compression-atmosphere composition, suggesting a new set of symptoms culminating in death of the animal (23). It is not clear whether these changes represent true cellular damage attributable to the high pressure environment, or a consequence of the effects of pressure upon other tissue components, notably the cardiovascular system and perhaps fluid compartmentation in the brain.

With regard to more extended pressure exposures, observations on crustaceans are interesting: when young crayfish were allowed to grow up in a high-pressure aquarium system at a pressure of 100 ATA, growth rate was reduced to approximately half the value observed in controls maintained in the same system and the same water—but at a pressure of 1 ATA. The effect involves delays in molting and distortion of the molting cycle suggestive of alterations of the endocrine balance controlling this particular process (Roer and Shelton, unpublished data, 1982).

Our own experiments designed to test the hypothesis advanced previously involved the technique of replicate exposure, using the same clinical endpoint as a determinant of the stress imposed and as a measure of residual injury. This technique was developed originally in conjunction with the study of residual effects of ionizing radiation exposure and was shown to be a powerful technique for developing pilot data (24). Two measures were employed: correlation of individual convulsion-threshold pressures in successive exposures, separated by a predetermined recovery interval, and comparison of mean convulsion-threshold pressures for the group in the same successive exposures. Both in mice (25) and in squirrel monkeys (26), these experiments showed high degrees of correlation of individual convulsion thresholds and no measureable displacement of mean convulsion thresholds—if compressions were carried out in heliox and recovery intervals were 2 weeks or more for CD-1 mice and four months for squirrel monkeys. By contrast, if the compressions were carried out in He-$N_2$-$O_2$ containing 12% $N_2$, correlation of individual convulsion-threshold pressures for mice in successive exposures became negligible and the mean convulsion-threshold pressure for the group on second exposure was raised significantly to a value 8% above that for the first exposure. Lengthening the recovery period to 4 weeks resulted in disappearance of this effect (Table III). The effect does not seem to result from a change in the effectiveness of $N_2$ as an HPNS antagonist: If the exposure sequence He-He/$N_2$-He was used, comparison of the results of first and second heliox exposures showed the charac-

TABLE III

Correlation of Individual Convulsion Threshold Pressure ($^{I}P_c$) in Successive Compression Experiments with CD-1 Mice

| % Nitrogen Content in Compression Mixture | Comp. Rate atm/h | Recovery Interval/days | $^{I}P_c$ – ATA 1st Exp/2nd | | $\Delta P_c$ atm | $P$ | $r$ 1st/2nd | $P$ |
|---|---|---|---|---|---|---|---|---|
| 0 | 60 | 14 | 90 | 92 | 2 | 0.2 | 0.79 | 0.001 |
| 12 | 60 | 14 | 121 | 129 | 8 | 0.05 | 0.18 | 0.5 |
| 12 | 60 | 28 | 122 | 122 | 0 | 0.5 | 0.11 | 0.5 |

teristic loss of correlation and upward displacement of convulsion threshold; control experiments showed that these effects were not observed if the exposure sequence was He-He-He and comparison made of first and third heliox exposure results.

In the squirrel monkey, loss of correlation of individual convulsion-threshold pressures and increase in mean convulsion-threshold pressure were observed when two exposures to HPNS seizures—one in He-$N_2$-$O_2$ containing 12% $N_2$ and one in He-$O_2$ at $PO_2$ = 2 ATA—were interposed between two normoxic heliox exposures. This result was not expected originally and at present the design of the experiment does not permit us to specify the stress factor involved; the necessary control experiments to analyze this situation are still in progress.

Thus, the data available fail to demonstrate any significant residual injury following one or a few high-pressure exposures to heliox. They do indicate a significant increase in the amount of detectable residual change when exposures are carried to the point of convulsions at the higher pressures made possible by the presence of $N_2$ in the compression atmosphere; to this extent they are compatible with the results of the analysis presented previously. Finally, the data indicate that whatever residual effect is detectable by this means is dissipated within about 4 weeks.

## SUMMARY AND CONCLUSIONS

Briefly to recapitulate, we have attempted to summarize evidence to define the effective stress factors unique to the deep-diving environment. We have also reviewed the time relations affecting the appearance of specific manifestations of high-pressure effects on the CNS; we have shown that these can be conveniently subdivided into three levels: the multi-generation adaptation level, the multi-day acclimation level, and the short-term accommodation level underlying compression-rate effects. We have suggested an approach to the problem of scaling to allow transposition of data from animal experiments to the prediction of results of human exposures. We have concluded that the most likely scaling procedure indicates that the time coordinates used in many ex-

periments with small rodents should be quite comparable to those encountered in current diving practice. Indeed, application of these factors to repetitive-diving experiments suggests that some degree of residual change from previous pressure exposures may endure for periods of time that are significantly longer than the recovery intervals usually interposed between deep dives in commercial practice. Finally, we turned to the question of mechanisms that might give rise to residual effects of such pressure exposures and have concluded that presently available data do not permit one to exclude the probability that at least four different sources of residual injury could be elicited by high pressure exposures. In particular, there is reason to suspect that the likelihood of development of significant residual change increases rapidly as the total pressures attained in deep diving are increased by use of modified compression atmospheres and extended compression profiles. On theoretical grounds, we would furthermore surmise that certain types of residual injury, notably those involving pressure-induced changes in cell population dynamics, might become more prominent as duration of pressure exposure is lengthened. Finally, we have reviewed the currently available evidence bearing on this subject and have concluded that at the present time this evidence is compatible with, but not probative of, the occurrence of residual injury under the conditions deduced from the theoretical analysis. We believe that the available evidence, together with the theoretical considerations advanced, point to a need to undertake systematic investigations that are aimed at detection and identification of residual effects of high-pressure exposures which utilize exposure conditions specifically designed to elicit such effects and endpoints that are more sensitive, and perhaps more selective, than those hitherto employed. Examples of such approaches include the use of appropriate behavioral measures applied to primates and the study of effects of repetitive or prolonged exposures to high pressure upon growth and development of immature animals.

## Acknowledgment

The study reported here has been jointly funded by the Office of Naval Research and the Naval Medical Research and Development Command through the Office of Naval Research Contracts N00014-75-C-0468 and N00014-75-6-0727.

## References

1. Brauer R. Irreversible changes of environmental stress. In: Edholm OG, Bacharach AL, eds. The physiology of human survival. New York and London: Academic Press, 1964:491–521.
2. Petersen RE, Wright WB. Pulmonary mechanical functions in man breathing dense gas mixtures at high ambient pressures–predictive studies III. In: Lambertsen CJ, ed. Underwater physiology V. Proceedings of the fifth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1976:67–78.
3. Halsey MJ, Daniels S, eds. Techniques for diving deeper than 1500 ft. Bethesda: Undersea Medical Society, Inc., 1980:68.

4. Brauer RW, Hogan PM, Hugon M, Macdonald AG, Miller KW. Patterns of interaction of effects of light metabolically inert gases with those of hydrostatic pressure as such—a review. Undersea Biomed Res 1982:9:353–396.
5. Smith RA, Miller KW. Amelioration of the high pressure neurological syndrome (HPNS) by anesthetic gases. Undersea Biomed Res 1978:5(Suppl):48.
6. Brauer RW. Mansfield WM Jr, Beaver RW, Gillen HW. The HPNS as a composite entity—consequences of an analysis of the convulsion stage. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology, Undersea Medical Society, Inc., 1981:391–400.
7. Brauer RW, Beaver RW, Hogue CD III, Ford B, Goldman SM, Venters RT. Intra- and inter-species variability of vertebrate high pressure neurological syndrome. J Appl Physiol 1974:37:844–851.
8. Sidelyova VG. Seismosensornaya sistema i ekologiya Baikalskykh podkamenschikoyvkh ryb (*Cottidae*). (Izd. Nauka—Sibirskoye otdeleniye, Novosibirsk, 1982).
9. Brauer RW, Sidelyova VG, Dail MB, Galazii GI, Roer RD. Physiological adaptation of cottoid fishes of Lake Baikal to abyssal depths—the role of hydrostatic pressure. Comp Biochem Physiol 1984;77A:669–705.
10. McCall RD, Frierson D Jr. Evidence that two loci predominantly determine differences in susceptibility to Type I HPNS seizures in mice. Genetics 1981;99:285–307.
11. Rahmann H. Brain gangliosides are involved in thermoregulation and adaptation of vertebrates. In: Lomax P, Schöbaum E, eds. Environment, drugs, and thermoregulation, Basel: S Karger, 1983:19–22.
12. Bennett PB, Coggin R, Roby J, Miller JN. Prevention of HPNS in man by rapid compression with trimix to 2132 ft (650 m). In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:345–355.
13. Brauer RW, Beaver RW, Lahser S, Venters R. Comparative physiology of the high-pressure neurological syndrome—compression rate effects. J Appl Physiol 1979;46:128–135.
14. Brauer RW, Beaver RW, Sheehan ME. The role of monoamine neurotransmitters in the compression rate dependence of HPNS convulsions. In: Shilling CW, Beckett MW, eds. Underwater physiology VI. Proceedings of the sixth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1979:49–59.
15. Brauer RW, Beaver RW, Lahser S, Mansfield WM, Sheehan ME. Time, rate, and temperature factors in the onset of high pressure convulsions. J Appl Physiol: Respir Environ Exercise Physiol 1977;43:173–182.
16. Brauer RW, Mansfield WM, Beaver RW, Gillen HW. Stages in the development of the HPNS in the mouse. J Appl Physiol: Respir Environ Exercise Physiol 1979:46:756–765.
17. Brauer RW, Beaver RW, Mansfield WM, O'Connor F, White LW III. Rate factors in the development of the high pressure neurological syndrome. J Appl Physiol 1975:38:220–227.
18. Stahl WR. Scaling of respiratory variables in mammals. J Appl Physiol 1967:22:453–460.
19. Sacher GA, Jones ML. Life span: Mammalia. In: Altman PL, Dittmer DS, eds. Biological data book, vol 1, Bethesda: Federation of American Societies for Experimental Biology, 1972:229–230.
20. Harris DJ, Bennett PB. Effect of trimix on reflex excitability in man at pressures up to 66 bar (Atlantis II). Undersea Biomed Res 1981;8(Suppl):16.
21. Anderson JC, Bennett PB. Hematologic change caused by deep trimix diving: the Atlantis II experience. Undersea Biomed Res 1981:8(Suppl):43.
22. Baden JM, Doré CJ, Green CJ, Monk S, Halsey MJ, Wardley-Smith B. Reduction of fertility of male mice after exposure to high environmental pressure. J Appl Physiol: Respir Environ Exercise Physiol 1982;53:1207–1212.
23. Rostain JC. Neurophysiological problems with nitrogen at pressure. Proc XXVIII Internat Cong Physiol Sci, Symposia and Lecture, Akademiai Kiado, Budapest 1981;18:189–196.
24. Krebs JS, Brauer RW, Kalbach H. The estimation of the non-recuperable injury caused by ionizing radiation. Radiat Res 1959:10:80–88.
25. Brauer RW, Beaver RW, Gillen HW. Physiological basis of variability in response to environmental stress—correlation of individual susceptibilities to different components of the high pressure neurological syndrome. J Appl Physiol: Respir Environ Exercise Physiol 1981:50:272–278.
26. Brauer RW, Way RO, Jordan MR, Parrish DE, Beaver RW, Goldman SM. Neurologic effects of repeated exposures to high-pressure heliox atmospheres. Undersea Biomed Res 1974:1:239–250.

# EVOLUTION OF HPNS IN 16 DIVERS BREATHING He-$N_2$-$O_2$ DURING LONG STAYS AT 45 BARS

*J. C. Rostain, C. Lemaire, M. C. Gardette-Chauffour, and R. Naquet*

Study of the neurophysiological effects of breathing He-$N_2$-$O_2$ under high pressure on the baboon *Papio papio* has allowed the development of a method of compression in this animal (1,2). This method allows us to use the beneficial effects of nitrogen on some clinical symptoms of the high pressure neurological syndrome (HPNS) reported by several authors (3–7) and to avoid the enhancement of synergistic effects of nitrogen with the hyperbaric environment on other HPNS symptoms that we have reported in man and baboon (2,6–9).

The extrapolation of this method has been extended to man and has been used during 3 dives to 450 m (10–12). The compression effects were limited by this method and in these conditions we have been able to follow the effects of long stays at a pressure of 45 bars in the He-$N_2$-$O_2$ breathing mixture in 16 subjects.

## METHODS

Three experimental series were carried out to 450 m (*DRET 79/131*, ENTEX V and VIII) with the following protocol:

1) Three- to 4-day predive in the hyperbaric facilities (with a slight increase in pressure (0.8 to 1 bar)) immediately before the dive.

2) Compression in 38 h to 450 m with (Fig. 1):

a) compression rates decreasing every 100 m following an exponential function of the depth;

b) 150-min stages, each 100 m;

c) nitrogen injections before each stage so as to reach 4.8% (2.2 bars) at 450 m;

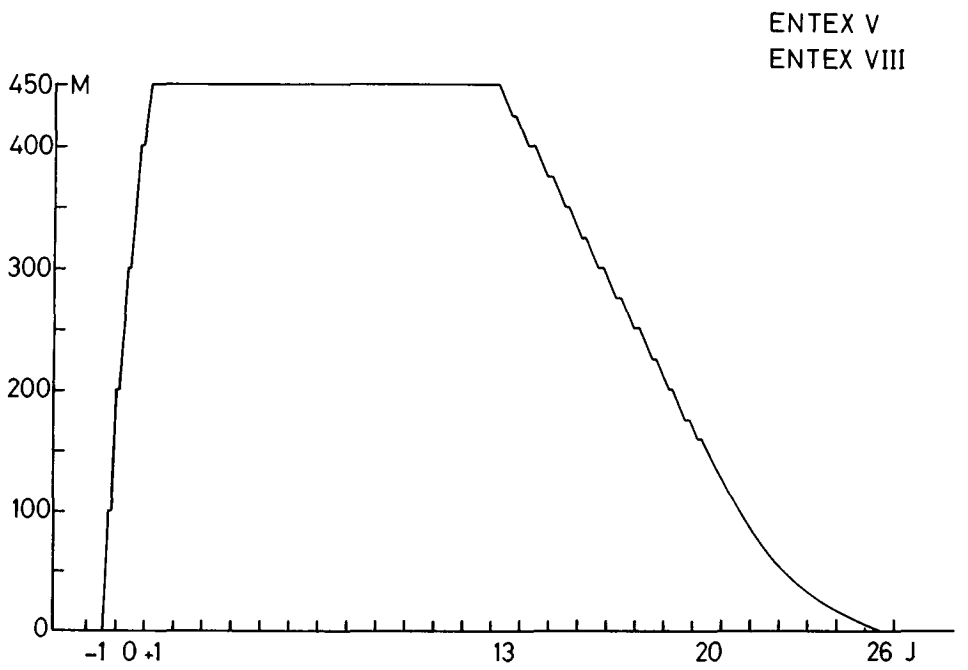



Fig. 1. Profile of ENTEX dives.

d) an oxygen partial pressure of 0.4 bar, a temperature of 31°C, and 50 to 70% relative humidity.

3) A 50-h stay (*DRET 79/131*) or 12-day stays (ENTEX V and VIII) at 450 m with a He-$N_2$-$O_2$ breathing mixture ($N_2$ = 4.8%; $PP_{O_2}$ = 0.4 bar; T°C = 32° ± 1°C; $H_2O$ = 50–70%).

Sixteen divers were compressed in these 3 dives. The subjects were professional divers (COMEX commercial divers or French Navy divers).

During these dives, the subjects were fitted with EEG electrodes mounted in the scalp and fixed for the duration of the dive ("hook"-type electrode for the *DRET 79/131* dive and platinum wire for the ENTEX dives). The electrodes were placed in fronto-polar, central, mid-temporal, and occipital regions of the right hemisphere. For the 180-m test dives the electrodes were of "anestho" type.

The tremor was measured by accelerometry. The data have been recorded, interpreted, and then analyzed by computer according to the methods described in preceding works (7,13). During all the dives, the recordings were carried out several times a day at the same hours. The psychometric tests were performed during predive and at 450 m, on the 1st, 2nd, 11th, and 12th days

of the stays. The tests consisted of two sensori-motor tests and two intellectual tests:

1) Manual dexterity (MD), which consisted of putting 50 pegs in holes, first with the right hand, then with the left hand.

2) Visual-choice reaction time (VCRT), which was studied by a series of 31 red or green light stimulations.

3) Number ordination (NO), which consisted of ordering series of 7 numbers listed out of order.

4) The second intellectual test, which consisted of symbol crossing (SC) or letter recognition (LR).

For the *DRET 79/131* dive, 8 subjects were selected in relation to the increase of the power of the theta frequency bands in EEG activities after a rapid compression to 180 m (14,15): 3 from *Group 0* (less then 10% increase); 2 from *Group 1* (10 to 100% increase); 3 from *Group 2* (more than 100% increase). The ENTEX V dive was performed by four *Group 1* subjects and, finally, the ENTEX VIII dive was performed by 4 subjects, one subject from *Group 0* and 3 subjects from *Group 1*.

## RESULTS

### Clinical Symptoms

During the compression, there was no dysmetria, fasciculation, or myoclonia, and the tremor was seen only in two subjects. These subjects showed a 50–100% increase at the end of the compression and during the first hours of the stay. This tremor was lessening 24 h later. In most subjects during the long stays, tremor had a tendency to appear (or to increase again) with an intensity that followed a 24-h cycle but was never more than a 100–150% increase. The average of the 8 subjects who stayed 12 days at 450 m showed an oscillatory evolution of their tremor. The tremor was maximum around the 4th day, minimum around the 9th day, and increased again until the 11th day (*see* Fig. 2).

### EEG Modifications

The intensity of EEG modifications was dependent on individual susceptibility: a 500 to 1000% increase in the 3 subjects of *Group 2* and less than a 300% increase in the subjects of the other two groups. It is important to note that the increase of theta activities in the frontal region of the scalp did not appear systematically during the compression and that the evolution during the stay at 450 m was different depending on the subjects. We recorded:

1) An increase of EEG modifications at the end of the compression and during the first hours of the stay, and a decrease 24 h later (10 subjects). Subsequently, 3 of these subjects who stayed more than 50 h at 450 m showed an

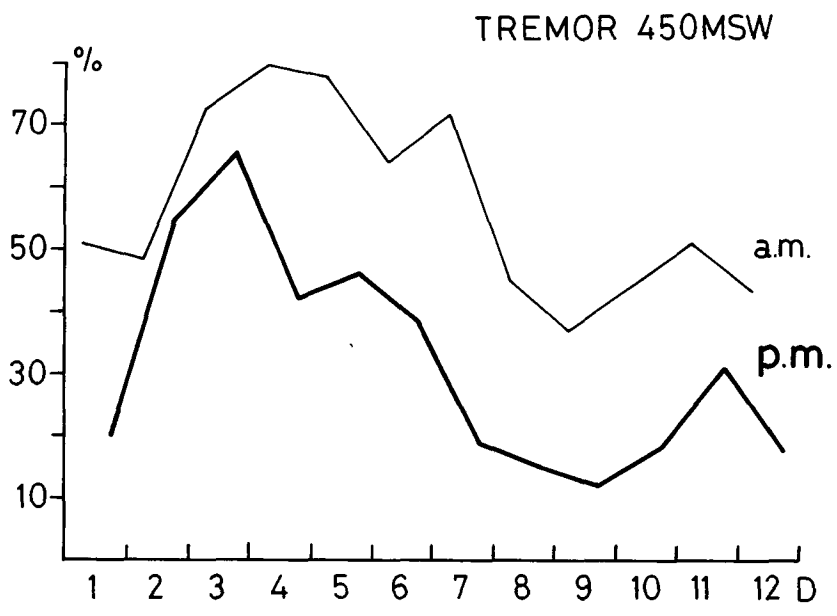



Fig. 2. Mean values of tremor for the 8 subjects of ENTEX dives during the 12-day stays at 450 msw. Means are calculated for the morning recording (a.m.) and the evening recording (p.m.). They are expressed as percentage difference from control values.

oscillatory change with a periodicity of 4 to 5 days, which included a 24-h cycle.

2) A progressive increase in EEG modifications during the stay of 12 days (1 subject).

3) A tendency to an increase in theta activities at the end of the 12-day stay (1 subject).

4) A progressive decrease in the power of the theta frequency band during the 12-day stay (2 subjects).

5) A stabilization of the increase in theta activities at the same level during the 12-day stay (1 subject). In all cases, the maximum increases recorded during the stays were never more than 400%.

A mean evolution of the power of the theta band during the stays at 45 bars for the 8 subjects is shown in Fig. 3. After the increase at arrival, the power decreased progressively to reach a minimum value between the 4th and the 5th day and increased again to reach a maximum the 11th day.

## Psychometric Performance

The psychometric tests always showed a decrease in performance on arrival at 450 m, but the variability among subjects was great according to their

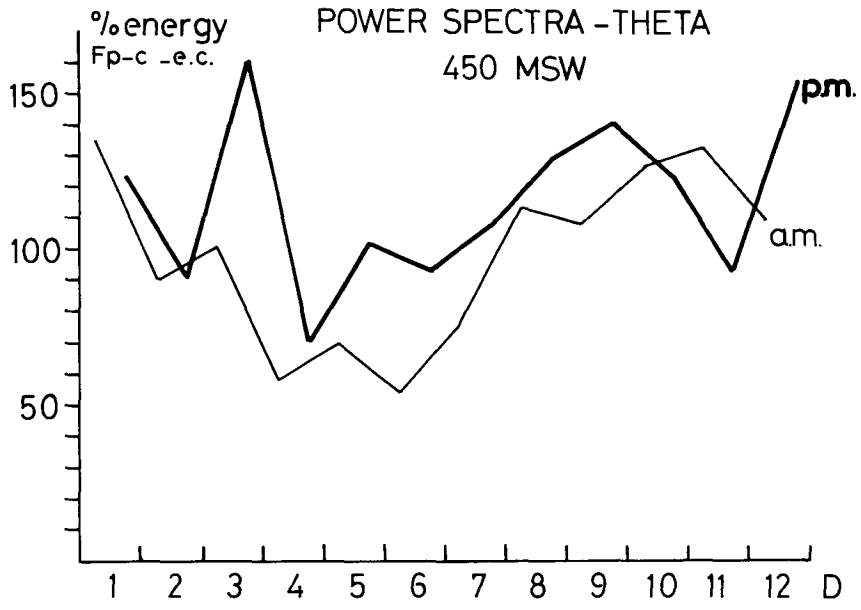



Fig. 3. Mean values of power of EEG theta band calculated for the 8 subjects of ENTEX dives, during the stays at 450 msw. Means are calculated for the morning (a.m.) and the evening recording (p.m.). They are expressed as percentage difference from control values.

individual susceptibility. For example, the impairment varied from −1 to −20% in manual dexterity and from −4 to −30% in number ordination. One day later, the performances were better for all the subjects and the decrease on the first day probably was a result of compression effects. Ten days later, the recovery was complete for the vigilance (VCRT) and intellectual tests (NO and SCII or LR). Only manual dexterity was affected throughout the stay (−4%) (Fig. 4). The means of these changes are represented in Table I.

## COMMENTS

The stays of long duration at 45 bars show a large variability in the changes at the individual level as well as at the symptom level. Some of these symptoms improved (vigilance and intellectual test); others appeared or persisted (tremor and manual dexterity); others, again, had different evolution depending upon individual subjects (EEG changes). Moreover, it is possible for one to visualize a cyclic evolution of tremor and EEG modifications during the long stays at 450 m; such cyclic evolution is not seen with psychometric tests. These facts confirm, once again, the heterogeneity of HPNS, the diversity of the mechanisms involved, and the different thresholds of sensitivity according to the nervous structures, which are themselves different depending upon the subjects.

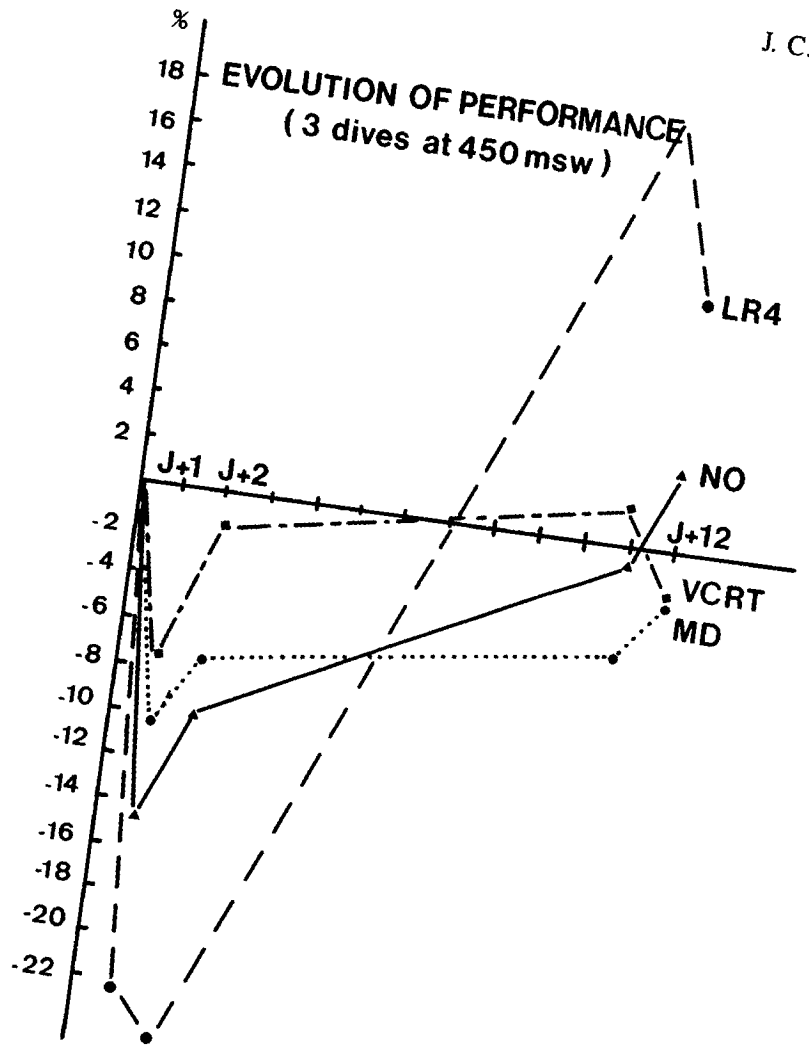



Fig. 4. Evolution of performance during the three dives at 450 msw. At arrival on the bottom ($J+1$), the decrease in performance is found for the four tests. One day later, the vigilance measured by VCRT comes back to the normal values. The performances on the other tests improve during the stay except for MD, which keeps values below control values.

TABLE I

| Manual Dexterity | | | Visual Choice Reaction Time | | | Number Ordination | | |
|---|---|---|---|---|---|---|---|---|
| D1 | D2 | D11 + 12 | D1 | D2 | D11 + 12 | D1 | D2 | D11 + 12 |
| −10% | −8% | −4% | −7% | −2% | 0% | −15% | −10% | +1% |

These different evolutions during the stays of long duration at constant pressure, also reported before for stays of long duration in He-$O_2$ breathing mixture (16), raise the problem of acclimatization to life at high pressure.

One must also note that the changes recorded during these three experimental series are in general not very important, and these results must be put in context with the method of compression developed in the monkey and extended to man, which allows us to attenuate the HPNS and to avoid most of the clinical symptoms. Nevertheless, the method used does not avoid completely the effects of compression because it provokes the appearance of EEG changes in at least 10 subjects and a decrease in performance in all the subjects. The partial recovery, 24 h later, is proof of that.

Even though these experiments raise several questions, we can say that dives performed with this method of compression and with stays of long duration at 45 bars can be carried out operationally with selected professional divers.

## Acknowledgment

We acknowledge grants from DRET and technical support from GISMER and COMEX.

## References

1. Rostain JC, Imbert JP, Gardette B, Lemaire C, Dumas JC, Naquet R. Compression methods: II. Study of the effects of profiles and $N_2$ injections on HPNS of the baboon *Papio papio*. Undersea Biomed Res 1978;5(Suppl):46.
2. Rostain JC, Gardette B, Gardette MC, Dumas JC. HPNS of baboon *Papio papio* during slow exponential compression with $N_2$ injection to 1000 msw and beyond. Undersea Biomed Res 1979;6(suppl):45.
3. Miller KW. Inert gas narcosis and animals under high pressure. In: Sleigh MA, MacDonald AG, eds. The effects of pressure on organisms. Symposia of the Society for Experimental Biology. Cambridge: Cambridge University Press, 1972:363–378.
4. Bennett PB, Blenkarn GD, Roby J, Youngblood D. Suppression of the high pressure nervous syndrome in human deep dives by He-$N_2$-$O_2$. Undersea Biomed Res 1974;1:221–237.
5. Brauer RW, Goldman SM, Beaver RW, Sheehan ME. $N_2$, $H_2$, and $N_2O$ antagonism of high pressure neurological syndrome in mice. Undersea Biomed Res 1974;1:59–72.
6. Rostain JC, Naquet R, Fructus X. Study of the effects of "trimix" and "heliox" mixtures during rapid compression. Undersea Biomed Res 1976;3:A13–A14.
7. Rostain JC, Gardette-Chauffour MC, Naquet R. HPNS during rapid compression of men breathing He-$O_2$ and He-$N_2$-$O_2$ at 300 m and 180 m. Undersea Biomed Res 1980;7:77–94.
8. Naquet R, Rostain JC, Fructus X. High pressure nervous syndrome: clinical and electro physiological studies in man. In: The strategy for future diving to depths greater than 1000 feet. UMS report number WS:6-15-75. 1975:62–65.
9. Naquet R, Rostain JC. A new appraisal of high pressure nervous syndrome. Arch Ital Biol 1982;120:201–211.
10. Rostain JC, Gardette B, Gardette-Chauffour MC, Naquet R. HPNS in human during 38 hours compression to 450 m with $N_2$ injections. In: Program, abstracts, and mini-papers, 7th symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1980:16.
11. Rostain JC, Lemaire C, Naquet R. HPNS in man during a 12 day stay at 450 m in He-$N_2$-$O_2$ breathing mixture. Undersea Biomed Res 1982;9(Suppl):22–23.

12. Gardette B, Rostain JC. Compression technique in deep sea dives, for man and animals in He-$N_2$-$O_2$ mixture. Undersea Biomed Res 1981;8(Suppl):15–16.
13. Rostain JC, Naquet R. Le syndrome nerveux des hautes pressions: caractéristiques et évolution en fonction de divers modes de compression. Rev Electroencephalogr Neurophysiol 1974;4:107–124.
14. Rostain JC, Lemaire C, Gardette-Chauffour MC, Doucet J, Naquet R. Criteria analysis of selection for deep diving: EEG and performance. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:435–443.
15. Rostain JC, Lemaire C, Gardette-Chauffour MC, Doucet J, Naquet R. Estimation of human susceptibility to the high pressure nervous syndrome. J Appl Physiol: Respir Environ Exercise Physiol 1983;54:1063–1070.
16. Rostain JC. Le syndrome nerveux des hautes pressions chez l'homme et le singe *Papio papio*. These de doctorat es Sciences Naturelles. Université d'Aix-Marseille I. 1980;1:195;2:130.

# NEREIDE I: AIR SATURATION DIVE TO 15 METRES (49 FT) WITH EXCURSIONS TO 42 (138 FT), 60 (199 FT), AND 75 METRES (246 FT)

*B. Gardette, F. Martin-Chave, Ph. Cavenel, and X. Fructus*

Compared to the number of deep dives using synthetic gas mixtures, air saturation diving at shallow depths has been used relatively little. In France the first operation of the *house-under-the-sea* type, PRECONTINENT I, goes back to 1962. This was followed by PRECONTINENT II in 1963 and PRECONTINENT III in 1965.

A dry-air saturation dive was carried out in October 1980 aboard the French Navy's ISM TRITON at a storage depth of 15 m (49 ft) and 20-min incursions to 50 m (164 ft). And for the past 10 years COMEX's scientific division has been working on air-diving tables. It was this team that developed the professional air-diving tables brought into force by the French governmental decree of July 11, 1974.

The French technique of monitoring decompression of divers by ultrasonic detection of circulating bubbles was extensively tested and developed by CERTSM (Centre d'Etudes et de Recherches Techniques Sous-Marines, Toulon, France).

Used by COMEX since 1975, this technique has made it possible to compare different air-diving tables with the actual physical reactions of divers being decompressed from air or nitrox saturation. Some 30 operations, for the most part American, have been carried out with excursions of up to 60 or 75 m, and even 91 m (300 ft) in hyperbaric chambers (SCORE, *Phase I*). But none of the decompressions were monitored by Doppler bubble detectors. As for narcosis, the effects have been evaluated only by diver self-observation during excursions.

The purpose of our air saturation dive, NEREIDE I, was to perfect air excursion dives from a saturation depth of 2.5 ATA (15 metres of sea water

[msw], or 49.2 feet of sea water [fsw]) with a tolerable degree of narcosis and safe decompression procedures. This dive took place at COMEX from April 19 to 30, 1982.

## METHODS

NEREIDE I constituted a rational, ergonomic approach to the problem, in which two main lines of research were developed.

### Evaluation of Narcosis

1) *Psychometrics (OCTARES)*
   Manual dexterity; visual-choice reaction time; figure crossing; estimating time (at 15-m storage depth and during excursions to 42, 60, and 75 m).
2) *Electroencephalography (GIS)*
   EEG; evoked potential at storage depth and during excursions.
3) *Stability Measurement (GIS)*
   Measurements at storage depth and during excursions.
4) *Ergonomics (COMEX, Cantini Hospital)*
   Cardiac monitoring (Holter) of muscular effort during excursions on a cyclorower in the water.

### Decompression Research

1) *Doppler Monitoring of Bubbles Circulating in Bloodstream (CERTSM/COMEX)*
   During both intermediate and final decompressions.
2) *Blood Biology (CERB)*
3) *Monitoring of Overall Health and Behavior—Fatigue Study*
   By COMEX and GISMER medical services, throughout the entire operation.
4) *Lung and Bone Monitoring (CERB)*
   Ventilation tests; lung diffusing capacity; scintiscanning of lungs and bones before and after.

### Protocol (Fig. 1)

*Saturation Dive*

| | |
|---|---|
| Saturation depth | 15 m (49 ft) |
| Breathing mixture | Air |
| $PI_{O_2}$ | 0.5 bar (7.25 psi) |

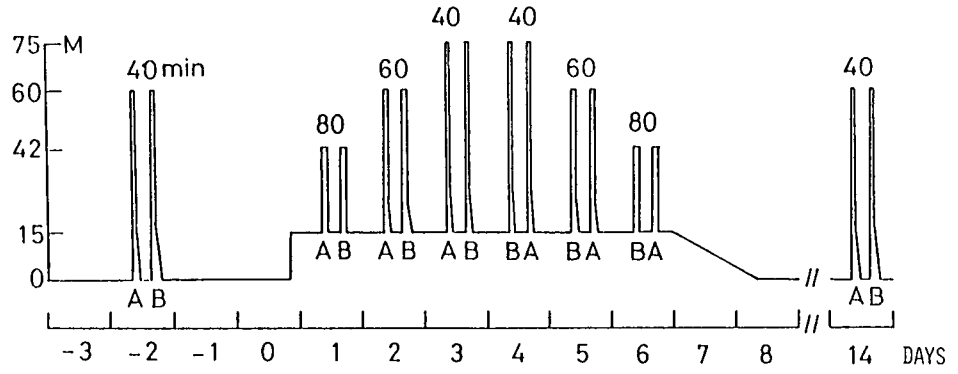



Fig. 1. Profile of dives carried out in the course of NEREIDE I (April 1982). The two control bounce dives using air were made before and after saturation at 15 m (40-min dives from surface to 60 m depth). Twelve excursion dives were carried out from the 15-m storage depth, to 42 m (80 min); 60 m (60 min); and 75 m (40 min). The 8 divers were divided into two 4-man teams, A and B.

*Air Excursion Dives*

Two per day, one per team

Depth/time: 42 m (138 ft)/80 min
60 m (199 ft)/60 min
75 m (246 ft)/40 min

Breathing mixture Air

Final decompression breathing mixture: Nitrox (23–24% $O_2$)

Duration of experiment: 12 days (*J*) as follows:

*J3*, *J2*, *J1*, and *J0*: surface references

*J1* to *J6*: saturation and excursions

*J7*: final decompression

*J8*: return to surface references

During each excursion, in the order shown in Table I, there were:

two divers in the dry (temperature 24° C or 75.2° F)

two divers in the water (temperature 24° C or 75.2° F)

Final decompression to begin at least 8 h after the divers returned to storage depth from the last excursion. *See* Table II for Excursion Decompression Tables.

## RESULTS AND COMMENTS

We shall limit ourselves to commenting on the results obtained by each of the research groups.

TABLE I

Order of Divers in Daily Excursions

| Daily Excursions (J:day) | Divers A.M. Dry | A.M. Wet | P.M. Dry | P.M. Wet |
|---|---|---|---|---|
| *J1* 42 m / 80 min | A1 A2 | A3 A4 | B1 B2 | B3 B4 |
| *J2* 60 m / 60 min | A3 A4 | A1 A2 | B3 B4 | B1 B2 |
| *J3* 75 m / 40 min | A1 A2 | A3 A4 | B1 B2 | B3 B4 |
| *J4* 75 m / 40 min | B3 B4 | B1 B2 | A3 A4 | A1 A2 |
| *J5* 60 m / 60 min | B1 B2 | B3 B4 | A1 A2 | A3 A4 |
| *J6* 42 m / 80 min | B3 B4 | B1 B2 | A3 A4 | A1 A2 |

*n*: 8 divers; 4 men each team; teams designated as A and B. *Also see* Protocol in text.

## Evaluation of Narcosis

*Psychometrics—OCTARES: C. Lemaire*

Interpretation of these tests is rendered difficult because of individual variations and the limited sample involved. We are, however, able to assert that deterioration is proportional to the bottom depth: it was moderate at 42 and 60 m and markedly greater at 75 m depth. Response time is slower at the end of the bottom time than at the beginning. Because there was less deterioration for the second excursion to 60 m and a better performance level, we suggest that it is possible to develop a certain tolerance to narcosis. It should also be noted that the diver achieves a better score at a given depth if he has already been exposed (the day before, for instance) to a greater depth. Repeated exposure to high partial pressures of $N_2$ and accomplishing the same task both contribute to improvement of performance. Repeated exposure may be obtained by departure either from the surface or from a shallow saturation depth.

*Electroencephalography—GIS: J. C. Rostain*

The EEG and visual-evoked-potential (VEP) study did not produce significant differences that could demonstrate a state of narcosis.

TABLE II

Decompression

| EXCURSION DECOMPRESSION TABLES | Time in Minutes |
|---|---|
| 42 metres (m) (138 ft) | |
| Bottom time | 80 |
| Ascent to first stop | |
| Stop: 80 m (59 ft) | |
| TOTAL ASCENT TIME | 3 |
| 60 metres (199 ft) | |
| Bottom time | 60 |
| Ascent to first stop | 4 |
| Stop: 24 m (79 ft) | 7 |
| 21 m (69 ft) | 10 |
| 18 m (59 ft) | 20 |
| TOTAL ASCENT TIME | 41 |
| 75 metres (246 ft) | |
| Bottom time | 40 |
| Ascent to first stop | 5 |
| Stop: 30 m (98½ ft) | 2 |
| 27 m (88½ ft) | 6 |
| 24 m (79 ft) | 6 |
| 21 m (69 ft) | 11 |
| 18 m (59 ft) | 18 |
| TOTAL ASCENT TIME | 48 |
| FINAL DECOMPRESSION TABLE | |
| From 15 m (49 ft) to surface | Nitrox 24% $O_2$ mixture<br>Rate 0.5 m/h or 120 min/m |

*Stability measurement—GIS: M. Hugon*

Loss of balance with the eyes open (eo) at 15 m increases with bottom time. It also increases during incursions, although it cannot be said that a *pressure factor* exists independent of the *time factor*. Closing the eyes increases loss of balance at all depths and throughout the experiment. The increase diminishes, however, when the eo instability increases. We were not able to demonstrate that a saturation depth of 15 m for excursion dives was more beneficial than dives from the surface. The divers themselves reported that their dynamic stability and bearing when walking were affected much more than their postural stability.

*Ergonomics, heart rate before and during dives—COMEX, Cantini Hospital: Dr Faugère*

The two divers in the water wear Holter boxes during an excursion dive. Analysis of the average heart rate (CF) curves showed a decrease in heart rate during the various phases of the dive: before diving, on the bottom, and during decompression (Fig. 2).

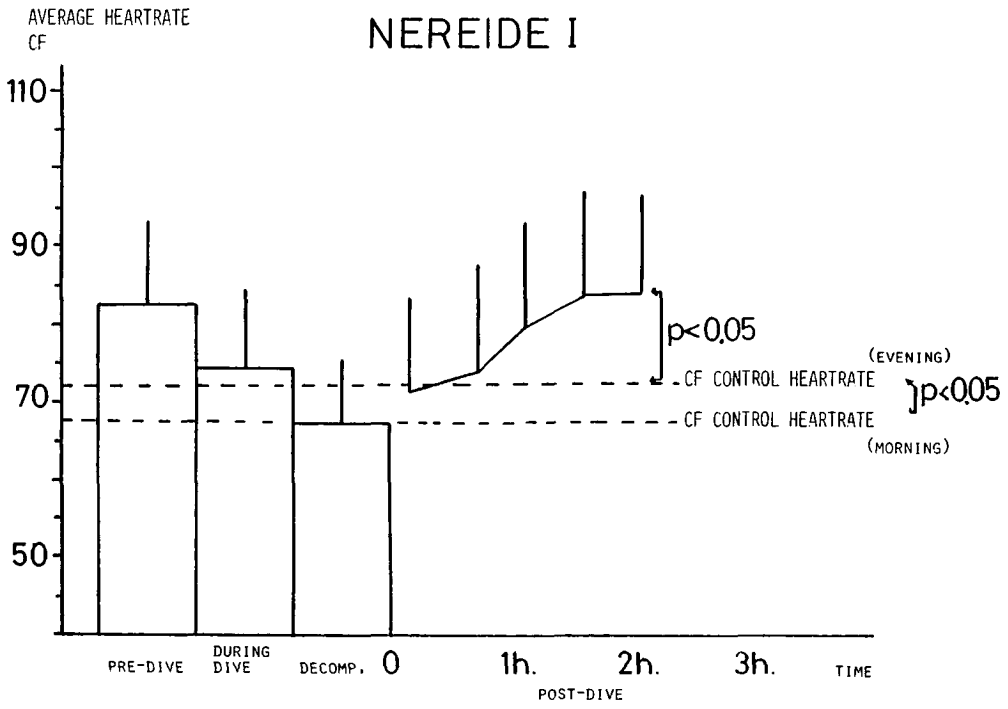



Fig. 2. Analysis of average heart rate (CF) curves before diving, on the bottom, and during decompression.

*Before diving.* In general, the heart rate is fairly rapid, about 82 beats/min, with activity corresponding to surface control tests.

*On the bottom.* During work periods on the cyclorower the CF increases less than at the surface (it reaches 100 beats/min in some subjects). The heart rate decreases during the second half of the dive (mean CF = 74). Sometimes deceleration does not occur during bottom time but appears during decompression (average CF = 67 beats/min).

*During decompression.* The heart rate is stable and slow. The Holter curve often goes to 50 beats/min and even 47 beats/min. Thus, bradycardia may occur during decompression.

*Postdive heart rate.* The cardiac rate remains fairly slow for about 1 h after the end of decompression, then gradually increases until it reaches a normal level or, depending on the individual diver, a tachycardiac level (see postdive CF curve).

## Decompression Research

*Doppler monitoring of bubbles circulating in the bloodstream—*
*CERTSM: G. Masurel; COMEX: H. Trellu*

The Doppler detector remained silent during nearly all of the excursion dives (to 42, 60, and 75 m), as well as during final decompression from 15 m to the surface, where no bubbles were detected.

Decompression from the control bounce dives carried out before and after saturation, on the other hand, produced a considerable number of bubbles. It would therefore appear highly preferable for prolonged dives (40–60 min) at these depths to apply saturation procedures, even for a fairly shallow storage depth.

*Blood biology—CERB*

Blood samples taken on days *J0*, *J4*, and *J8*, with results of six individuals. Measurements carried out were: blood red and white cell count, ferritin, glycemia, ion concentration, urea.

Substantial variations were found in the platelet count, the white cell count, and the ferritin rate.

*Platelet count (Fig. 3).* All six divers showed a marked decrease in the blood platelet count between the beginning of saturation and the return to the surface. The average decrease was 48 500, or 19.7%. This drop appears to indicate the presence of bubbles undetectable by the Doppler bubble detector.

*Leucocyte count.* The number of white blood cells increased in all six divers. The lymphocytes varied very little in absolute values, whereas the polynuclear neutrophils increased.

*Serous ferritin rate.* This rate increased by 96.2%, on the average.

*Clinical surveillance: Postdive heartbeat and fatigue study—*
*COMEX: Y. Giran, M. Comet; GISMER: D. Lamy*

The results of fatigue evaluation are based on forms filled in morning and evening by the divers. Evaluation of one's condition or sleep is, of course, subjective. It should be noted, however, that when one has not slept as well as usual the blood pressure (BP) rises and the person's overall condition is slightly under par. Temperature and relative humidity during the night have a definite effect on sleep. Vital capacity (VC) increases with bottom time. Peak flow increases or remains stable. Some divers report fatigue during the 2 to 3 h following excursion dives.

Heart rate is measured after the dive, at the same time as Doppler bubble detection. The diver is standing, immobile. The effort preceding fitting of the detector consists either of getting up from the bunk and walking 2 m or getting up from a chair and walking 1 m. This gives an orthostatic control frequency. The subject has not begun to do any bends. The mean heart rate (Fig. 2) changes

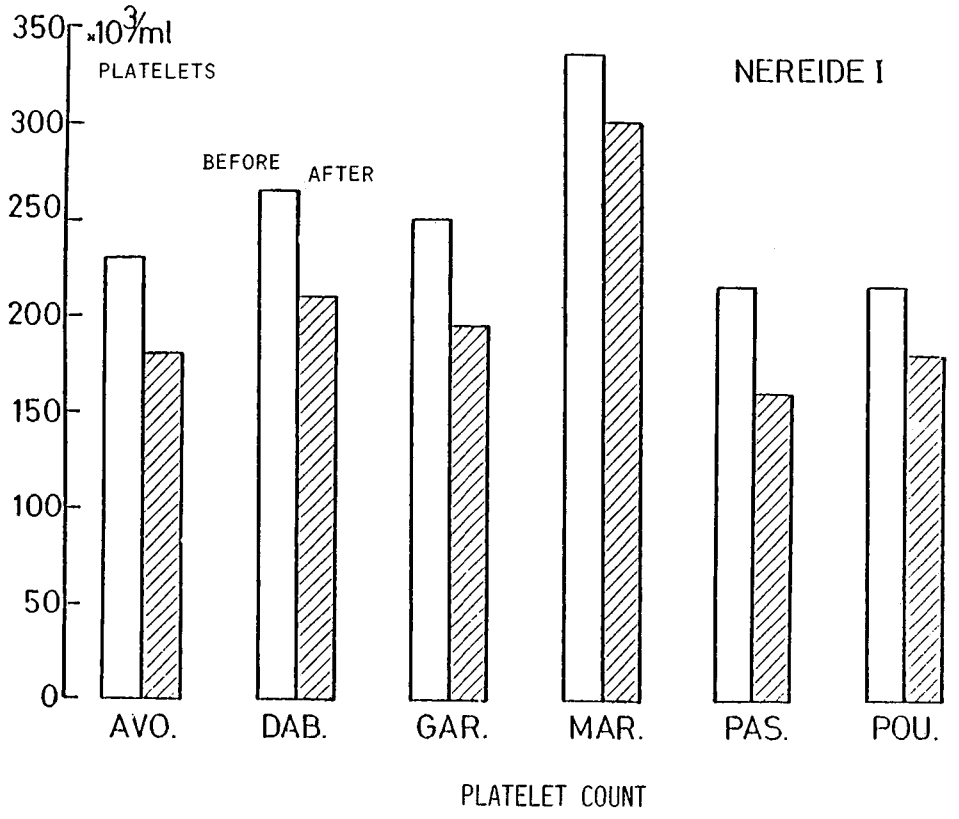



Fig. 3. Bar graph of the number of circulating platelets, diver by diver, before and after saturation (with excursion dives). The average drop in platelet count after saturation is 19.7% ($P < 0.05$).

after arrival at the surface or at storage level (15 m or 48 ft). It becomes normal within a quarter of an hour after arrival at the surface. Then there is a gradual acceleration of the mean heart rate ending in tachycardia 2 or 3 h after the dive (mean CF 80–90 beats/min). This increase in CF is statistically significant ($P < 0.05$) when compared to the control heartbeat taken in the evening.

There does not appear to be any correlation with the number of bubbles that can be detected by the Doppler method, because tachycardia occurs even when the Doppler detection is negative (excursion dives). When does it subside? It does not seem to last more than 5 to 6 h after the dive, because the measurements taken in the evening show normal heart rate. This tachycardia may be caused by the presence of gaseous microemboli, which may or may not be detectable by the Doppler method, with an accumulation of the emboli in the pulmonary circulation.

Together with the drop in platelet count the heart rate appears to constitute a subclinical biological index of bubble degassing, which cannot be measured by the Doppler method.

*Lung and bone monitoring—C.E.R.B.: R. Hyacinthe, P. Giry, J. L. Morcellet, A. Elizagarai*

Lung monitoring of the divers showed discrete reversible functional disturbances.

Scintiscanning of bones before and after saturation showed no difference.

## CONCLUSIONS

1) *The psychometric tests* permitted a more objective evaluation of the effects of narcosis. Narcosis is proportional to the depth and bottom time; for depths of 42 and 60 m it was moderate. During the excursions to 75 m (246 ft) the 12% drop in performance level constituted a handicap and a risk for the diver, a risk which should not be underestimated because a repetitive task performed on the bottom improves with the number of excursions. Indeed, the deterioration will show up in an unforeseen situation demanding quick, sure judgment and intelligent decision, which the diver will not be able to furnish.

The various and sundry improvements observed do not suffice to prove that saturation to 15 m improves narcosis. At most, one can say it is not more pronounced than in diving from the surface. The improvements would appear to suggest the possibility of adapting, or building up a tolerance, to narcosis. The stability measurements show a change as a function of depth but also as a function of the number of days spent in saturation. This change is, on the whole, moderate and does not prevent the subject from standing up. But, it is mainly on the dynamic level that several minor locomotive anomalies were observed.

The repetitive effect combined with habituation explain the all-important role of training, which permits a developed awareness of the hostile environment and, in turn, makes for improved control and behavior on the part of the diver.

2) *The excursion dives and saturation time* were well tolerated on the physical level. The Holter cardiac monitoring showed:

a) asymptomatic bradycardia developing during the second half of the dive, predominant during decompression and persisting until 1 h after arrival at the surface;

b) moderate to serious tachycardia, experienced by the diver himself. This usually appears three-fourths of an hour after arrival at the surface. After each excursion we noticed the onset of fatigue after a reaction time of about an hour.

3) *NEREIDE I* confirmed the validity of the intermediate decompression tables in use. Air saturation diving at a relatively shallow storage depth, which avoids the necessity for the diver to return to the surface, permits him to work on the bottom for longer periods. The advantages of saturation appear quite

clearly in the intermediate decompression procedure, which becomes safer and shorter (no decompression accidents or Doppler-detected circulating bubbles).

4) *The postdive biological examinations* revealed an average decrease in the blood platelet counts of 20% and an increase in the serous ferritin rate. Lung examinations showed only a discrete decrease in ventilation, and bone scintiscanning showed no change.

## Acknowledgments

Grants: DRET; Technical support: COMEX and GISMER.

# WORKING IN WATER AT 500 MSW BREATHING HELIOX: AN ANALYSIS OF DIVER PERFORMANCE AS A FUNCTION OF HPNS AND BODY TEMPERATURE

*R. Vaernes, A. Påsche, S. Tønjum, and R. Peterson*

In hyperbaric research it has been found that both high pressure (1–3) and hypothermia (4–6) cause impaired diver performance. Previous studies, however, have concentrated only on the high pressure and hypothermia effects separately, and for hypothermia mainly in shallower dives. The present study concentrates on the combined effects of high pressure and hypothermia in simulated dives in cold water at 500 msw.

Divers are often required to work in cold water situations. In temperatures less than 10°C, as in the North Sea, it has been difficult to provide the diver with adequate thermal protection when conventional diving equipment has been used. Previous studies (4–6) have shown that diver performance is significantly impaired in cold water. This impairment has been related directly to the water temperature (7). Bowen (8) has found that diver performance deteriorates progressively with continued exposure to the cold water. In deep saturation diving, equipment has been developed that significantly lengthens the period during which the diver may be exposed to cold water. This equipment consists of heated diving suits and breathing-gas heaters. If such equipment is not provided, body core temperatures will drop to critically low levels, which impair diver performance and endanger the health of the diver.

Furthermore, deep heliox saturation diving below 150 msw leads to the problem of the high pressure neurological syndrome (HPNS). The syndrome includes tremor in the hands and arms, increased slow-wave activity (2–7 Hz), and depression of alpha waves (8–13 Hz) in the electroencephalogram (EEG), dizziness, nausea, and vomiting. At depths greater than 300 msw (31 ATA), lapses of consciousness may occur (9). The symptoms of HPNS become more

severe with increasing depths and during fast rates of compression. Previous studies, especially in animals, have shown that the HPNS can be counteracted by addition of a mild narcotic agent, such as an increased partial pressure of nitrogen (10–12). Bennett (13,14) examined the effects of pressure exposure equivalent to 460 msw using 5% $N_2$ (*Atlantis I*) and 10% $N_2$ (*Atlantis II*). Whereas, the first dive with 5% $N_2$ caused symptoms of HPNS, the dive with 10% $N_2$ and the same compression rate caused no nausea, dizziness, or other clinical symptoms of HPNS. Performance tests indicated impairment of motor and cognitive skills, however, which partly can be explained as an effect of nitrogen narcosis and partly as an effect of HPNS.

In the present study we wanted to investigate the possible effects of heliox on diver in-water performance at 500 msw, as well as how gas and water temperatures served as interacting factors on the performance. One group of divers were compressed on trimix (10% $N_2$) and underwent a gradual gas change to heliox after 4 days before entering the water on heliox. A second group of divers were compressed on heliox and worked in water after 24 h at saturation depth. Despite heliox breathing for both groups when performing in-water work, a valid question to raise was whether there were group differences in diver in-water performance because of longer saturation time and change of gas at depth for one of the groups.

## METHODS

### Subjects

Six males participated in the study. The average age was 32 years (range = 4). Four of the subjects had participated in a previous dive (3) to 300 msw at the Norwegian Underwater Technology Center (NUTEC). Five subjects were commercial divers and one was an engineer.

### Experimental Design

The divers were divided into two groups, both of which were compressed to 500 msw (51 ATA). One group ($n$ = 3) breathed trimix with 10% $N_2$ during the compression (*T Group*), and the other group ($n$ = 3) breathed heliox (*H Group*). The planned and actual compression profiles are presented in Vaernes et al. (15). The holding period at 400 msw had to be extended for the *T Group* because of severe symptoms of HPNS (15).

The *T Group* stayed at 500 msw for 13 days. The *H Group* was compressed 5 days after the trimix compression and stayed at 500 msw for 9 days.

On the fourth day a gas change from trimix to heliox was started for the *T Group*. The gas change started at 7 p.m. and was completed on the next day at 4 p.m., when the nitrogen concentration was reduced to about 2%.

At 500 msw 15 heliox wet chamber dives were performed. When the diver was in the water, he went through a check procedure administered from

the chamber control. The diver was then told to stand by until breathing temperature was comfortable. When the diver and the chamber control had completed these check procedures, primary communication was relinquished to the investigator.

## Instrument Description

*The Compression Battery*

The following five tests were administered throughout the compression and during the saturation phase: a) *Electroencephalography (EEG)* with bipolar EEG recording from both hemispheres (C3-F3 and C4-F4)—data were analyzed in 2-s epochs by Fast Fourier Transform (FFT) computer; b) the *Static Steadiness Test* for recording of postural tremor; c) the *Finger Oscillation Test* for recording finger tapping speed; d) the *Dynamometer Test* for recording hand grip strength; and e) the *Trails Test* for recording visuomotor coordination and speed. For further description of the tests *see* Vaernes et al. (15).

*In-Water Performance Tests*

A battery of motor, visuomotor, and cognitive tests was administered in water at 500 msw. Except for broad manual work, the Performance Measurement System (PMS) was used. The PMS is a compact test system including a microcomputer that is programmed to control testing and log data. The following in-water performance tests on the PMS were also administered during compression and saturation in the living chamber (15,16).

*Finger dexterity.* For evaluation of arthralgia, finger dexterity was tested. The subjects were required to insert the square and circular ends of a key insertion device alternately into round and square "asterix" and plus cells on the PMS panel. This procedure required rapid, controlled manipulation for 60 s, using the dominant hand.

*Manual dexterity.* This test was included to assess the ability of manual dexterity, i.e., rapid, skillful manipulation of relative large objects. The subject was required to use the wrench to alternately expose the round and square ends of the cylinder into alternating square asterix and the round plus panel cells. Scores obtained were the number of responses completed in a 120-s period.

*Hand-wrist speed.* To test the ability of making rapid, repeated hand movements, we used a tapping test. The subject held a stylus firmly between the thumb and first two fingers of his dominant hand. The task was to tap with the stylus in a marked panel cell for 30 s as fast as possible.

*Visual reaction time test.* The ability to respond to a discrete stimulus was assessed by means of this test. The subject placed the stylus into the panel cell. This initiated a randomly varying delay of 1 to 3 s before the cell was illuminated. The subject withdrew the stylus from the cell as rapidly as possible upon seeing

the light. There were 20 trials or 600-s maximum, and the score was the mean reaction time for the completed trials.

*Visual digit span test.* Within each presentation of this test a trial began as a series of one-digit numerals ordered randomly. They were shown on the top panel cell on the PMS at the rate of one numeral per second. The series length increased in one-digit steps. The subject was asked to reproduce the numerals using the stylus and the display-respond panel.

*Operational test.* This is a test of the subject's ability to select rapidly the correct arithmetic operation for solving a problem without using time to do the actual computation. A trial began by showing a number at the left on the display, a number at the right, and a number on the keyboard. The task was to indicate the required operation to go from the number at the upper left, using the number on the keyboard, to arrive at the number at the upper right. The score was the number of trials correct during a 120-s test session.

In addition to the tests on the PMS, the diver had to perform some broad manual work:

*Test rig.* To test his capacity for broad manual work, the diver learned to mount a rig consisting of elements that should be assembled in a fixed sequence. Each element was marked with a number. The result was then independent of the way the individual diver chose to put it together. The score was the total time used.

*Heavy flange.* To test his ability for heavy work, the diver was required to mount a flange (weight: 100 kg) using two commalongs for security reasons: the heavy work consisted of moving the flange up to the mating flange on the rig. It was then mounted with eight bolts, and the score was the total time used.

## Physiological Monitoring during In-Water Performance

Core temperature was accessed directly by thermistor rectal probes. Mean skin temperatures were calculated from four skin thermistors placed on the chest, the lower arm, the thigh, and the front of the calf. For this calculation, the four skin-temperature readings were rated equally. Inspired gas temperature and hot water temperature to the diver were also monitored. The thermistors used were YSI series-700 probes accurate within 0.1°C of the correct temperature value.

## Diver Equipment

Three different breathing systems, yielding no significant difference in performance during control dives, were compared at 500 msw: A modified GSD 400, modified Superlite 17B, and Rat Hat (*see* NUTEC report 10B-1982).

The suit used during the 500-msw dive was the NRV II hot water suit and Thinsulate underwear manufactured by Diving Unlimited International, Inc.

For breathing-gas heating a prototype heater, developed at NUTEC was used.

## RESULTS

### Compression

Compression to 500 msw in 41 h, 20 min with the trimix breathing gas, which contained 10% $N_2$, did not prevent signs and symptoms of HPNS. Power-spectrum analysis of EEG showed changes from baseline EEG recording for all trimix divers (Fig. 1).

There was a slowdown of visuomotor speed during compression, and this impairment was pronounced on reaching 500 msw (Fig. 2).

Performance testing at intermediate depths showed pronounced impairment, particularly in cognitive functions. On the motor and visuomotor tests, manual dexterity and static control were impaired. Postural tremor and hand grip strength, however, were unaffected (*see* Ref. 15).

Compression to 500 msw in 26 h, 45 min using heliox as breathing gas also caused signs and symptoms of HPNS. Power-spectrum analysis of EEG showed increased theta activity and inhibition of activity in the alpha band during compression (Fig. 1). There was significant increase in postural tremor and reduction of hand grip strength (Fig. 2). The performance tests showed impaired reasoning and long-term memory and increased error score on the Static Control Test. There was, however, some tendency toward recovery in EEG and visuomotor and cognitive tests during the late phase of heliox compression (*see* Ref. 15 for a complete description of the compression results).

The subjective symptoms reported by the divers differed between and within groups. One diver in the *T Group* experienced severe HPNS symptoms. The two others reported dizziness, stomach trouble, and euphoria. The most commonly reported symptoms in the *H Group* were dizziness and tremor. One diver felt very dizzy, but none of the divers in the *H Group* was as much affected as the impaired diver in the *T Group*. One diver in each group was relatively symptom-free, one diver showed symptoms, and one, at periods, showed severe symptoms. For both groups the symptoms occurred below 300 msw, with recovery after 3–4 h at stable depths (15).

### Saturation

During saturation on trimix there was some recovery in the EEG, but no diver from the *T Group* had returned to predive values by the third day of saturation. The same was seen in the *H Group*. For postural tremor there was still a significant difference between the two groups, but there was some recovery in the *H Group* on the third day of saturation. The same tendency was

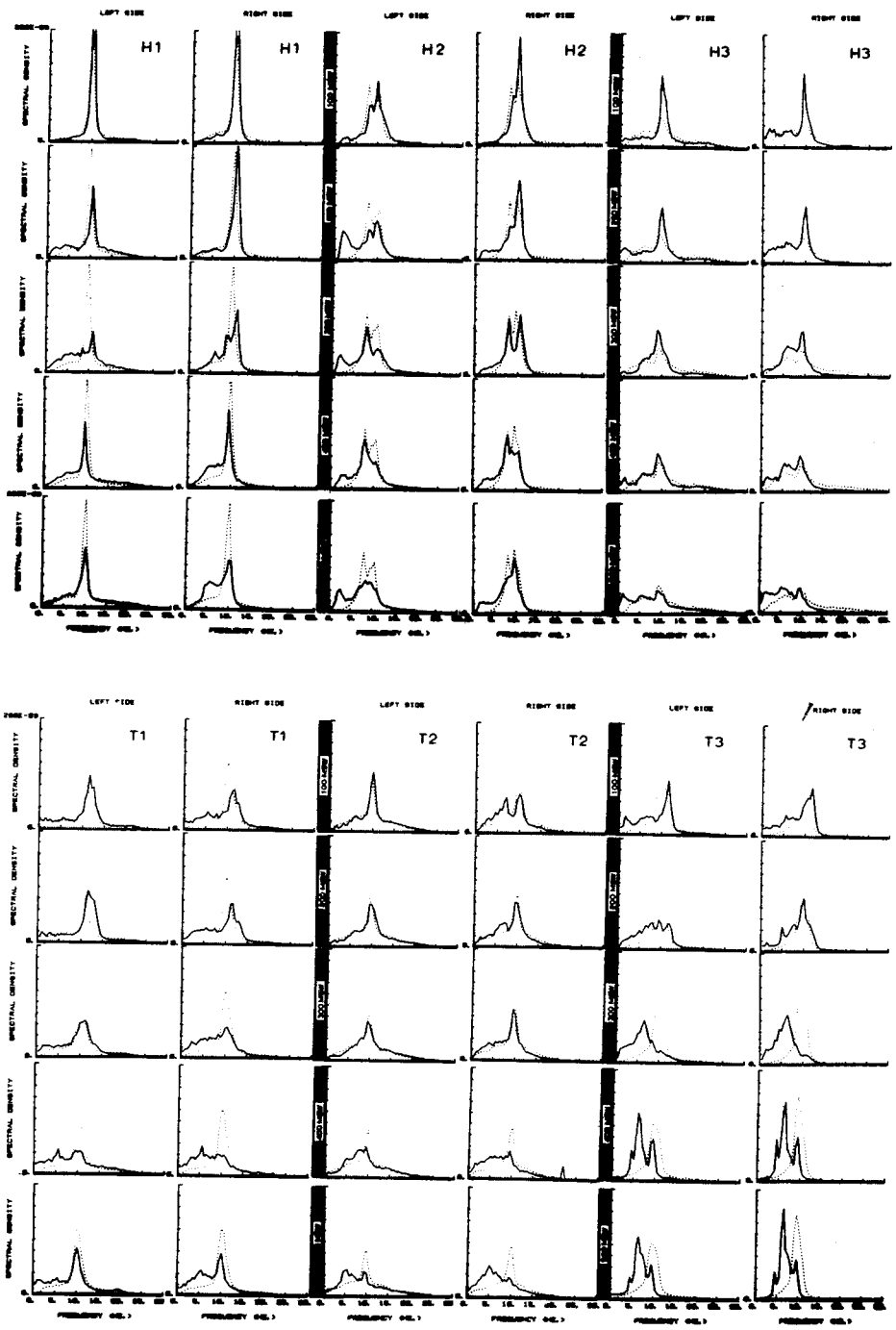



Fig. 1. Relative power-spectrum EEG during the compression to 500 msw on heliox (*Divers H1*, *H2*, and *H3*) and trimix (*Divers T1*, *T2*, and *T3*). The *stippled curve* is the mean predive power-spectrum EEG.

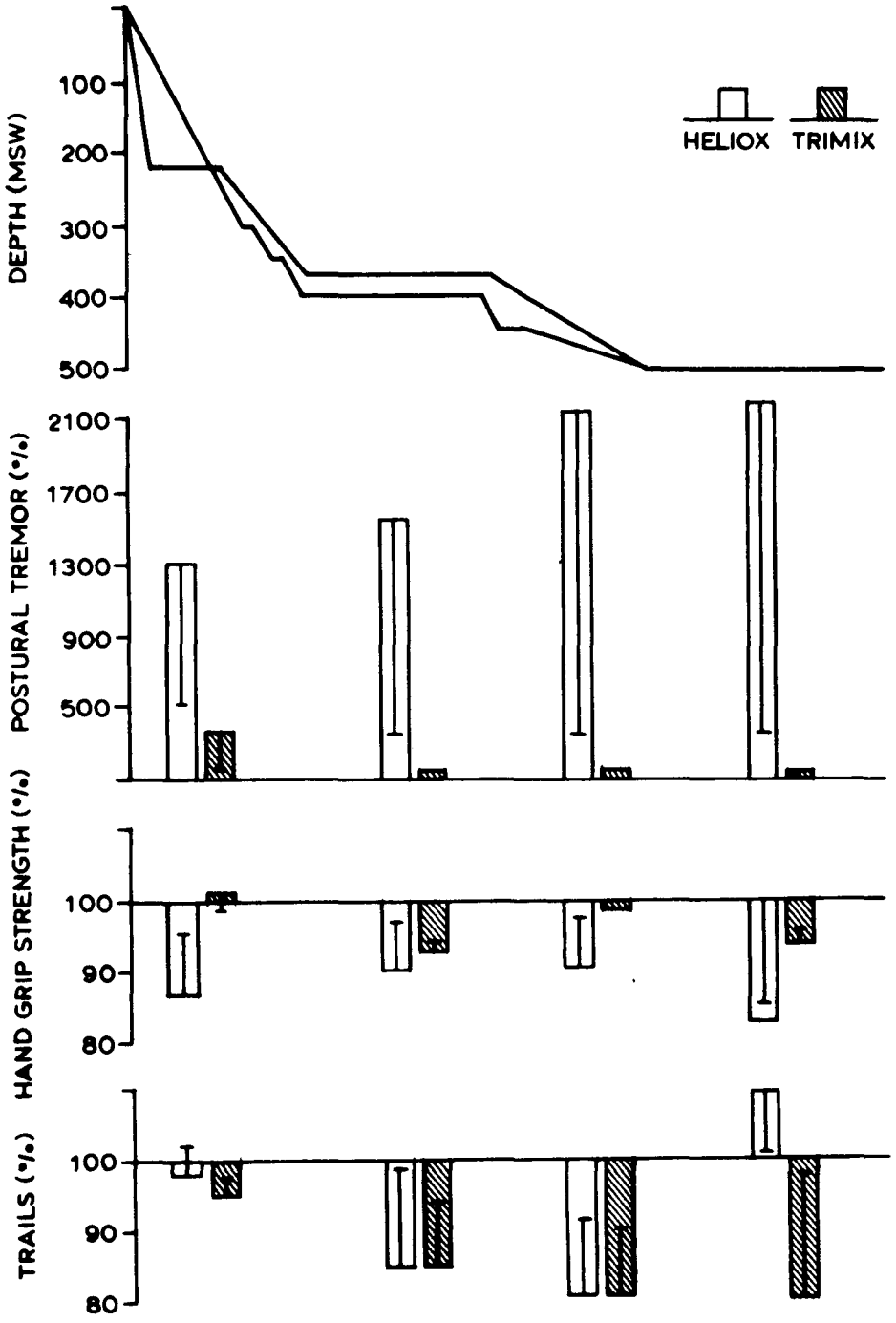



Fig. 2. Relative change in postural tremor, hand grip strength, and visuomotor speed during the compression to 500 msw on heliox and trimix. 100% is the mean predive control level.

found for hand grip strength. The most striking difference between the two groups was seen in visuomotor and cognitive functions. Whereas the *H Group* performed close to predive levels on the third day of saturation, the *T Group* was significantly impaired on several tests on the third day of saturation.

Both groups reported symptoms on the second and third days of saturation. The main symptoms in the *H Group* were tremor, clumsiness, and dizziness; the *T Group* also reported concentration difficulties, upset stomach, euphoria, and reduced appetite.

There were minor changes in the EEG during the gas-change from trimix to heliox; one diver was affected the most. There was, however, a marked increase in postural tremor for two of the subjects, an indication that the nitrogen had exerted an inhibitory effect on the tremor. For the majority of cognitive tests, there was a recovery correlating with elimination of the nitrogen. This recovery was particularly true for long-term memory. During the early phase of gas-change there were no severe symptom changes. In the evening, when the gas-change was completed, however, some symptoms occurred, and during the 12 h after the gas-change severe symptoms were reported by two of the divers. The symptoms were visual and auditory hallucinations and myoclonic jerks—they were suggestive of a withdrawal syndrome. In general, it seems that narcosis was eliminated and that some HPNS-related symptoms occurred, but the main problem at this stage of the dive were the symptoms that occurred during the 12-h period after the completion of the gas-change (*see* Ref. 16).

## In-Water Performance

### *Manual Work*

*Testrig*. At 500 msw all divers showed a decreased work rate, as compared to the control condition, in completing the testrig (Fig. 3). The mean was 40%.

All six divers had an increase in work time completing the assembly. This was most marked for the *T Group* divers (Fig. 4). In the *H Group*, only *Diver 1* had a marked increase, but this diver only performed one wet chamber dive at this depth.

For *T Group Divers 1* and *3* and for *H Group Diver 3*, there was no decrease in work time correlating with numbers of dives performed at 500 msw. This finding indicates that the divers had reached a ceiling level of performance on this test in the predive period.

*Heavy flange*. The divers performed more or less at predive level on this task, except for the *H Group Diver 1* who only had one dive (Figs. 3 and 4).

### *Motor Tests*

*Finger dexterity*. There was a mean impairment in finger dexterity of 25% for the whole group at 500 msw. At group level all three divers in the *T Group*

performed below one standard deviation of control value. In the *H Group*, *Divers 2* and *3* performed at predive level, while *Diver 1* was markedly impaired (Figs. 3 and 4).

*Manual dexterity*. There was a mean impairment of 11% on manual dexterity. Individually, there were minor differences with only *T Group Diver 1* performing at predive level (Figs. 3 and 4).

*Hand-wrist speed*. For manual dexterity, the mean impairment on the hand-wrist speed test was 11%. Two of the *T Group*, *Divers 1* and *2*, had a more marked impairment (Figs. 3 and 4).

*Visuomotor and Cognitive Tests*

*Visual reaction time*. The mean impairment was 23% on the visual reaction time test (Fig. 3). As for previous tests, there was a difference between the *T Group* and the *H Group*. All three divers in the *T Group* were markedly impaired in visual reaction, while only one diver in the *H Group* was below one standard deviation of predive control value (Fig. 4).

*Visual digit span*. Short-term memory was not markedly impaired compared to control values at 5 msw. The mean impairment was 9% (Fig. 3), and only the two divers who had one dive in the wet pot performed below one standard deviation of the control value (Figs. 4 and 5).

*Operational test*. The mean impairment was only 7% on this test (Fig. 3). All three *T Group* divers performed at predive level, while two of the *H Group* divers had a 20% impairment (Fig. 4).

Performance in water was compared to performance in the living chamber at 500 msw, and it was found that finger dexterity was 10% and visual reaction 12% more impaired in water; whereas, manual dexterity, operational testing, and visual digit-span performance was similar under the two conditions. Therefore, one can say that the mean impairment in water at 500 msw was mainly caused by the HPNS. Variance in performance was, however, greater in the in-water condition. The divers in the *T Group* showed a more pronounced impaired work rate on the testrig and on the majority of the performance tests than the divers in the *H Group*. This was probably the effect of longer time to exposure to high pressure in combination with the aftereffects of the harder compression and the gas change.

## Temperature Monitoring During In-Water Performance

In addition to these factors, the HPNS, longer saturation time, and gas change, a relationship between peformance in water and temperature data was observed in some of the dives.

When the DUI NRV II suits were used in conjunction with appropriate breathing-gas heating, the divers were kept in thermal balance, even in 6°C

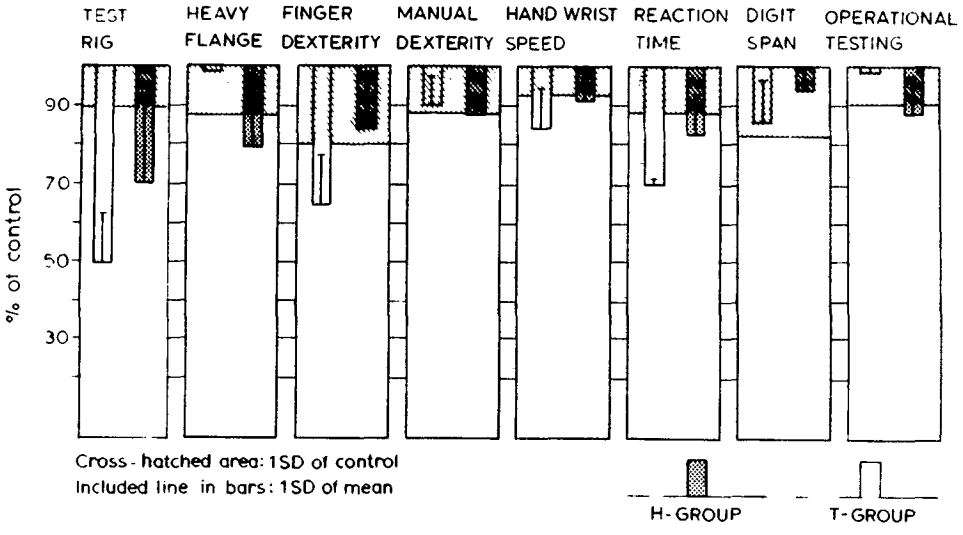



Fig. 3. Mean performance levels in water at 500 msw breathing heliox for the *Trimix Group* and the *Heliox Group*.

water at 500 msw. The experiment showed how critical breathing-gas temperature is at this depth. An inspired gas temperature of 30–32°C was considered by the divers as comfortable. Gas temperatures of 28°C or lower were too low, while 34°C was uncomfortably hot.

During the first lock-out dive at 500 msw, a short trial dive using trimix as breathing gas, the hot-water hose to the gas-heating system became disconnected. Within the following two breaths the diver sensed the gas getting colder and headed for the dry section of the chamber. He was out of water 15 to 20 s later, but had already started to shiver. Breathing-gas temperature had meanwhile dropped from 30°C to 13–14°C, while skin temperatures were unchanged.

A distinct difference was observed in the heating of the upper and lower parts of the body. Generally, the lower-body skin temperatures were 2–3°C lower than the upper-body skin temperatures. This difference was increased markedly if the diver was not moving. For instance, when the diver was doing the cognitive performance tests he had to remain relatively still, and the skin temperature difference between upper and lower body would increase as much as 5°C.

A relationship between performance in water and temperature data was observed in some of the dives. In the majority of dives, body core, breathing gas, and hot-water suit temperatures were adequate, but the effect of temperature drop on performance could be evaluated in two dives. In the first case, one diver had two consecutive dives. In the first dive hot-water suit temperature was normal; performance was normal as well under this condition. During

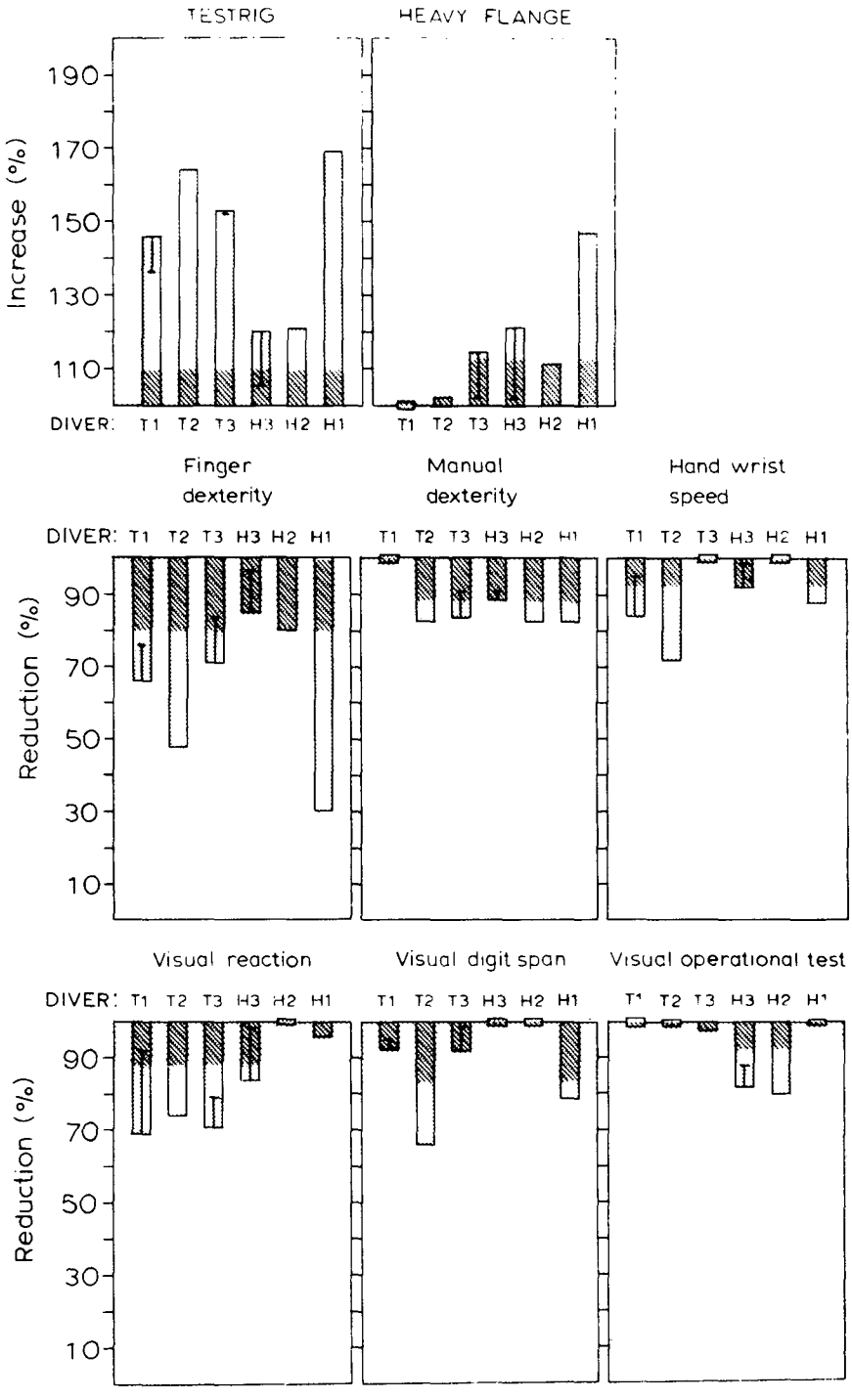



Fig. 4. Individual performance levels in water at 500 msw breathing heliox.

the second dive hot-water temperature to the diver was reduced by 3°C; performance was normal as long as the diver worked on the testrig and no drop in body temperature was observed. Performance on the PMS was, however, significantly impaired. A 26% impairment was found on the visual reaction time test and the cognitive tests had to be omitted because the diver became very cold. There was a 0.3°C reduction in body core temperature, and the skin and breathing-gas temperature both had a 1.0°C drop (Fig. 5).

In the second case, where hot-water suit temperature was kept normal, a diver showed a 0.9°C drop in body core temperature, which caused shivering and a subjective feeling of coldness. Normalization of body core temperature was slow after completion of the dive. On the performance tests, there was a 54% decrease in work rate on the testrig and a 40% increase in time on the heavy flange. On the motor tests, finger and manual dexterity were further impaired compared to the diver's 500-msw dives with normotemperature (17% and 11% further impairment, respectively).

## DISCUSSION

Compression to 500 msw in 41 h, 20 min with a trimix breathing gas containing 10% $N_2$ did not prevent signs and symptoms of HPNS. Power-spectrum analysis of EEG showed changes from baseline EEG recordings for all trimix divers. There was a slowdown of visuomotor speed during compression, and this impairment was pronounced on reaching 500 msw. Compression to 500 msw in 26 h, 45 min with the heliox breathing gas also caused signs and symptoms of HPNS. Power-spectrum analysis of EEG showed increased theta

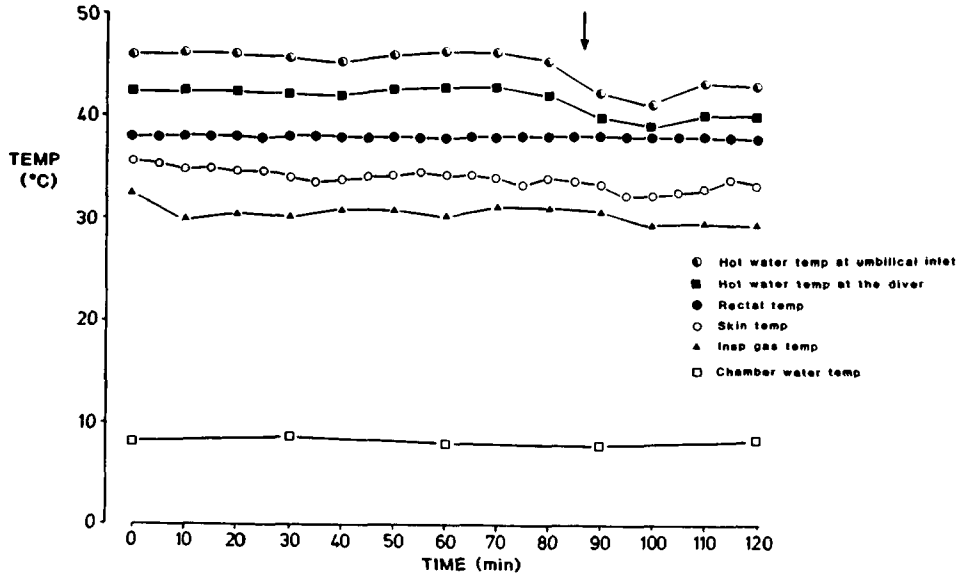



Fig. 5. Temperature recordings during a dive in water of 8–9°C at 500 msw. The *arrow* indicates the time where the hot-water temperature to the diver was reduced in an attempt to produce hypothermia.

activity and inhibition of activity in the alpha band during compression. There was a significant increase in postural tremor and reduction of hand grip strength. There was, however, some tendency toward recovery in EEG and visuomotor and cognitive tests during the late phase of heliox compression. For a further discussion of the compression, *see* Ref. 15.

The divers did not perform at predive level while working on the testrig. But there was a clear tendency that the *T Group* divers were more impaired than the *H Group* divers. This was not found on the Heavy Flange. Analyzing the finer motor functions, the Finger Dexterity was markedly impaired, especially for the *T Group* divers, and this may explain the increased work time on the testrig for the divers in this group.

Broad manual dexterity and Hand Wrist Speed were mildly impaired. The normal cognitive capacity further showed that there were no added central nervous effects compared to dry chamber testing at 500 msw. Generally speaking, only the Finger Dexterity and Visual Reaction seemed to be the functions most impaired, especially for the *T Group* divers.

In addition to these factors, a relationship between performance in water and temperature data was observed in some of the dives. In the majority of dives, body core, breathing gas, and hot-water suit temperatures were adequate, but the effect of a temperature drop on performance could be evaluated in two dives.

In the two dives where the effect of temperature drop on performance was evaluated, a significant change in performance was observed for minor temperature changes. Therefore, one cannot exclude the possibility that even smaller temperature changes, which did occur, could have had some effects on the performance of the divers.

## CONCLUSION

Performance was impaired in divers working in water at 500 msw compared to control measures at 10 msw. There were generally minor additive defects in water compared to the percent performance level in the dry conditions at 500 msw. Therefore, the impairment on in-water performance was mainly caused by the HPNS. However, there was an additive effect on some functions because of a general fatigue in the *Trimix Group*, and for the dives involving freezing, the hypothermia further impaired diver performance. For all dives the diver preferred to have a higher temperature for the hot water when he was performing finer motor and cognitive tasks compared to when he was working on the testrig.

## References

1. Bennett PB. Performance impairment in deep diving due to nitrogen, helium, neon, and oxygen. In: Lambertsen CJ, ed. Underwater physiology III. Proceedings of the third symposium of underwater physiology. Baltimore: Williams & Wilkins, 1967.

2. Lemaire C, Murphy EL. Longitudinal study of performance after deep compressions with heliox and He-$N_2$-$O_2$. Undersea Biomed Res 1981;8 (Suppl):A13.
3. Vaernes R, Bennett PB, Hammerborg D, Ellertsen B, Peterson RE, Tønjum S. Central nervous system reactions during heliox and trimix dives to 31 ATA. Undersea Biomed Res 1982;9:1–14.
4. Davis FM, Baddeley AD, Hancock TR. Diver performance: the effect of cold. Undersea Biomed Res 1975;2:195–213.
5. Biersner RJ. Motor and cognitive effects of cold water immersion under hyperbaric conditions. Hum Factors 1976;18:299–304.
6. Vaughan WS. Distraction effect of cold water on performance of higher-order tasks. Undersea Biomed Res 1977;4:103–116.
7. Stang PR, Wiener EL. Diver performance in cold water. Hum Factors 1970;12:391–399.
8. Bowen HM. Diver performance and effects of cold. Hum Factors 1968;10:445–464.
9. Bennett PB, Roby J, Simon S, Youngblood D. Optimal use of nitrogen to suppress the high pressure nervous syndrome. Avait Space Environ Med 1975;46:37–40.
10. Lever MJ, Miller WD, Paton WDM, Smith EB. Pressure reversal of anesthesia. Nature 1971;231:368–371.
11. Brauer RW, Goldman SM, Beaver RW, Sheehan ME. $N_2$, $H_2$, and $N_2O$ antagonism of high pressure neurological syndrome in mice. Undersea Biomed Res 1974;1:59–72.
12. Zaltsman GL. Hyperbaric epilepsy and narcosis. I.M.Sechenov Inst Evolutionary Physiol Biochem Leningrad: Academy of Sciences USSR, 1968.
13. Bennett PB, Blenkarn GD, Roby J, Youngblood D. Suppression of the high pressure nervous syndrome in human deep dives by He-$N_2$-$O_2$. Undersea Biomed Res 1974;1:221–237.
14. Bennett PB, Coggin R. Relationship between compression rate and trimix 10 in amelioration of HPNS in man. Undersea Biomed Res 1981;8(Suppl):15.
15. Vaernes R, Hammerborg D, Ellertsen B, Peterson RE, Tønjum S. Central nervous system reactions during heliox and trimix dives to 51 ATA, DEEP EX 81. Undersea Biomed Res 1983;10:169–192.
16. Vaernes R, Hammerborg D, Ellertsen, B, Peterson RE, Tønjum S. Saturation at 51 ATA on heliox. An analysis of recovery from HPNS and narcosis at depth. In: Elliott DH, ed. Proceedings of the EUBS annual scientific meeting Travemunde/Lübeck 1982;213–248.

# DEEP DIVING: TRIMIX AND HELIOX COMPARED BY EEG AND TREMOR CRITERIA

*Z. Török*

Hyperbaric physiological studies are needed in support of divers exposed to high pressures in the sea. The electroencephalogram (EEG) in such studies is useful for two reasons: first, in animals, exposure to high pressures may lead to convulsions, thus a lookout is kept for early EEG signs of activation (destabilization) whenever human experimental subjects are used. Second, cerebral hypoxia at cellular level may theoretically develop at extreme pressures. The EEG is therefore monitored with the basic principle that no change from control values is good news. This simple approach has to suffice, as there are no theories postulating cellular or integrative mechanisms accounting for, for example, the increased theta activity found in hyperbaric experiments. The hippocampus, a theta generator in some animals, is likely to be irrelevant here (1).

The high pressure neurological syndrome (HPNS) consists of a variety of symptoms and signs resulting from exposing a subject to too much pressure or bringing on pressure too fast (2). Changes in EEG are regarded as an HPNS component; the following changes are sufficiently constant in hyperbaric exposures to constitute one set of criteria along with cognitive and psychomotor tests or tremor measurements for comparing objectively, different compression profiles or techniques:

1) An increase of delta and theta frequency power in the EEG is best obtained when a subject's alertness is monitored by the simultaneous performance of a task (3). This latter precaution tends to control some of the variability resulting from the subject's state of mind, usually unknown. Frontal and central scalp electrodes show this increase best (4), where it may be up to 40 times normal (3).

During a well-documented dive in 1968, *Physalis III* (5), compression was halted at 1189 fswg and stay at depth was curtailed to 4 min when the large amount of slow-wave activity seen (especially after a few minutes voluntary hyperventilation) was interpreted as possible impending cellular asphyxia (5–7). The occurrence of large-amplitude delta waves is well known in some diseases, e.g., those involving metabolic changes severe enough to disturb brain function.

2) A tendency of the subjects to fall asleep in the absence of external stimuli is mirrored by large increases of power in the delta band, as in stage I sleep. They can be roused easily. This phenomenon, best termed somnolence, may be variously referred to as inattention or drowsiness (or microsleep when supported with EEG evidence).

3) A decrease in alpha activity, though often apparent on the "alert" EEG record, is best regarded as an impaired alpha response, expressed as the ratio of power in the alpha band upon closure of the eyes, to alpha power with eyes open. This response shows large individual variability under normal circumstances, can be as large as 6, and may decrease at depth. Alpha activity is best seen in occipital regions.

4) Slowing of the alpha activity by 2 Hz may occur in severe HPNS (3,4). In clinical practice the alpha frequency is sufficiently stable in a given individual to regard a change of 0.5–1.0 Hz as abnormal. This slowing may also be apparent in the late components of the visual evoked response (3).

5) Decrease of power in the beta band at the high-frequency end of the spectrum is occasionally noted by authors (8) and appears to form part of the picture of generalized slowing of the EEG in hyperbaric experiments.

6) Paroxysmal EEG activity consists of short (a few seconds) high-amplitude fast bursts that may be localized to one or two channels of the recording. They are rarely seen, e.g., in dives *Coraz I* and *II* at 300 m (9), and provided they are not due to muscle contractions, may represent synchronous electrical activity of cortical neurons.

The use of nitrogen in an attempt to counteract HPNS is currently one of the most significant endeavors in hyperbaric research. The origin of the technique is in observations on tadpoles (10), newts (11), and lipid bilayers (12) concerning antagonism between narcosis and hydrostatically applied high pressure. The resulting critical volume hypothesis (13) refers to the volume or active surface area of cell membranes, especially in the central nervous system. This hypothesis permits a calculation of the optimal concentration of a narcotic gas like nitrogen or nitrous oxide expected to counteract the effects of high pressure (14). It is central to this theory that the narcotic agent is preventive, blocking the mechanism of HPNS so ideally it never becomes manifest. Were it merely therapeutic in nature, that action could take place at several different levels in biochemical or physiological processes and lead to a symptom or sign of HPNS. The fundamental process constituting HPNS could thus either be corrected or merely hidden from view as in symptomatic treatment of a disease.

The history of using trimix in diving experiments from the earliest manned dives in 1961 was previously reviewed by Bennett (15,16), and some of the more recent work by Török in this volume (17).

## METHODS

To dissociate the roles nitrogen and pressure per se may play in the development or suppression of HPNS, we performed three simulated dives at AMTE Physiological Laboratory, Alverstoke, UK. The trimix dive used 10% $N_2$ in helium to 660 m. The heliox dive was performed to 540 m with helium alone as the inert gas constituent, the third dive with nitrogen alone to 61 m (nitrox). Oxygen was constant at 0.4 bar. To test the interaction of two variables in three dives, we employed a triangular design: raised inert gas pressure alone (ideally) was present in heliox (*AMTE Dive 13*, 1981); nitrogen, the second independent variable, alone in nitrox (*AMTE Dive 14*, 1982); and both present in trimix (*AMTE Dive 12b*, 1980). The rate of increase of nitrogen partial pressure was identical in the two dives using it, so time-variable adaptation effects were kept the same. The ideal of using the same two dive subjects in all three experiments could only partially be realized: the same two men took part in the trimix and heliox dives, but two different ones participated in the nitrogen exposure. The compression profile was different in the two "deep" dives: on consecutive days 420, hold 420, 540, and 660 m was reached in trimix with the data recorded on *Days 4 (MG)* and *9 (ME)*, whereas 180, 300, 420, hold 420, 480, and 540 m were daily compression stages in heliox with the data recorded on *Day 6*. Compression in the trimix dive was carried out with premixed inert gases.

The most reliable hyperbaric change of the EEG is undoubtedly the increasing power in the spectral band 4–8 Hz, traditionally designated as theta. Somewhat less constant is the decrease of alpha activity. The use of a combined index, the theta-to-alpha power ratios (18), when both are obtained from the same spectrum, has the advantage of emphasizing the magnitude of the numerical change because the two variables are expected to move in opposite sense in hyperbaric experiments. Furthermore, the arithmetic brings the advantages of dealing with dimensionless "normalized" quantities, one of which is that amplifier gain or electrode impedance changes cancel out on the assumption that they affect equally components in the 4–8 Hz and 8–13 Hz wavebands. Similarly, the bandwidth resulting from relevant parameters of the computational process yielding the power spectrum will be cancelled, to facilitate comparisons. A disadvantage of the ratio is its increased variability when compared to the summed power approach, reflected in the confidence intervals almost doubling for the worst case, given the same quantity of data. Biological variability may also be higher if either the theta or the alpha band does not change as expected.

The EEG was recorded while subjects performed a tracking task. The objective here was to keep the level of arousal or attentiveness constant between and also during the experiments, because large changes in the EEG power spectrum may occur in response to this variable alone. Single-channel bipolar recording from location P3 against the vertex with mastoid reference (10–20 system) was obtained—all subjects were right-handed. This location on the scalp shows low-frequency shifts well and early during their development, is well placed to record alpha activity, and has been used extensively in hyperbaric research in the past.

Anterior regions on the scalp show theta band power increase much more than the occipito-parietal location chosen here (3). The compromise was made in favor of early detection of this change (3,19) during compression. Ag–AgCl electrodes with 0.1 Hz −3dB point of the amplifier enabled efficient recording of low-frequency activity, but also required visually directed rejection of artifacts before data analysis.

Eight minutes of recorded EEG was used for frequency analysis on each occasion. The stretch of data was discontinuous for two reasons. First, the tracking task was stopped after 2–3 min and its parameters changed for the next segment. The EEG was discarded during these rest periods of about 2-min duration. Second, if artifacts were seen on visual inspection, the data processing was interrupted for some seconds until "clean" data were again forthcoming. The sampling interval of 19.5 msec permitted a Nyquist frequency of 25.6 Hz, the power spectrum was only made use of up to 20.0 Hz. Occasional checks up to 50.0 Hz confirmed the absence of significant power above 20.0 Hz.

Eight hundred spectral estimates to 20.0 Hz were computed in epochs of 20.5 s and 20, or so, spectra averaged per measurement. The spectral estimates were summed to give total power in the delta, theta, alpha, and beta bands, yielding by chi-squared methods approximately 0.8 and 1.3 as coefficients for 95% confidence intervals (20). The figures given for EEG in Table I

TABLE I

Measures of EEG and Tremor Power

| Dive | | Subject | Theta/Alpha Ratio | | Tremor Power (arbitrary units) | |
|---|---|---|---|---|---|---|
| | | | Control | Dive | Control | Dive |
| Heliox | (430 m) | *MG* | 0.55 | 4.35 | 33.1 | 85.6 |
| | (540 m) | *ME* | 0.53 | 2.86 | 42.4 | 126.8 |
| Trimix | (600 m) | *MG* | 0.67 | 6.46 | 32.9 | 95.9 |
| | (D:560 m) | *ME* | 1.69 | 5.23 | 47.7 | 136.7 |
| Nitrox | (50 m) | *JF* | 1.02 | 0.40 | 49.8 | 44.6 |
| | (60 m) | *JT* | 0.71 | 1.07 | 48.1 | 43.5 |

Theta-to-alpha ratios (dimensionless) of occipital EEG. Power summed up to 20.0 Hz ($k\ V^2_{rms}$/Hz, where $k$ = undefined constant; V = output of accelerometer in volts) measuring the magnitude of postural hand tremor. Coefficients for 95% confidence intervals are 0.6 and 1.6 for EEG; 0.8 to 1.3 for tremor (*see* text).

are theta-to-alpha power ratios; using these, we found for the worst case that the confidence intervals widened to 0.6 and 1.6.

Unlike the slowing of the EEG, increased postural hand tremor, another HPNS component, is of immediate operational significance to the diver, and, historically, was the first to be described. It is a steady-state tremor present in limbs when muscles oppose the force of gravity without additional load. We collected postural tremor data from the middle finger of both outstretched arms using accelerometers weighing 17 g. Lengths of 80–120 s of the FM tape-recording were subjected to power-spectral analysis of the same parameters used for EEG. The figures for tremor given in Table I measure total power 1–20 Hz and each is the mean of tremor of both hands. Most of the spectra had large peaks at about 8–10 Hz, the well-described physiological tremor frequency, and a smaller one at 2–3 Hz. Increase of power occurred by increased height of both peaks and apparent overspill of power to neighboring frequencies of the spectrum.

## RESULTS

The delta band was large and variable, and, in general, some slowing of the EEG was found in all three dry dives. Significant increases in the beta band were found in the nitrox dive, whereas the larger hydrostatic pressures applied in the other two experiments were associated with inconsistent decreases in both the alpha and beta bands. The theta-to-alpha ratios are given in Table I.

The theta-to-alpha ratio was successful in indicating hydrostatic pressure effects inasmuch as nitrogen at a partial pressure (dose) high enough to cause behavioral changes usually described as *narcosis* did not cause the theta-to-alpha ratio to change significantly from control values.

The summed power tremor data (in Table I) describing the nitrox experiment indicate that no significant change occurred in either subject from his own control values. Data from both of the other two simulated dives show an almost three-fold increase in the power of postural hand tremor, a highly significant result. The patterns shown by the EEG and the postural tremor data were thus similar.

In terms of depth and nitrogen partial pressure, the data obtained in the three experiments were not recorded at exactly equivalent points—this is readily apparent from the first column of Table I. The 10-m difference in the nitrox experiment was simply because of priorities given to mutually exclusive tasks and success, or otherwise, of data recording. In the trimix dive these factors were further compounded by the subjects' inability at the maximum pressure of 660 m to cope successfully with tasks like applying EEG electrodes. Thus, data obtained at points nearest the optimal had to be used in the above analysis; in the case of *Subject ME* that point was at 560 m during decompression. All the other data were recorded during compression or shortly on arrival at the depth stated.

Another factor limiting the validity of direct comparison is the different lengths of the trimix and heliox compression profiles. Thus, we were testing not so much the effectiveness of the two gas mixtures but rather whether or not trimix permits compression to these pressures at half to two-thirds the time of heliox compression.

Inspection of EEG power spectra obtained in the two deep simulated dives supports the impression that though the theta band power was increased in both, this increase was larger in trimix at all pressures than in heliox at that pressure and in the same subject. The alpha response seemed to be higher in trimix and was not inhibited by increasing pressure. As is often the case, however, only one of the two subjects was a good alpha responder in predive control experiments. At depth the trimix exposure produced a marked showing of the frequency of the dominant alpha activity in this man amounting to 2 Hz.

The triangular design (nitrogen only in nitrox, high hydrostatic pressure only in heliox, both in trimix) permits the following conclusions. First, the high hydrostatic pressure (or helium) present in two simulated dives was accompanied by increases in tremor and changes in EEG. Second, nitrogen at partial pressures up to 5.6 bars, high enough to modify behavior in the sense of *nitrogen narcosis*, did not cause significant changes in either tremor or EEG. Third, nitrogen at approximately the same partial pressure did not decrease the magnitude of these changes (when compared to the heliox dive), nor did it stop them from becoming manifest.

## DISCUSSION

These findings are borne out in another dimension by Baddeley's work, which obtained performance scores for cognitive and visuomotor coordination in the same simulated dives at Alverstoke (21,22). On trimix deeper than 300 m subjects were less efficient on all tests than on heliox at the same pressure. At 660 m in trimix the subjects were unable to perform the well-practiced tests at all; they were affected by lassitude, somnolence, nausea, vomiting, myoclonus, and dyspnea as well as poor concentration and performance. On the second day at 660 m decompression was started ahead of plans—even so, no improvement occurred for several days. Clearly, the 6.7-bar $N_2$ alone would have been incapable of producing these effects, demonstrated by the minimal or no decrements found in the nitrox dive of the trio. As in the case of tremor and EEG changes, nitrogen gas-pressure interaction or HPNS with rate-of-compression factors predominating remain likely potential explanations. In the second case nitrogen is either insufficient or irrelevant: the subject's safety clearly demanded concentrations exceeding 10%—not to be used at these pressures.

In the heliox dive (*AMTE Dive 13*) there were decrements of around 30% on arrival at 540 m, for example, in the arithmetic adding test (22), and symptoms and signs of HPNS were minimal; therefore, the consensus of the subjects and dive-control team at the time was that lockout for work would have

been possible on arrival in the sea, or indeed later, because there was no deterioration in the subjects' condition (23). The wisdom of this subjective judgment may be questioned in light of performance test scores or EEG findings; however, it contrasts the trimix and heliox dive results rather well.

It is difficult to collate the marked success of *Atlantis II* (24) with the subjects' poor condition and performance in the *AMTE Dive 12b*. Higher oxygen content in the *Atlantis II* experiment (0.5 bar vs. 0.4) was linked to decreasing nitrogen concentration from 460 m (7.8% at 650 m vs. 10.0% in *Dive 12b*). Intersubject variability would have to be large indeed to explain the differences. The remaining potentially significant variable was compression rate, which was much faster from 420 m in *Dive 12b*. *Deep Ex 81*, the Norwegian 500-m dive using trimix with 10% $N_2$, was also less successful in inhibiting HPNS than *Atlantis II* or *III* (25). Nitrogen narcosis was present, yet their subjects' condition was markedly better than in *Dive 12b*.

Postural hand tremor that is two to three times larger than its normal value in young men is not very large; it may or may not be apparent to an observer. Several reports indicate that trimix prevented development of large tremors of HPNS (24–27,29). Others show 300% tremor in 7.8% $N_2$ at 650 m (*Atlantis III* [27]). The time course of the increased tremor in *Dive 12b* also agreed with that in the French dive *DRET 79/131* (29) and earlier in *Janus IV* (30), where once this sign was established; it remained stable until some days into decompression. Thus, both the magnitude and persistence of the postural hand tremor in the trimix *Dive 12b* might have been expected on the basis of earlier work.

What is of special interest about the tremor data here emerges on comparison with the heliox dive: tremor was not less in trimix than it was in heliox at about the same depth in the same two subjects.

Previous manned experimental dives also illustrate that hyperbaric EEG changes were present (25,28,29) at levels that were no better, or were in fact worse, in trimix than in heliox (31). In other trimix exposures no EEG changes were reported (27). The *Coraz* series was designed as a comparative experiment using identical 4-h compressions to 300 m, with some overlap of subjects as well. Anterior scalp electrodes yielded increases in theta-band power of over 10 times in trimix, more with 9% $N_2$ than with 4.5% $N_2$, while increases in heliox did not exceed 800% of control values. Paroxysmal EEG activity prominent in the *Coraz* dives using trimix was not seen either in the *Atlantis* series (28) or in the *AMTE Dive 12b*. Slowing of the alpha activity noted here was seen before in *Physalis VI* (3,4). Interpretation of this or, indeed, of most of the other hyperbaric EEG changes remains unknown.

## CONCLUSIONS

Findings obtained in this comparative study using power-spectral analysis of postural hand tremor and EEG, reinforced by performance test scores ob-

tained at the same time and by observation of physical signs and symptoms, support the following conclusions:

First, the concentration of nitrogen used (10%) was predicted to be optimal by the critical volume hypothesis of pressure vs. narcotics antagonism. The absence of improvement in the subjects' condition is incompatible with the critical volume hypothesis, certainly in its present form. The validity of this conclusion is subject to the influence of those relevant experimental variables that were not kept constant in the trimix and heliox dives at AMTE, notably compression rate, availability of data, and the susceptibility of the dive subjects to nitrogen.

Second, nitrogen (or the above variables) seemed to compound HPNS, modifying rather than diminishing it. In the 61-m nitrox exposure, nitrogen at the same partial pressure and time course did not produce effects that were present in the trimix dive but absent in the heliox dive. An additive rather than subtractive interaction may exist between nitrogen and pressure. This possibility has been raised before from fundamental work (31,32).

No general validity is claimed for the above conclusions on the basis of three dives with four subjects. The first approximation to the desired nitrogen concentration, namely 10%, did not provide the benefits hoped for. Until further work using trimix is carried out, especially about coordinating nitrogen concentration with compression profile, the adding of nitrogen in deep dives seems to offer little to offset the inherent disadvantages.

## References


1. Bennett TL. Hippocampal theta activity and behaviour. Comm Behav Biol 1977;6:37–48.
2. Török Z. The symptomatology of HPNS—a proposed classification. In: Elliott DH, ed. Eighth annual congress of EUBS—Report of the proceedings. Lubeck: European Undersea Biomed Society, 1982:104–119.
3. Fructus X, Agarate C, Naquet R, Rostain JC. Postponing the HPNS to 1640 feet and beyond. In: Lambertsen CJ, ed. Underwater physiology V. Proceedings of the fifth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1976:21–33.
4. Rostain JC, Naquet R. Human neurophysiological data obtained from two simulated He-$O_2$ dives to 610 m. In: Shilling CW, Beckett MW, eds. Underwater physiology VI. Proceedings of the sixth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1978:9–19.
5. Fructus P. Deep diving. In: Proc. Conference Oceanology International, Brighton UK. London: Society for Underwater Technology, 1969.
6. Brauer RW. Seeking man's depth level. Ocean Ind 1968;3:28–33.
7. Fructus X, Brauer RW, Naquet R. Physiological effects observed in the course of simulated deep chamber dives to a maximum of 36.5 atm in He-$O_2$. In: Lambertsen CJ, ed. Underwater physiology IV. Proceedings of the fourth symposium on underwater physiology, New York: Academic Press, 1971:545–550.
8. Bennett PB, Towse EJ. The HPNS during a simulated He-$O_2$ dive to 1500 ft. EEG Clin Neurophysiol 1971;32:383–393.
9. Rostain JC, Gardette-Chauffour MC, Naquet R. HPNS during rapid compression of men breathing He-$O_2$ and He-$N_2O_2$ at 300 m and 180 m. Undersea Biomed Res 1980;7:77–94.
10. Johnson FH, Flagler EA. Hydrostatic pressure reversal of narcosis in tadpoles. Science 1950;112:91–92.
11. Lever MJ, Miller KW, Paton WDM, Street WB, Smith EB. Effects of hydrostatic pressure on mammals. In: Lambertsen CJ, ed. Underwater physiology IV. Proceedings of the fourth symposium on underwater physiology. New York: Academic Press, 1971:101–108.

12. Bennett PB, Papahadjopoulos D, Bangham AD. The effect of raised pressures of inert gases on phospholipid model membranes. Life Sci 1967;6:2527–2533.
13. Miller KW, Paton WDM, Smith RA, Smith EB. The pressure reversal of general anaesthesia and the critical volume hypothesis. Mol Pharmacol 1973;9:131–143.
14. Simon S, Katz Y, Bennett PB. Calculation of the percentage of a narcotic gas to permit abolition of the high pressure nervous syndrome. Undersea Biomed Res 1975;2:299–303.
15. Hunter WL Jr, Bennett PB. The causes, mechanisms, and prevention of the high pressure nervous syndrome. Undersea Biomed Res 1974;1:1–28.
16. Bennett PB. The HPNS in man. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving. 3rd ed. London: Baillière Tindall, 1982:262–296.
17. Török Z. Invited review: Behavior and performance in deep experimental diving with man—A review of recent work. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:739–760.
18. Török Z. Power spectral analysis of EEG in experimental diving: The use of the theta to alpha ratio. Electroencephalogr Clin Neurophysiol 1983;55:40P.
19. Fructus X, Vigreux J. Les plongees tres profondes—point des resultat acquis. Bull Med Sub Hyp 1970;2:23–25.
20. Jenkins GM, Watts DG. Spectral analysis and its applications. London: Holden Day, 1968.
21. Logie RH, Baddeley AD. A trimix saturation dive to 660 m: Studies of cognitive performance, mood and sleep quality. Medical Research Council (UK) Report RNP 2/82. London: MRC, 1982.
22. Logie RH, Baddeley AD. Simulated deep sea diving in trimix, oxyhelium and oxynitrogen: studies of cognitive performance, sleep quality and mood. In: Coombes K, ed. Proc Ergonomics Society Conference 1983:177–180.
23. Török Z. Recent experience in deep diving research with man. In: Oceanology international. London: Society for Underwater Technology, 1982:182/8.1–8.8.
24. Bennett PB, Coggin R, Roby J. Control of HPNS in humans during rapid compression with trimix to 650 m (2132 ft). Undersea Biomed Res 1981;8:85–100.
25. Vaernes R, Hammerborg D, Ellertsen B, Peterson R, Tønjum S. Central nervous reactions during heliox and trimix dives to 500 msw, Deep Ex 81. Undersea Biomed Res 1982;9(Suppl):22.
26. Vaernes R, Bennett PB, Hammerborg D, Ellertsen B, Peterson RE, Tønjum S. Central nervous system reactions during heliox and trimix dives to 31 ATA. Undersea Biomed Res 1982;9:1–14.
27. Bennett PB, Roby J, Simon S, Youngblood D. Optimal use of nitrogen to suppress the HPNS. Aviat Space Environ Med 1975;46:37–40.
28. Bennett PB, Coggin R, McLeod M. Effect of compression rate on use of trimix to ameliorate HPNS in man to 686 m (2250 ft). Undersea Biomed Res 1982; 9:335–351.
29. Rostain JC, Lemaire C, Naquet R. HPNS in man during a 12-day stay at 450 m in He-$N_2$-$O_2$. Undersea Biomed Res 1982;9(Suppl):22–23.
30. Lemaire C, Charpy JP. Efficacité sensorimotrice et intellectuelle après une compression à 400 m en 24 h. Med Aero Spat et Med Sub Hyp 1977;16:296–298.
31. Roth SM. Anaesthesia and pressure: antagonism and enhancement. In: Fink BR, ed. Molecular mechanisms of anaesthesia. Progress in anaesthesiology. New York: Raven Press, 1975;1:405–420.
32. Hsia JC, Boggs JM. Protein perturbation hypothesis of anaesthesia. In: Fink BR, ed. Molecular mechanisms of anaesthesia. Progress in anaesthesiology. New York: Raven Press, 1975;1:327–339.

# NEUROPSYCHOLOGIC SEQUELAE OF A DEEP-SATURATION DIVE: A THREE-YEAR FOLLOW-UP

*B. Becker*

The neurological and psychological manifestations of decompression sickness and diving accidents have been documented by using standard neuropsychological tests and neurological examinations. Peters, Levin, and Kelly (1) studied 10 divers with a history of decompression sickness involving the central nervous system and found that 8 had unequivocal neurological deficits that implied multiple supraspinal lesions. The investigators concluded that cerebral disturbance following decompression sickness in divers is more common than they previously believed. In recent years, anecdotal reports have been received regarding neuropsychological symptoms following deep dives in which no accident or decompression sickness occurred. These reports have prompted some members of the diving community to express concern about the possibility of subtle long-term effects of such hyperbaric exposure and to acknowledge the need for careful studies involving neuropsychological testing before diving and at various intervals after deep-saturation dives. Such anecdotal reports of post-dive symptoms had been received from participants in a 40-day, 1800-foot helium-oxygen saturation dive conducted at the Naval Experimental Diving Unit, Panama City, Florida, in November 1979. Although these divers had received no previous neuropsychological tests, investigators decided early in 1982 to interview the six participants carefully and to administer neurological and neuropsychological examinations to assess possible sequelae at that point.

No accidents or decompression sickness were reported as a result of the experimental dive, but records and anecdotal accounts do include vertigo and nausea associated with the high pressure neurological syndrome (HPNS), as well as weight loss and difficulty performing tasks at depth.

Several of the divers reported that in the days, weeks, and months following the dive they experienced a variety of behavioral symptoms of neuropsychological disorder. These divers reported that simple tasks required more time and more effort to accomplish and that fatigue set in earlier and more often. Cognitive operations such as mental arithmetic calculation were more difficult or actually impossible because they required use of paper and pencil. Attention span was reduced and reaction time was lengthened. One subject described actual failure to react at all when traffic lights changed from green to red.

Divers experienced problems in finding words, putting thoughts together, and expressing thoughts easily and articulately. They also experienced a variety of memory problems. Altered states of consciousness were described: feeling "odd," sluggish, confused, depressed. Inconsistency was reported: feeling bright, sharp, and capable one day, but slow and ineffective the next.

Not all the symptoms were described by every subject, and one subject reported no symptoms at all. Improvement in the months following the dive was reported consistently, and almost all subjects felt they were back to normal by the time this study was conducted, some 3 years after the dive. The residual symptoms reported included mild concentration and memory difficulties and the inability to do calculations as well as before.

## PROCEDURES

The subjects were all male: five Navy enlisted divers and one diving physician. They ranged in age from 31 to 40 years (mean: 34.2). Estimates of intelligence quotients based upon classification test scores (GCT/ARI) of the enlisted divers ranged from 101 to 121 (mean: 112).

Educational levels (also excluding the physician) ranged from 12 to 15 years of school (mean: 13). All were in good health at the beginning of the dive, and all the enlisted divers were still on active duty at the time of this study.

In addition to being interviewed about their postdive experiences, the subjects were given a physical neurological examination, a clinical waking electroencephalogram (EEG), and the Halstead-Reitan battery of neuropsychological tests. A brief historical note and a description of this battery of tests will help to clarify the rationale for its selection for use in this study.

Clinical neuropsychology, a relatively new specialty area concerned with the measurement and description of brain function and dysfunction, has grown enormously in the past three decades. The assessment of brain damage in humans by use of a battery of objective tests has its basis in Halstead's (2) attempt to differentiate between biological and psychometric intelligence. But an empirical basis for using such a battery was not firmly established, and the use of this approach was not accepted until Wheeler, Burke, and Reitan (3) showed that a battery of neuropsychological tests used in a systematic fashion could accurately predict different aspects of brain damage.

## The Halstead-Reitan Battery

The battery of tests that Wheeler, Burke, and Reitan used has been refined during the past 20 years and is now widely known as the *Halstead-Reitan* battery. It is still regarded as the most thoroughly standardized and documented battery of its type, and its application in the diving industry has been strongly advocated (4). The battery consists of seven subtests used to calculate the Halstead Impairment Index and is commonly supplemented by the Wechsler Adult Intelligence Scale (WAIS), the Minnesota Multiphasic Personality Inventory (MMPI), and several allied procedures (5).

*The Halstead Impairment Index*

The Halstead Impairment Index is a summary score ranging from 0.0 to 1.0, and is calculated using cutoff scores on the following measures:

*Halstead category test.* This test consists of 208 patterns of figures divided into 7 subsets. The subject's task is to abstract a single concept for each subset, a job requiring attention, concentration, and memory as well as conceptual and problem-solving ability.

*Tactual performance test (time, memory, and localization).* Using a modified Seguin-Goddard Form Board, the blindfolded subject is required to fit 10 variously shaped blocks into their proper spaces. This subtest assesses several abilities, including motor speed and the use of tactile and kinesthetic cues to enhance psychomotor coordination, learning, response to the unfamiliar, and incidental memory.

*Rhythm test.* This test is a measure of nonverbal auditory perception that requires the subject to indicate whether pairs of tone sequences are the same or different. Presumedly, it also measures attention and sustained concentration as well as auditory perception and comparator functions.

*Speech sounds perception test.* This task requires the subject to identify 60 nonsense syllables presented on an audiotape. In this way, the test taps auditory-verbal perception, auditory-visual coordination of language processing, and sustained attention and concentration throughout a relatively complex task.

*Finger oscillation test.* This is a measure of simple motor speed of the right and left index fingers.

*Supplemental Tasks*

In addition to the tasks listed previously which contribute to the Impairment Index, the Halstead-Reitan battery includes the following measures:

*Wechsler Adult Intelligence Scale (WAIS).* This is an individually administered set of 11 subtests divided into Verbal and Performance Scales that measure

the subject's store of general information. It also measures common-sense judgment, verbal abstract reasoning, mental arithmetic calculation, immediate auditory memory, vocabulary, visual-motor associative learning, constructional praxis, visual-perceptual accuracy and judgment, nonverbal reasoning, and recognition of logical sequence.

*Trail-making test.* This paper-and-pencil test requires the subject to connect circles in number and in alternating letter-number sequence. It is used as a measure of perceptual-motor speed, flexibility, immediate recall, attention, and performance under time pressure.

*The sensory-perceptual examination.* This is a series of tests designed to evaluate intactness of sensory input and ability to identify the source of that input. Unilateral and bilateral auditory tests, visual and tactual stimulation tests, and tests for suppression under simultaneous stimulation are also included.

*Aphasia screening test.* This test, a modification of the Halstead Wepman Aphasia Screening Tests (6), is designed to determine the presence of both receptive and expressive language problems, and related disabilities.

*Minnesota Multiphasic Personality Inventory (MMPI).* This screening instrument is included to provide some measure of the additional dimension involved in coping and adjustment, even though no unique personality profile is associated with organic impairments of the brain.

*The Wechsler Memory Scale (WMS).* This standard scale assesses orientation, mental control, immediate and short-term recall of verbal and figural material, as well as the initial learning of word associations.

Utilization of the Halstead-Reitan Battery provides the opportunity to assess the overall level of adequacy of an individual's performance across a broad range of higher-level mental abilities and lower-level functions, such as motor and sensory skills. In addition, specific signs of impairment or disorder of function are elicited—such as those obtained on the aphasia test—as well as observed through examination for constructional dyspraxia and sensory suppressions in the areas of vision, hearing, and touch. The relationships between areas of adequate performance and areas of deficient performance are also examined with respect to their implications for adequacy or impairment of related brain function. Finally, the comparative efficiency of the two sides of the body is evaluated according to perceptual, sensory, and motor functions. Utilization of these four complementary inferential methods provides both a series of checks and balances against which the individual test can be examined and a series of screens to evaluate the data before a conclusion can be reached.

## RESULTS

The results of the physical neurologic examinations were within normal limits on all but one subject. The mild sensory dysfunction in this subject was thought to be the result of earlier trauma, unrelated to the dive. All the EEG's were read as normal by the neurologist-electroencephalographer.

The results of the neuropsychological tests were generally within normal limits. Only one subject's Impairment Index was in a range suggesting mild cognitive impairment (almost entirely because of problems with concentration, attention, and incidental memory). All the others showed no evidence of impairment at this point. The modal Impairment Index was 0.1, well within normal limits. Of the 42 Halstead-Reitan subtest scores obtained on the six subjects, nine fell beyond the standard cut-off score. Seven of these were on tasks requiring sustained attention and concentration. Essentially no sensory/perceptual, motor, or language problems were seen. Memory as sampled on the Wechsler Memory Scale Becker was in the superior range (mean MQ:129).

The mean Full Scale IQ as measured on the WAIS-R (110) showed no decline for the enlisted divers from the IQ estimated by their original classification tests scores (112). No prior score was available for the physician, but his present IQ fell in the very superior range.

The MMPI profiles were within normal limits, with only a few scattered elevations and no common personality pattern.

## SUMMARY

In summary, these test results fail to document significant impairment of cognitive function in the 6 subjects some 3 years after an 1800-foot dive of 40 days' duration, although minor problems with sustained attention and concentration were elicited in one subject. Despite these reassuring findings, all but one had reported significant cognitive symptoms in the weeks and months immediately following the dive. These symptoms might have been reflected in testing directly following the 40-day dive. Such verbal reports underscore the need for more careful study of subtle changes in cognition, with testing before, during, and at various intervals after each dive. The reports and the test results obtained here suggest that tasks requiring sustained attention, vigilance, memory, and continuous performance should be included and are likely to show changes. Current and proposed studies do include such tasks; Gronwall's Paced Auditory Serial Addition Task (PASAT) (7), Smith's Symbol Digit Modalities Test (8), and Mirsky and Rosvold's Continuous Performer Test (9) are among these. Because even transitory impairments may well have a cumulative effect on cognitive efficiency, caution should certainly prevail until such careful studies can be completed.

## Acknowledgments

This study was supported by the Naval Medical Research and Development Command, Research Task No. MF58524.02D.0004. The opinions and 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.

The contributions of CDR Joseph A. Miller, MC, USN, as neurologist/examiner and electroencephalographer are gratefully acknowledged.

## References

1. Peters BH, Levin HS, Kelly PJ. Neurologic and psychologic manifestations of decompression illness in divers. Neurology 1977;27:125–127.
2. Halstead W. Brain and intelligence. Chicago: Chicago Press, 1947.
3. Wheeler L, Burke C, Reitan R. An application of discriminant functions to the problem of predicting brain damage using behavioral variables. Percept Mot Skills 1963;16:417–440.
4. Townsend RE, Hall DA, Knippa J. Clinical applications of neuropsychological tests in the diving industry. Presented at the International Diving Symposium, Association of Diving Contractors, New Orleans, 1979.
5. Boll TJ. The Halstead Reitan Neuropsychology Battery. In: Filskov SB, Boll TJ, eds. Handbook of clinical neuropsychology. New York: Wiley, 1982:557–607.
6. Halstead WC, Wepman JM. The Halstead-Wepman Aphasia Screening Test. J Speech Hear Dis 1959;14:9–15.
7. Gronwall D. Performance changes during recovery from closed head injury. Proc Austr Assoc Neurol 1967;13:143–147.
8. Smith A. The symbol-digit modality test: A neuropsychologic test of learning and other cerebral disorders. In: Helmuth J, ed. Learning disorders. Seattle: Special Child Publications, 1968.
9. Rosvold HE et al. A continuous performance test of brain damage. J Consult Psychol 1956;20:343–350.

# *NISAHEX*: DEEP NITROX SATURATION WITH NITROX AND TRIMIX EXCURSIONS

*A. Muren, J. Adolfson, H. Örnhagen, M. Gennser, and R. W. Hamilton*

Depth limitations to diving with air as the breathing gas are well known; the properties of air that limit its use are its oxygen content, density, narcosis, and requirements for decompression. Oxygen can be set properly for the depth in a nitrox (nitrogen-oxygen) breathing mixture. Density limitations result from both human and equipment characteristics and at the moment appear not to be the highest priority. Limited experimental work has suggested that management of both the narcosis and decompression problems can be improved by the use of saturation-excursion diving techniques (for reviews *see* 1–3).

*Nisahex* was designed to add to the experimental basis for such activities. It was a saturation exposure to a stressful level of hyperbaric nitrogen; it looked at performance and physiology in that environment and the accommodation or "adaptation" that might take place after several days of exposure. It also investigated excursions to greater pressures with both heliox and nitrox mixtures.

## METHODS

### The Exposure

*Nisahex* was a 6-day saturation of 6 diver-subjects at a pressure of 7 atm or 60 msw (*see* Units, next paragraph). Oxygen partial pressure was mildly hyperoxic at 0.4 atm. The experiment began with a simulated "lost bell" survival study (4). Two divers—*Team A*—spent 2 days at 150 msw (~16 atm) and were decompressed to 50 msw, where they switched to nitrox and met two other 2-man teams, *B* and *C*. During compression *B* and *C* held 2 h at 40 msw, and all 6 divers held 4 h at 50 msw before going to 60 msw (*see* Fig. 1).

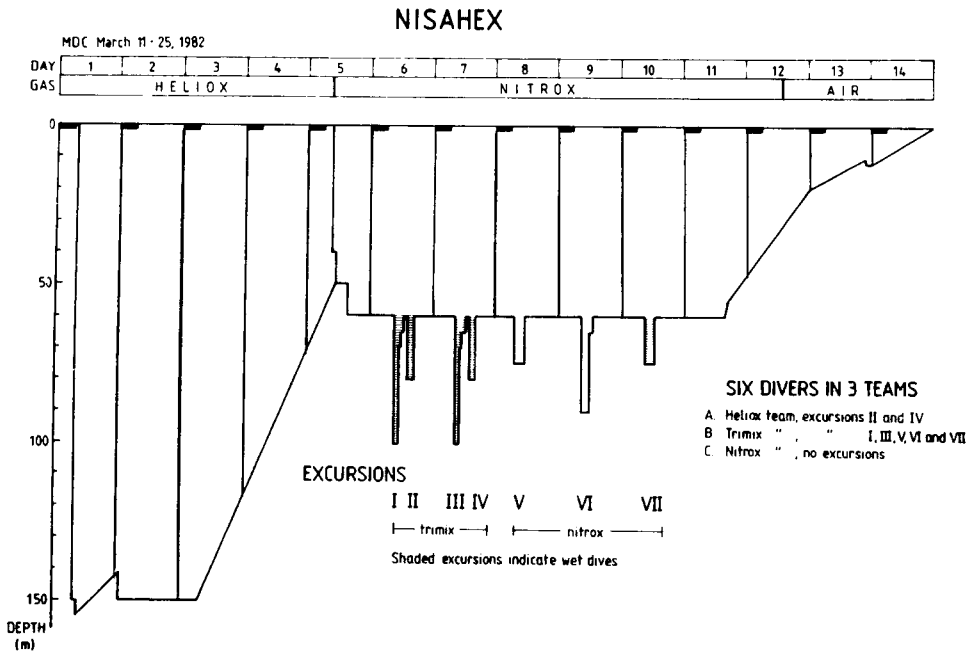



Fig. 1. Overall profile of *Nisahex*. Initial deep heliox excursion *at left* was for survival equipment test with *Team A*. *Teams B* and *C* were compressed on *Day 5*, and all divers were in nitrox thereafter. $P_{O_2}$ was 0.4 atm during bottom time at 60 msw; 0.5–0.55 during decompression until *Day 12*; then oxygen fraction was held at 0.21. Oxygen on excursions was 10%; trimix excursions had the same $P_{N_2}$ as the saturation gas.

## Units

Pressure measurements were made in metres of sea water (msw). The msw is commonly defined as 1/10 bar, or more precisely 10.00 kPa. For convenience, the overall environmental exposure levels are given here in atmospheres (absolute); 1 atm = 101.3 kPa and about 10 msw.

## Operations

The experiment was conducted at the Swedish Naval Diving Center, Hårsfjärden, near Stockholm, during March of 1982. A four-compartment chamber system with an internal volume of 35 $m^3$ was used. Four of the divers slept in the main chamber and two in an attached PTC (personnel transfer chamber) mounted on the top of the entry lock. The entry lock served as a toilet room with wash basin and a thermoregulated shower. The lower part of the work chamber was filled with water and in this chamber the dry and wet excursions were made.

The daily routines started at 0630 with recording in bed. After this recording, the morning hygiene and blood sampling (5) were done. The morning meal was served 0730–0830.

Activities such as excursions, wet dives, and physiological and psychometric investigations were usually scheduled for the three working periods 0900–1200, 1300–1600, and 1800–2100. Hot meals were served at lunch and dinner. There were no restrictions in the amounts of food and beverages served. During leisure time radio, taped music, TV, and video sound could be enjoyed via headsets; TV was displayed in one of the ports. After the late evening snack at 2130 the personal hygiene and cleaning of the chamber were performed. One of the divers stayed up to conduct the in-bed electrocardiographic recording (ECG) just before the lights were put out at 2230.

The life-support system for the living chambers kept the temperature at 25°C, the $P_{O_2}$ between 0.40 and 0.45 atm, and the relative humidity between 50 and 70% throughout the stay at pressure, except for short periods during excursions and pressure changes. The water temperature in the wet dives was approximately 7°C.

The nitrox compression was started so as to meet the decompressing helium divers (*Team A*) at 50 msw, and then continued to storage depth at 60 msw. This procedure gave a safety margin in gas loading for *Team A*. When the chambers met, the two *Team A* divers transferred from the work chamber to join the others and the work chamber was flushed with nitrox. The result was an initial 6–7% helium in the system; this amount decreased to 2% by the start of decompression.

## Subjects

Two of the six divers (*Team C*) were last-year medical students and graduate students of physiology, with earlier experience from sports diving and scientific work in pressure chambers. This was their first saturation dive. They stayed all the time at 7 atm nitrox, making no excursions. *Teams A* and *B* were experienced Navy divers. The two divers in *Team A* started the *Nisahex* dive with a 150-msw heliox saturation. They also made *Excursions II* and *IV* on trimix to 80 msw. *Team B* was compressed on nitrox along with *Team C*. They made *Excursions I*, *III*, *V*, *VI*, and *VII* on trimix and nitrox to different depths (Table I). All divers were physically fit (*see* Table I).

TABLE I

Description of Diver-Subjects

| *Team* | *Diver* No. | Age (yr) | Height (cm) | Weight (kg) | Max $O_2$ (mL/kg) |
|---|---|---|---|---|---|
| *A* | *1* | 39 | 188 | 90 | 67 |
| | *2* | 41 | 179 | 76 | 55 |
| *B* | *3* | 38 | 176 | 72 | 60 |
| | *4* | 47 | 181 | 72 | 64 |
| *C* | *5* | 27 | 173 | 62 | 60 |
| | *6* | 31 | 175 | 66 | 61 |

## Experimental Procedures and Monitoring

Psychomotor performance tests were performed daily during the "bottom time" at 7 atm by all subjects, as were isometric muscle measurements. Exercise performance was done every other day. Operational requirements precluded proper predive and immediate postdive measurements of many parameters.

Psychomotor testing consisted of an optokinetic readaptation time (RAT), the Stroop colored-word test, a cooperative tracking task performed simultaneously by both members of a team, and statometry (a balance or standing-steadiness measurement).

Physical performance testing consisted of an exercise period on the bicycle ergometer. Muscle measurements were of grip and elbow-flexion strength and included maximum voluntary contractions, endurance, and recovery.

Heart rates and electrical activity (ECG's) were monitored both at rest and during the exercise regimens. Indications of narcosis were obtained by daily interviews with the divers and by subjective observations. During decompression back to saturation depth following excursions, bubble detection using Doppler ultrasound was attempted. Microbiological and hematological studies were also performed (5).

The Stroop test and RAT measurements were made in the entry lock, which could be completely darkened. For the RAT a 3-wire differential-lead electrooculographic recording was made on a Mingograph 800 after amplification by an EMT 17. This recorder was also used for ECG's.

The main chamber was equipped with penetrators for force transducers and feedback instruments used in the muscle and statometry projects. The force transducers were tested and found free from pressure artifacts. They were calibrated daily with standard weights. The signals were amplified in a bridge circuit. The signals from statometry and force measurements were processed by amplifiers and recorded on a Tandberg FM tape recorder and Houston and Mingograph ink recorders. Later, frequency analysis was made of the tapes by computer. Student's $t$ and paired $t$ tests were used for statistics.

The working chamber above the wet pot was used for experiments regarding physical work capability. Divers exercised on an electronically braked bicycle ergometer (Siemens Elema); its electrical parts were purged with pure $N_2$ to reduce the fire hazard. Heart rates were counted manually by an assisting diver and reported to outside. The subject was breathing through a low-resistance one-way valve system; a sampling catheter just downstream of the exhalation valve led a sample gas stream to a Centronics mass spectrometer. During the wet dives no physiological or psychometric recordings were made.

## Excursions

Descending excursions were simulated on the second through sixth days of nitrox saturation by compressing a team of divers in the working chamber (Fig. 1). In most excursions one or two divers worked in the water. Gas mixes and other details of the excursions are given in Table II.

TABLE II

*Nisahex* Excursion Summary

| Excur. No. | Dive Day | *Team* | *Divers* | Wet Diver | Depth msw | Bottom Time, hr | Percent $O_2$/He/$N_2$ | Decompression Stops, msw/min |
|---|---|---|---|---|---|---|---|---|
| I | 6 | *B* | *3 & 4* | 3 | 100 | 2 | 10/30/60 | 70/35, 65/80 |
| II | 6 | *A* | *1 & 2* | 1 | 80 | 3 | 10/15/75 | — — |
| III | 7 | *B* | *3 & 4* | 4 | 100 | 2 | 10/30/60 | 70/35, 65/80 |
| IV | 7 | *A* | *1 & 2* | 2 | 80 | 3 | 10/15/75 | — — |
| V | 8 | *B* | *3 & 4* | — | 75 | 4 | 10/0/90 | — — |
| VI | 9 | *B* | *3 & 4* | — | 90 | 3 | 10/0/90 | 65/40 — |
| VII | 10 | *B* | *3 & 4* | 3 & 4 | 75 | 4 | 10/0/90 | — — |

## PSYCHOMOTOR PERFORMANCE

Predive performance measurements were made with the subject in the chamber at normal atmospheric pressure 8–9 days before the dive. Standing steadiness and RAT were recorded and the Stroop colored-word test was performed by *Teams B* and *C* during the compression stops at 5 and 6 atm. All six subjects were tested again at 7 atm where a tracking task was also performed. The test procedure was repeated on the following 6 days. A control measurement was performed at 1.5 atm while subjects breathed air towards the end of the decompression phase on the evening of the 10th day at pressure. Postdive controls at 1 atm were performed about 2 months after the dive.

### Ocular Readaptation Time

Optokinetic nystagmus is used to monitor the eye's RAT after a bright exposure to a light flash. The light sensitivity of the eye adapted to a low luminous level decreases abruptly when the eye is exposed to a bright flash of light. As a result the nystagmus disappears but will reappear after a few seconds.

The optokinetic stimulation apparatus with the flash lamp was mounted on the outside over a circular window in the entry-lock wall. The subject inside the chamber wore three electro-oculographic electrodes, one attached to each temple and one to the forehead. The subject was dark adapted with red goggles. He looked through the porthole into a white "dome." An image projected on the dome moved from left to right or from right to left at a fixed rate. The time between the flash and the moment steady eye movements resumed was considered the RAT and could be determined from the recording; scores are the mean of 10 trials.

In previous studies it has been shown that the RAT changes as a function of $Pa_{O_2}$ (6). The effect of alcohol intake showed an average RAT prolongation of 60–70% during the acute phase of intoxication (7). The central nervous system (CNS)-depressant drug Oxazepam changed the RAT with a maximal pro-

longation after 2 h for the studied group (8). Toxic welding fumes also produced a mark prolongation of the RAT (9).

The aim of the present experiment was to study the effects of a 7-day nitrox saturation at 7 atm on the RAT.

### The Stroop Serial Colored-Word Test

In this test the subject has to read colored words printed on a card in incongruent colors, naming the color instead of the printed word (10). A total of 40 words were read for each run with the speed of reading in seconds recorded as the result.

### Tracking

The tracking test required the subject to follow a 6-mm wide circuitous path using a pen held in a simple pantograph. As a special measure of "psychosocial" function (teamwork) a two-handle pantograph was used, with one handle controlled by each diver. The two divers in each team performed the test together and had to cooperate to complete the test. The score was based on the time to finish, with 1 s added for each time the line left the path. Tracks were often irregular, but there were relatively few errors. Divers had a 1-day training period before the experiment.

### Standing Steadiness

In previous studies it has been shown that the involuntary sway motion that man exhibits when standing upright with his feet together increases when the inspired nitrogen partial pressure is raised (11–13). The device for measuring these motions—the Statometer—gives two analog signals, which were recorded on tape and paper. The signals were analyzed by evaluation of variances in frequency and amplitude of the pattern of body sway, in both sagittal and lateral directions.

### Results of Psychomotor Performance Tests

Some initial prolongation of the RAT was seen at 6 atm ($P < 0.01$) followed by successively shorter RAT's (hence better performance) over the course of the dive. The prolongation was statistically significant also at 7 atm the first day at the saturation depth but not during the rest of the dive (Fig. 2, *solid line*).

The Stroop colored-word test showed the same pattern (Fig. 2, *interrupted line*). The results were similar to those of the RAT, with an initial disturbance at 5 atm followed by successively higher reading speeds (better performance) over the course of the dive. Nevertheless, none of the differences were statistically significant.

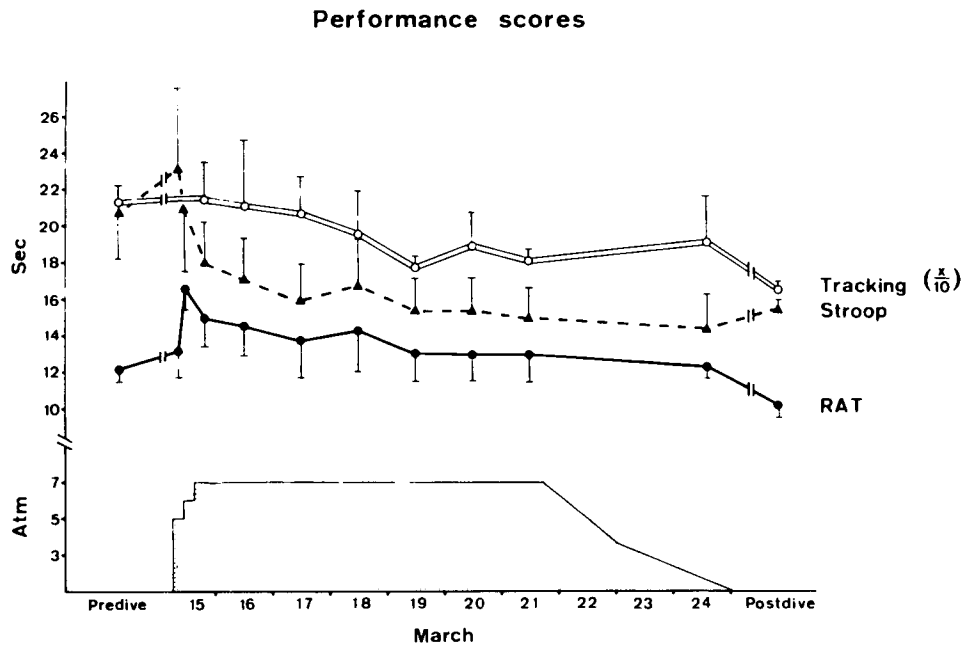



Fig. 2. Results of performance tests. Tracking (*double line*) is 1/10th of time in seconds to complete course by a dive team operating a 2-handle pantograph. Stroop is time to name 20 colored words. RAT (retinal readaptation time) is time delay following light flash before eye begins to track moving target. All scores show steady improvement throughout the dive (means ± SE; 4 divers at 5 and 6 atm; 6 divers at 7, 1.5, and 1 atm pre- and postdive).

The tracking test (Fig. 2, *double line*) showed few changes. As with the other tests, there was a general trend toward improved performance throughout the dive. There was no evidence of a deterioration of teamwork.

The variances in the standing steadiness (Fig. 3) showed a maximum effect on arrival at 7 atm. In the following days a tendency toward normalization could be seen.

Generally, the results show little variation from day to day. After initial adjustments there was no great decrement in performance as a result of breathing nitrox at 7 atm. The observed deterioration on arrival at 5, 6, and 7 atm the first day tends to confirm earlier suggestions that the narcosis is more pronounced during the first period of a dive and that there is an adaptation or acclimatization factor over time (14–16). Nevertheless, the number of subjects and amount of data collected do not allow definite conclusions.

Performance in the *Nisat* experiment (17,18,3) showed an initial decrement on reaching bottom, a slight recovery, then a secondary depression on the fourth day at pressure. This biphasic effect is not evident in *Nisahex* results.

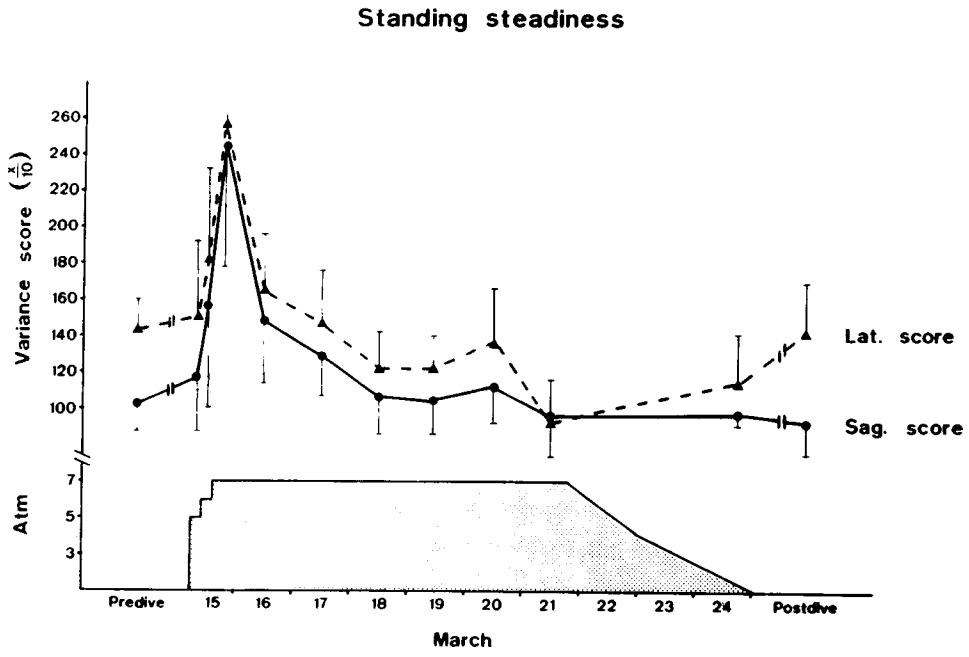



Fig. 3. Standing steadiness. Diver stands on statometer equipment with transducers on front-back (*Lat.*) and side-to-side (*Sag.*). Motions used to maintain balance are recorded on tape and their variances computed. Results show initial effect on variance of each stage of compression, with steady return to normal beginning the second day (means ± SE; 4 divers at 5 and 6 atm; 6 at 7, 1.5, and 1 atm pre- and postdive).

## Subjective Narcosis

The level of narcosis experienced by the divers was assessed by subjective observations.

At 60 msw the divers were clearly narcotized, but none got sick or complained of nausea. They described the feeling as like being mildly drunk. For *Team A* the narcosis came on during the first 5–10 min after switching to nitrox. The expected wide difference in susceptibility among six subjects prevailed throughout the exposure. The divers were cooperative and generally quite happy. They could perform their expected tasks, but more slowly and with some difficulty. New or unrehearsed tasks were much harder. Some had difficulty enjoying reading or even watching TV throughout the bottom time.

The second day in nitrox (*Day 6* in Fig. 1) the divers were still narcotized. Yet all experiments, including the excursion dives, were performed according to plan. During the first trimix excursion to 100 msw the divers felt more narcosis, even though the lockout mix gave the same $P_{N_2}$, 6.6 atm, as was in the living chamber. On the third day at 60 msw (some 48 h after starting the nitrox exposure) they still felt drunk, but the less affected divers felt

and acted essentially normal and were able to do complex tasks. On the fourth day at 60 msw some felt the narcosis level was now slight, others said it was still pronounced. By the sixth day in nitrox all felt more reliable and noticed that they could now think more easily than before (17,3). The *Nisat* experiment had about the same findings, but two of three divers in that exposure were nauseated for 2 days.

The divers scored their level of narcosis each day, on a scale where 3 is pronounced, 2 is noticeable, 1 is slight, and 0 is none. The average score the first 2 days was 2.0, and it gradually and steadily decreased to just over 1 on the day decompression began but did not reach 0 until the dive was over. Interestingly, the range of scores was narrow the first 2 days, with all divers scoring 2; for the next few days the range was wider, 1 to 2.5, then it narrowed as the mean diminished.

## PHYSICAL PERFORMANCE

### Dynamic Work

The divers performed exercise tests on alternate days on the bicycle ergometer. Workload was increased in steps of 50 watts (W) at 5-min intervals. The average peak value of $CO_2$ was estimated for each 5-min period. Workloads of 150–250 W were attained before reaching our arbitrary heart rate limit of 150.

Heart rate at specific workloads (100 and 150 W) gradually increased over the dive period (Fig. 4). The heart rate was considerably lower at 90 msw than at 60 msw. For both divers the depression was nearly 20% on all work levels. The increased pressure and the increased $P_{O_2}$ would be, at least in part, responsible for this depression (19).

The $CO_2$ level of the expired gas reached high levels during work but the increase at specific work levels was less pronounced toward the end of the period at 60 msw (Fig. 5). During the dry nitrox *Excursions V* and *VI* to 75 and 90 msw, workloads of up to 200 W were well tolerated. The divers did not experience much difference compared to similar work at 60 msw. The $P_{CO_2}$ at 60 msw approached 8 kPa (60 mmHg) at 200 W, and did not reach higher levels at 75 and 90 msw during excursions. Neither narcosis nor breathing resistance was considered disturbing.

### Static Work

To assess if the chamber environment affected the muscular performance of the divers, we measured voluntary isometric force daily. The muscle groups studied were those that comprise the left handgrip and the right elbow flexors. The instrumentation consisted of two Bofors strain-gauge dynamometers. One was used for the handgrip and the other for the elbow-flexion measurements. The subject was seated on a plywood board to give him a firm support. A

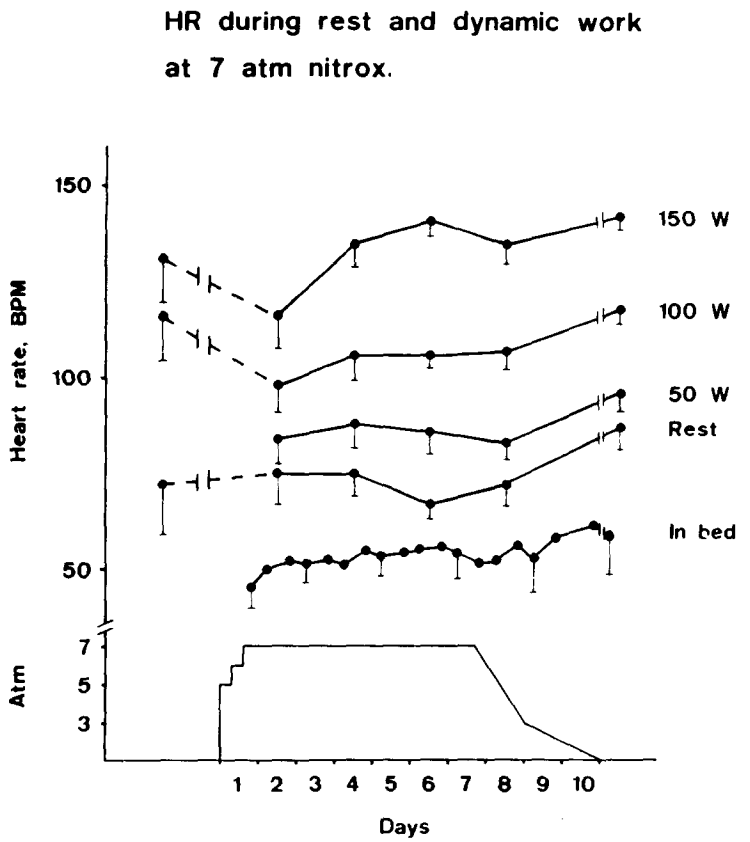



Fig. 4. Heart rates during rest and exercise. Each exercise run consisted of 5 min work on bicycle ergometer at each work level, or until heart rate reached 150 beats/min (BPM). Some individual runs reached 250 watts (W). Except for pre-exercise "rest" periods while sitting on ergometer, results show classical hyperbaric bradycardia, recovering after several days. Heart rates of divers resting in bed show gradual increase, but degree of initial decrease is obscured by lack of predive data.

nonelastic sling attached to the force transducer was placed over the right wrist, which was held in a semipronated position and adjusted to give the elbow an angle of 90°.

The handgrip strength was measured with the dynamometer held in the left hand and supported by the right hand or the knee. Precordial ECG electrodes and a cardiotachometer were used to monitor the heart rate during the experiments. Maximum voluntary contraction (MVC) of elbow flexion and handgrip were measured each evening to avoid interference with other measurements. The subjects made three trials, with 1-min intervals between successive contractions; the highest result was taken as the MVC.

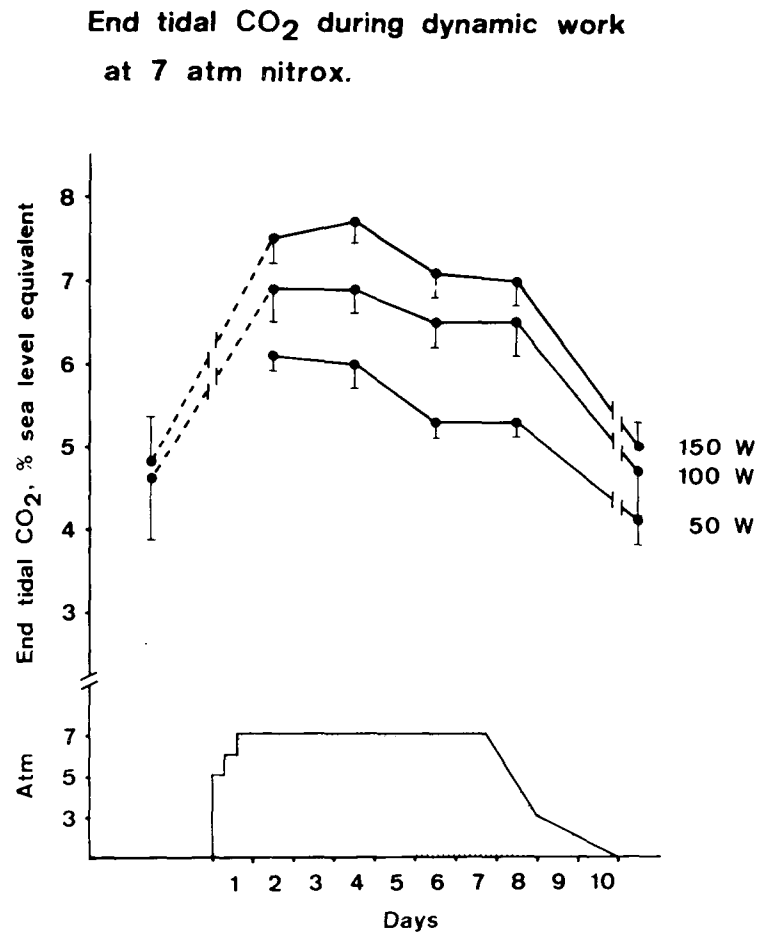



Fig. 5. End-tidal $CO_2$ during exercise. Runs the *same as in Fig. 4*: divers breathing chamber atmosphere and exhaling through a simple low-resistance valve with end-expiratory gas analyzed by mass spectrometer. Divers tolerated higher $CO_2$ levels at all workloads during first days at depth, returning toward a normal response during the next few days.

During measurement of endurance the divers were asked to pull a force of 50% of their MVC as shown on an analog instrument (which was disconnected during the MVC measurement). The elbow-flexion endurance time was measured at an arbitrary contraction level of 50% of the MVC from the previous evening. When the subject could no longer maintain the assigned level he rested for 1 min, then performed a second endurance period to assess the recovery taking place during the 1-min rest. After a 3-min rest the left handgrip endurance was timed and the level of recovery was measured with a second MVC maneuver 1 min later. The results of the muscle tests are shown in Fig. 6.

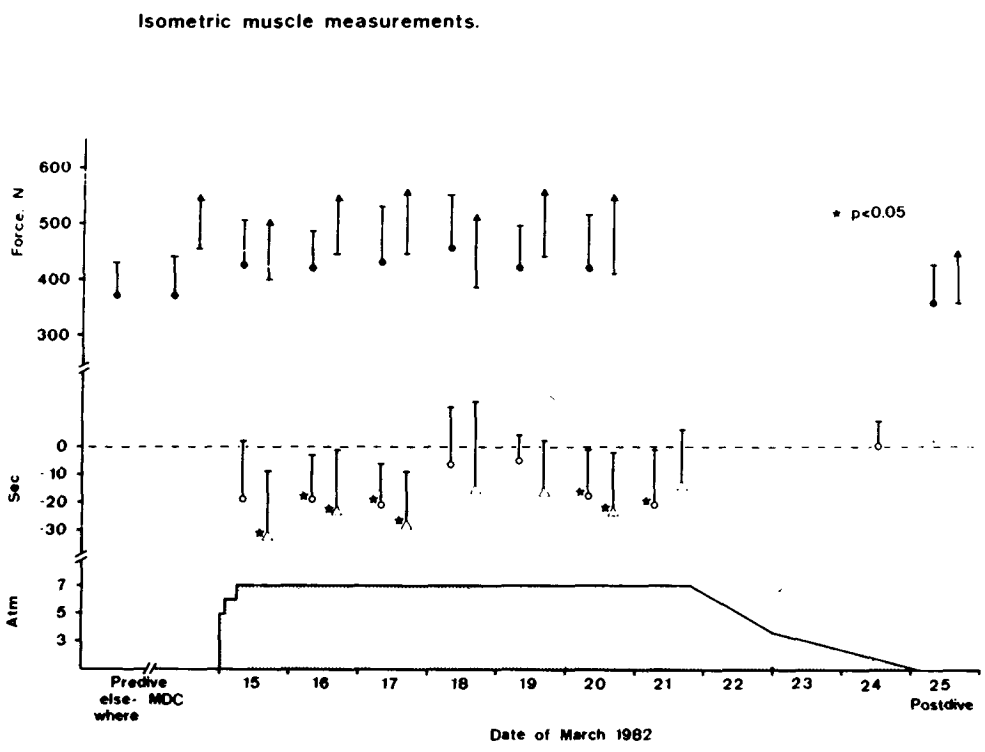



Fig. 6. Isometric muscle measurements. *Solid symbols* show maximum voluntary contraction (MVC) measured with strain gauges; *triangles* are left-hand grip strength; *circles* are right elbow flexion; *bars* are 1 SD, MVC was essentially unchanged. *Open symbols* are endurance, the time diver could maintain 50% of previous MVC, expressed as difference from surface control value. These were reduced significantly (*stars*) on all but the 4th and 5th days at pressure.

## Maximum Voluntary Contraction

During the nitrox saturation exposure the handgrip and elbow-flexion MVC of *Divers 1*, *3*, and *6* were unaffected. *Divers 4* and *5* seemed to have a gradual increase in elbow-flexion strength, and *Diver 2* had a significant rise in handgrip strength ($P < 0.05$). But none of these effects can be separated from a possible training effect. No significant change in MVC could be seen for the group as a whole.

## Muscular Endurance

The endurance times showed a statistically significant decrease during time at pressure and remained 20–30% low during all but the fourth and fifth days at 7 atm. Because the loads were varied (as a result of variations in MVC) the

raw values were compared with a Rohmert plot, a graph showing the standardized relationship between percent MVC and endurance time (20). The perpendicular distances from the measured data points to the curve were measured and compared to the surface values. When analyzed in this way we still found a decreased endurance during the first 3 days at pressure. During decompression, at 17 msw the elbow flexion had returned to surface values.

## Muscular Recovery

The "rate of recovery," measured as endurance after 1-min rest, did not change, either for elbow flexion or handgrip strength. Endurance tests for the handgrip were not done on some divers (at their request) at 17 msw because of fear of provoking bends by too much isometric work. Also, the postdive values were unreliable because the divers were mentally and physically exhausted at that time.

## Heart Rates

An in-bed ECG (standard leads) was taken before standing up each morning and after going to bed at night. No significant respiratory arrhythmias or changes in electrical axis (21) could be detected as a result of breathing dense gas. After the initial drop the heart rate increased over the dive; the lowest value was the first night at pressure. Because we lacked a good predive baseline, we compared individual means of first and second morning values with fifth and sixth morning values; this comparison gave a mean individual increase in heart rate of 5.5 $\pm$ 4.3 beats/min ($P < 0.05$). The same procedure for the evening values gave an increase in heart rate of 4.8 $\pm$ 2.1 beats/min ($P < 0.005$). The mean in-bed heart rates and standard deviations of all six divers are plotted in Fig. 4.

The resting heart rates while sitting waiting for the static muscle experiments show the same tendency, with an increase from 63 $\pm$ 7.9 the first 2 days at pressure to 76 $\pm$ 11.5 the fourth and fifth day at pressure. The first 2 days at pressure the mean increase in frequency during 30 s of endurance test (50% MVC) yielded 27 $\pm$ 16.2 beats/min increase in arm and 16 $\pm$ 11.5 beats/min in hand work. On the fourth and fifth day the corresponding values were 18 $\pm$ 9.3 for arm work and 11 $\pm$ 9.8 for hand work.

During dynamic work the high pressure bradycardia and its tendency to disappear over time at pressure is seen. The lack of a clear bradycardia at rest on the bicycle the first day at pressure might be caused by tension in the new situation, and that the divers were not fully at rest because of movements and equipment preparation. The high resting heart rate at surface after the decompression could be caused by lack of sleep; surface was reached in the middle of the night and all divers wanted to get out of the chamber.

In conclusion, the stay at pressure does not seem to have had any harmful effects on the physical performance. Surprisingly, high workloads were tolerated on the ergometer. Maximum voluntary contraction remained stable or slightly

increased. Endurance during static work decreased initially but recovered after a few days. Recovery after static endurance tests was not affected at all. Measurements of heart rate showed hyperbaric bradycardia both during rest and work. This bradycardia was less pronounced towards the end of the dive.

## DECOMPRESSION

Two separate decompression tasks were involved in *Nisahex*, the excursions and the decompression from saturation.

Decompression from the cold survival test was according to accepted rates for heliox decompression over the range covered, 2 msw/h from 150 msw to 50 msw, and was uneventful. The $P_{O_2}$ was 0.5 to 0.55 atm.

### Excursions

We calculated decompression from the excursions using our laboratory's established DCAP program based on commercial heliox and nitrox experience and other NOAA OPS-type dives (2,22). This program uses a neo-Haldanian approach with an 11-compartment model. Because the excursions were both deeper and longer than those of NOAA OPS, where most testing had been done, we made the ascent-limiting matrix more conservative, about halfway between the NOAA OPS matrix (1) and one established in commercial diving decompressions (23). The entire dive profile was considered in each calculation.

Details of the excursions are given in Table II, including bottom times, gas mixtures, and stops. There were no problems, and no bubbles were detected by Doppler ultrasonic monitoring after each excursion.

Experience in the use of helium for excursions from nitrox saturation is limited. We took the approach that counterdiffusion bubbles can be avoided by compressing enough to prevent development of supersaturation; because this point was far exceeded, the excursions offer no real test for this principle (24).

### Saturation Decompression

Decompression from air and nitrox saturations in the shallow depth range (15–20 msw) have become relatively routine (2). However, for saturation depths in the range of 30 msw and beyond there have been problems. Many of these difficulties have been noted in conjunction with saturation treatments of decompression sickness and embolism, with the result that data are difficult to interpret and are not usually reported. One thing does seem clear: that as the saturation depth increases the rate of ascent has to be slower.

The table used for *Nisahex* was developed in collaboration with Dr. R. E. Peterson, then of the Norwegian Underwater Technology Center. It was a conservative descendant of the British Navy's RN 71 (25); this is not intended to be a saturation table, and there is little published experience in its use (26). It was conducted with 1-msw stops, with planned "rates" of 15 min/msw to

56 msw, then 60 min/msw to 20 msw, and 120 min/msw thereafter. These rates were followed except for a recompression from 11 msw to 13 msw; the 120-min/msw rate was resumed after a 170-min hold for treatment. $P_{O_2}$ was 0.5–0.55 until reaching 21% oxygen; air was used from then on. Oxygen was breathed by *Divers 1*, *2*, *5*, and *6* at 16 msw, 6 cycles of 20 min on, 5 off; 3 cycles were breathed by *Divers 1*, *2*, *3*, and *4* at 8 msw.

On reaching 11 msw, *Diver 3* had mild muscle pain in the left shoulder, which was treated at 13 msw with 6 cycles of oxygen. The remaining ascent to surface was uneventful, but it became evident afterward that *Diver 6* had knee pain; he had felt at the time that it resulted from a previous sprain and that it was not strong enough to merit treatment.

A complex series of symptoms and treatments followed surfacing, which was on Thursday morning at 0110. *Diver 1* felt an aching in his upper arm on Friday evening; it resolved overnight without treatment. Also on Friday *Divers 3* and *5* had the beginnings of vague muscle pains and *Diver 6* still favored his knee. On Sunday *Divers 5* and *6* were treated with USN Table 6; *Diver 5* was relieved, *Diver 6* still had some feeling. Also, *Diver 2* noted elbow and axillary lymph node pain. On Monday *Diver 3* also developed pain in his same shoulder, and both *Divers 2* and *3* were given a Table 6 and were relieved. During the following week *Divers 5* and *6* again had vague symptoms and were treated again on Saturday.

Thus, it appears that the adjustments to the familiar nitrox saturation decompression were not sufficient for a dive starting this deep. In a more recent exposure at Duke University to 50 msw, the rates were slowed even further but there were bends nonetheless (27). In the *Nisat I* dive, Harvey decompressed at 54 min/ft or 177 min/msw with no problems, and it now looks as if that was perhaps not as excessively conservative as he had thought.

## CONCLUSIONS

Significant findings of *Nisahex* include demonstrating the feasibility of deep excursions, especially those using helium, and that they can be long enough to enable work to be done and to require decompression stops. Living in nitrox at 7 atm need not cause nausea or significant discomfort, but clearly causes narcosis. Some types of performance were reduced at first, but in most cases returned to near normal after 4–6 days; the narcosis did not completely resolve until decompression. Heavy exercise proved possible, and like other factors seemed to be tolerated better after some time at pressure. Maximum muscle tensions were unchanged, but isotonic endurance time seemed to be reduced.

We never believed that the depth chosen for saturation would be a model for operational work, and, indeed, it proved too narcotic and the recovery too slow to offer much practicality. Nevertheless, the principle of saturating and accommodating to the environment then accessing the work area by excursions seems feasible. The use of trimix as the excursion gas bears further study.

Another area clearly in need of further work is the final decompression from saturation. Possibilities here, in addition to significantly slower ascent rates, include the use of helium as a component of the decompression gas and reducing the bottom depth by more extensive use of excursions.

## References


1. Hamilton RW, Kenyon DJ, Freitag M, Schreiner HR. NOAA OPS I and II: Formulation of excursion procedures for shallow undersea habitats. UCRI-731. Tarrytown, NY: Union Carbide Corp, 1973.
2. Miller JW, Adams GM, Bennett PB, Clarke RE, Hamilton RW, Kenyon DJ, Wicklund RI. Vertical excursions breathing air from nitrogen-oxygen or air saturation exposures. Rockville, MD: National Oceanic Atmospheric Administration, 1976.
3. Hamilton RW, Adams GM, Harvey CA, Knight DR. SHAD-NISAT: A composite study of shallow saturation diving. Groton, CT: Naval Submarine Medical Research Laboratory, 1982. (NSMRL Rep No 985)
4. Magnusson J, Holmér I, Elnäs S, Hamilton RW. Metabolic rate of divers in a simulated "lost bell" survival test. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc. 1984:349–360.
5. Paciorek JA, Örnhagen H, Eiken O, Liner M. Red cell morphological and hematological studies in men during and after a 60-m nitrox chamber dive. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:301–308.
6. Tengroth B, Högman B, Linde CJ, Bergman H. Readaptation time after photo stress. Readaptation time as a function of oxygen concentration. Acta Ophthalmol 1976;54:507–516.
7. Högman B, Bergman H, Borg S, Eriksson T, Goldbert L, Jones AW, Linde CJ, Tengroth B. Readaptation time after photo stress. Alcohol-induced acute and post-alcohol "hangover" changes in ocular readaptation time. Psychopharmacology 1977;53:165–167.
8. Bergman H, Borg S, Högman B, Larsson H, Linde CJ, Tengroth B. The effect of Oxazepam on ocular readaptation time. Acta Ophthalmol 1979;57:145–150.
9. Linde CJ. The effect of welding fumes on ocular readaptation time. Scand J Work Environ Health 1980;6:135–145.
10. Stroop JR. Studies of interference in serial verbal reactions. J Exp Psychol 1935;18:643–661.
11. Adolfson J, Goldberg L, Berghage T. Effects of increased ambient air pressure on standing steadiness in man. Aerosp Med 1972;43:520–524.
12. Adolfson J, Fagraeus L, Hesser CM. Deterioration of standing steadiness in hyperbaric environment. Försvarsmedicin 1973;9:253–256.
13. Adolfson J, Bjerver K, Fluur E, Goldberg L. Balance disturbance in man at 10 ATA ambient air pressure. Försvarsmedicin 1974;10:148–156.
14. Moeller G, Chattin C, Rogers W, Laxar K, Ryack B. Performance effects with repeated exposure to the diving environment. J Appl Psychol 1981;66:502–510.
15. Schmidt TC, Hamilton RW, Moeller G, Chattin CP. Cognitive and psychomotor performance during NOAA OPS I and II. Tarrytown, NY: Union Carbide Corp, 1974. (Tech Memo CRL-T-799)
16. Adolfson J. Human performance and behaviour in hyperbaric environments. Stockholm: Almqvist & Wiksell;1967:52–53.
17. Weybrew BB. Diver adaptability during a nitrox saturation dive at 7 ATA. Undersea Biomed Res 1978;5:259, 273.
18. McKay CL, Strauss MS, Kinney JAS, Luria SM. Visual evoked responses, EEG's and reaction time during a normoxic saturation dive. NISAT I. Undersea Biomed Res 1977;4:131–145.
19. Flynn ET, Berghage TE, Coil EF. Influence of increased ambient pressure and gas density on cardiac rate in man. Panama City, FL: Navy Experimental Diving Unit, 1972. (NEDU Rept. 4-72)

20. Rohmert W. Ermittzung von Erholungspausen fur statische Arbeit des Menschen. Int Z Angew Einschl Arbeitsphysiol 1960;18:123–164.
21. Beckwith JR. Grant's clinical electrocardiology: The spatial vector approach. New York: McGraw Hill, 1970.
22. Hamilton RW, Kenyon DJ. DCAP: Decompression computation without a computer expert. In: Proceedings, International Diving Symposium '82. Gretna, LA: Association of Diving Contractors, 1982.
23. Retallack LJ, Lomnes RK, Kenyon DJ, Peterson RE, Hamilton RW. A specialized microprocessor system for decompression management of deep diving. Publ 77-WA/OCE-7. New York: American Society Mechanical Engineers, 1977.
24. Peterson RE, Hamilton RW, Curtsell I. Control of counterdiffusion problems in underwater dry welding. In: International Diving Symposium '80. Gretna, LA: Association of Diving Contractors, 1980.
25. Diving Manual. BR 2806. London: Ministry of Defence (Navy), 1972.
26. Buckingham IPB, Thalmann ED. Saturation therapy for dysbaric injury. Undersea Biomed Res 1981;8(Suppl):13.
27. Barry PD, Vann RD, Youngblood DA, Peterson RE, Andersen JC, Bennett PB. Decompression from a deep nitrogen/oxygen saturation dive: A case report. Durham, NC: FG Hall Environmental Laboratory, Duke University, 1983.

# ALTERATION OF SOLEUS ELECTROMYOGRAM SILENT PERIOD IN MAN AT PRESSURES UP TO 70 BARS

*D. J. Harris and P. B. Bennett*

Prominent among the physiological effects provoked by compression to depths in excess of 180 msw are disturbances of the motor system such as tremor, incoordination, brisk myotatic reflexes, spontaneous myoclonic twitches, and fasciculations (1–4). We are far from understanding the underlying causes of all these phenomena, since the answers often appear to lie beyond the limits of our present general neurophysiological knowledge. Nevertheless, spinal reflex tests have been used to study certain aspects of the peripheral motor control system, with some success (3,5–8). One such test concerns the *silent period* phenomena in the electromyogram (EMG).

When constant voluntary muscular activity is interrupted by the superimposition of a single-reflex twitch contraction, a silent period occurs in the EMG and coincides with the rising phase of the twitch contraction (9,10). This period of more-or-less total inhibition is followed by a period of enhanced EMG activity (rebound), which coincides with the relaxation phase of the twitch. The rebound activity usually produces a small secondary twitch, which in turn generates a second silent period. Between two and four silent periods separated by rebounds of quickly diminishing size usually can be observed (*see* Fig. 1). One explanation states that oscillations up and down the stretch reflex loop may be responsible for what is effectively a highly damped clonus, as the neuromuscular system attempts to restore the desired level of activity following the disturbance (11,12). If the loop were underdamped (lack of inhibitory feedback, excess reflex loop gain), unstable conditions would result in sustained clonus. If the loop were overdamped (excess inhibitory feedback, hyporeflexia), it would take too long to adjust to new sensory input. If a delay was introduced into the loop, the frequency of clonus would be expected to fall.

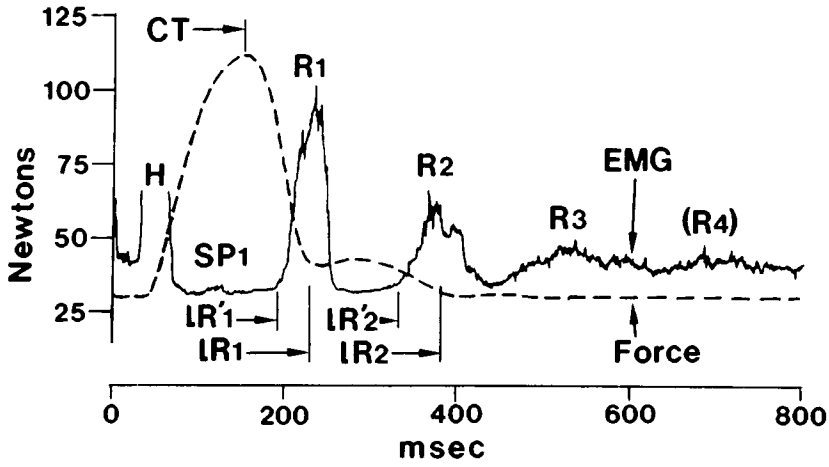



Fig. 1. Rectified, averaged ($n$ = 100) trace of soleus EMG activity during voluntary contraction of 30-newton force. *Solid line*: EMG; *dashed line*: force trace. Stimulus given at 0 msec produces superimposed H-reflex (H) followed by first silent period (SP1) in the EMG, then a rebound (R1) of EMG activity. Further diminishing silent periods and rebounds (R2-R4) may follow before steady voluntary activity is restored, as at end of trace. CT: contraction time of H-reflex twitch. tR'1: time from stimulus to start of R1 burst; tR1: time from stimulus to middle of R1 burst; tR'2: time from stimulus to start of R2 burst; tR2: time from stimulus to middle of R2 burst.

During the *Atlantis III* 70-bar simulated dive, stretch reflex responsiveness was increased, although normal alpha motor pool excitability was preserved, a condition implying a gamma motorneurone/muscle spindle mechanism for hyperbaric hyperreflexia (13). In addition, increases in the peak force and contraction time of the muscle twitch were observed, which were independent of reflex responsiveness (8). We report here the apparent consequences of these effects upon the frequency, amplitude, and duration of the damped clonic activity in the soleus EMG.

## METHODS

Observations were made during the *Atlantis III* simulated dive to 70 bars using trimix 10 (10% $N_2$, 0.5 bar $O_2$, rest He) carried out at Duke University Medical Center. Three healthy male subjects were studied: 5 times during an 8-week predive control period; 4 times during compression at 56 (*Day 4*), 63 (*Day 6*), 66 (*Day 8*), and 70 bar (*Day 13*); twice during decompression at 56 (*Day 18*) and 44 bar (*Day 26*); and twice postdive (+4, +14 days). Other details about the dive are given elsewhere (14).

In each session the subject was seated comfortably in a reflex chair with knee and ankle angles clamped at 115° and 90°, respectively (13). The subject was asked to maintain an isometric soleus plantar flexion of 30-newton force.

This was measured by a force transducer attached to the underside of the footplate and displayed to the subject on an analogue voltmeter. The methods used to stimulate and record Hoffmann (H-) reflex responses are described in detail elsewhere (3,13). For the present test, an H-reflex was elicited in the soleus every 5 s by electrical stimulation of medial popliteal nerve using 1-ms pulses ($n$ = 120). Electrical activity in the soleus was recorded with the aid of bipolar surface electrodes placed on the dorsal midline of the muscle about 4 cm below the bulge of the gastrocnemius. One hundred responses (EMG and force) were amplified, rectified (EMG only), averaged by a signal-averaging computer, and displayed in ink by an X-Y plotter.

A typical averaged trace is shown in Fig. 1. The following parameters were measured by hand or calculated from such tracings (*see* Fig. 1):

CT, twitch contraction time, from stimulus to peak tension
tR'1, time from stimulus to start of R1 burst
tR1, time from stimulus to middle of R1 burst
tR'2, time from stimulus to start of R2 burst
tR2, time from stimulus to middle of R2 burst
$f_1$, clonic frequency based on R1-R2 interval (1 cycle)
$f_3$, clonic frequency based on R1-R4 interval (3 cycles).

## RESULTS

The control values for latency and frequency of clonic components obtained from each subject are shown in Table I. Reproducibility was generally good and standard deviations were usually 5% or less of the mean. Intersubject variability due to differences in body shape, and the like, was often statistically significant though not important in itself. Nevertheless, for this reason and because the number of subjects was small (three), each subject was used as his own control when we calculated changes observed at pressure. The data

TABLE I

Latency and Frequency of Clonus Parameters During Surface Control

| | CT | tR'1 | tR1 | tR'2 | tR2 | $f_1$ | $f_3$ |
|---|---|---|---|---|---|---|---|
| *Subject* | msec* | | | | | Hz* | |
| *SP* | 160.4 | 203.8 | 253.0 | 350.1 | 414.0 | 6.23 | 6.32 |
| | ±7.1 | ±11.3 | ±6.5 | ±16.8 | ±11.6 | ±0.43 | ±1.25 |
| *LW* | 154.5 | 225.4 | 270.2 | 362.5 | 405.2 | 7.45 | 7.04 |
| | ±7.6 | ±3.1 | ±6.8 | ±14.8 | ±12.4 | ±0.53 | ±0.35 |
| *EK* | 157.6 | 187.8 | 233.6 | 316.8 | 353.0 | 8.27 | 7.87 |
| | ±3.4 | ±11.5 | ±4.5 | ±10.1 | ±7.4 | ±0.38 | ±0.36 |

*Mean ± 1 SD; $n$ = 5 sessions (× 100).

were then pooled and the means of the percent changes observed in the three subjects at each pressure were tabulated. Thus, Table II confirms that the H-twitch contraction time was slowed increasingly during compression to 66 bars, as we have previously reported (8). It is also evident that the latency of all measured rebound parameters increased during compression. In particular, there was an almost linear relationship ($r = 0.97$, $P \ll 0.001$, linear regression by least-squares method) between the mean increases in CT and in tR1. Indeed, the regression equation shows that a 25-ms increase in CT would be associated with a 23.6-ms increase in the latency of the R1 rebound, almost a 1:1 relationship. The latency of R2 was also correlated with the slowing of CT ($r = 0.94$, $P < 0.001$), an increase in CT of 25 ms associated with a 55-ms increase in tR2. The difference between the calculated increase in the latencies of R1 and R2 reflects the observed change in the $f_1$ frequency of clonus. Table II reveals that $f_1$ was slowed on average by 32%, from 7.3 Hz to 5.0 Hz, after compression to 66 bars. The $f_3$ frequency of clonus was similarly slowed. Coefficient of correlation between CT and $f_1$ was $r = -0.76$, $P < 0.05$; between CT and $f_3$ it was $r = -0.95$, $P < 0.001$.

The above effects of compression on the latency of clonus parameters were consistently seen in all three subjects. But the extent to which the amplitude and duration of clonus was affected varied considerably. One subject (*LW*) revealed normal damping of clonus throughout the dive; EMG and force trace patterns were unchanged from predive controls. Interestingly, this subject was assessed as showing the least decrement of the three subjects in psychomotor

TABLE II

Change in Latency and Frequency of Clonus Parameters During *Atlantis III*

| | CT | tR'1 | tR1 | tR'2 | tR2 | $f_1$ | $f_3$ |
|---|---|---|---|---|---|---|---|
| | mean difference (%) from control* | | | | | | |
| Compression (bars) | | | | | | | |
| 59 | 6.6 | −1.6 | 1.8 | 11.0 | 10.4 | −18.7 | −14.7 |
| 63 | 13.3 | 8.9 | 7.8 | 17.1 | 15.2 | −27.8 | −24.2 |
| 66 | 30.9 | 13.3 | 18.1 | 14.3 | 29.8 | −31.7 | −33.1 |
| 70 | 15.9 | 7.9 | 6.1 | 12.9 | 13.7 | −20.2 | −20.8 |
| Decompression (bars) | | | | | | | |
| 44 | 5.7 | 6.4 | 4.3 | 12.5 | 9.6 | −15.3 | −12.7 |
| 39 | 2.8 | 8.5 | 2.4 | 14.6 | 13.7 | −25.9 | −15.1 |
| Postdive (days) | | | | | | | |
| + 4 | −1.2 | −1.2 | −4.2 | 4.5 | 3.1 | −13.3 | −9.1 |
| +14 | −2.1 | −0.1 | −5.0 | 6.3 | 6.1 | −3.6 | −2.5 |

*Mean of 3 subjects' percent differences.

performance (14) and in psychiatric performance (Dr. P. Logue, personal communication). *Subject EK* exhibited a tendency towards clonus, occasionally sustained for several beats, which was noticeable at 59 and 63 bars. Waveforms recorded at 66 bars and during decompression appeared normal. The third subject (*SP*) showed an interesting range of pressure-related responses, examples of which are displayed in Fig. 2. At 59 bars, evidence of underdamping was observed with clonus persisting for an extra beat. At 63 and 66 bars the greatly slowed clonic contractions became less distinguishable from one another, a condition effectively overdamping the system slightly. This condition had improved slightly at 70 bars (after a 5-day pressure hold at 66 bars). Decompression was characterized by a marked increase in duration and amplitude of clonus, an effect suggesting a significant underdamping. These latter observations were coincident with a fall in the $N_2$ percentage from 10 to 5%. The EMG records reflect the different underlying neuromuscular activity at the various pressures.

## DISCUSSION

The $f_3$ clonic frequency of 6.2 to 8.2 Hz recorded from our subjects at the surface corresponds very closely with the 6 to 9 Hz *physiological tremor* measured at the statically flexed ankle joint by Pozos et al. (15). These workers also showed that during voluntary sinusoidal ankle movement, a tremor of larger amplitude but slower frequency (4–8 Hz) called *physiological action tremor* was predominant. It is important to distinguish the latter from the lowered frequency of damped clonus seen during compression of the dive reported here. In the present case the ankle remained statically flexed and yet the clonic frequency was lowered, even when the amplitude of the response remained unchanged. If the slowing of the clonus was caused by a slowing of muscle contraction-relaxation, as we have suggested, it follows that in the case of the ankle joint at least, the mechanical properties of the limb (resonance, inertia, and the like) are important in establishing the frequency of the alternating silent period and rebound phenomena. It is difficult therefore to escape the probable importance of the re-activation of the stretch receptors during the preceding relaxation when one determines the onset and amplitude of the rebound bursts in a voluntarily contracted muscle. The reflex loop appears to have an important role in ankle clonus.

Some investigators have suggested that such oscillations in the stretch reflex loop are important in the generation of the normal 8 to 12-Hz physiological tremor (this frequency is recorded at the finger [11,12,16]). Others include reflex loop oscillations among a number of factors, any of which may be involved in different forms of tremor (17,18). It is too early to say whether our findings can be applied to tremor in general since these observations are so far confined to the compression of one experimental dive with the breathing gas 10% trimix. Nevertheless, one implication for the hyperbaric physiologist may be suggested. After a compression in trimix 10 to depths of 550 msw or deeper,

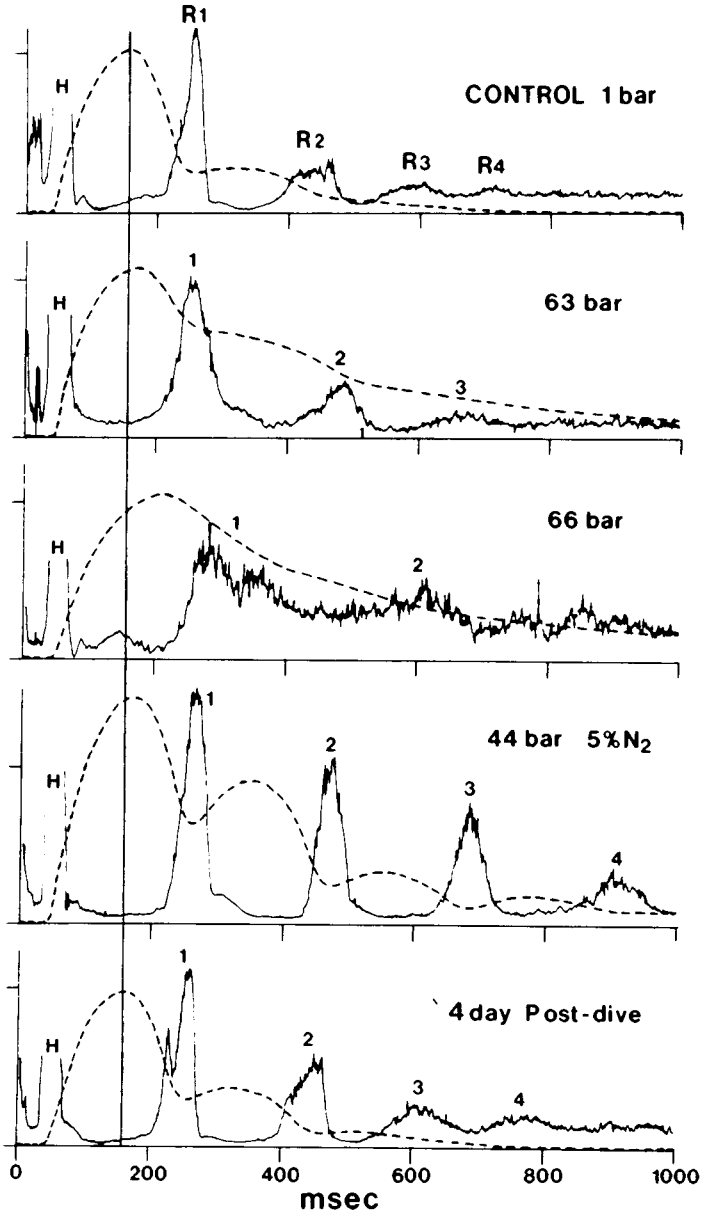



Fig. 2. Sequence of records from *Subject SP* showing variations in frequency, duration, and amplitude of soleus clonic activity with pressure. *Vertical connecting line*: control value of CT, contraction time of H-reflex twitch. *Vertical calibration*, 100 newtons. H: H-reflex; R1: rebound of EMG activity; R2, R3, R4: further rebounds of EMG activity (*also see* Fig. 1).

a temporary slowing of ankle clonus and possibly tremor need not of itself imply an underlying central neuropathological condition. It might be caused by a muscle-contraction effect acting via the reflex loop. It is not known whether the observed slowing is a feature only of deep trimix-10 compression, or whether it could be provoked in any deep compression.

The other findings in this study concern the amplitude of the rebound bursts (and hence the clonic muscular contractions) and the number of clearly definable silent periods and rebounds (the damping). Changes in these parameters were quite different among the subjects, underlining the considerable individual variability of response to a given set of environmental conditions. There was evidently a tendency for the strength and duration of clonus to increase at times in certain subjects, while others appeared normal. Without having the advantage of comparable measurements made in helium-oxygen with which to compare our results, it is not possible to determine the benefit, or otherwise, of nitrogen in these particular events. Only future studies will show whether the absence of nitrogen or a lesser quantity would result in a greater incidence of increased clonus.

## Acknowledgments

We acknowledge, with considerable gratitude, the dedication and skill of the three subjects: Stephen Porter, Leonard Whitlock, and Eric Kramer. These experiments would not have been possible without the willing participation and professionalism of all the technical staff of the F. G. Hall Laboratory, Duke University Medical Center, whom we thank sincerely. Thanks are also due to James Parmentier and Zoltan Török for helpful discussion and to Val Rees for typing the manuscript.

Funding support was provided by NIH grant HL07896, Shell Oil International, U.S. Navy Bureau of Medicine and Surgery, Oceaneering International, and the United Kingdom Ministry of Defence Procurement Executive.

## References

1. Hugon M. Mammalian nervous system at depth: Introduction to a round table. In: Obal F, Benedik G, eds. Adv Physiol Sci, vol 18. Environmental Physiology. Academiai Kiado, Budapest; Pergamon Press, 1980:135–141.
2. Török Z. Recent experience in deep diving research. Proc Soc Underwater Technology Conference, Brighton, UK. London: Society for Underwater Technology, 1982.
3. Harris DJ. Hyperbaric hyperreflexia: Tendon jerk and Hoffmann reflexes in man at 43 bar. Electroencephalogr Clin Neurophysiol 1979;47:680–692.
4. Bennett PB. The high pressure nervous syndrome. In: Bennett PB, Elliott DH eds. The physiology and medicine of diving, 3rd ed. London: Baillière Tindall, 1982:262–296.
5. Roll JP, Lacour M, Hugon M, Bonnet M. Spinal reflex activity in man under heliox conditions (31 and 62 ATA). In: Shilling CW, Beckett MW, eds. Underwater physiology VI. Proceedings of the sixth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1978:21–27.
6. Harris DJ. Observations on the knee-jerk reflex in oxygen-helium at 31 and 43 bar. Undersea Biomed Res 1979;6:55–74.

7. Hugon M, Fagni L, Rostain JC, Seki K. Somatic evoked potentials and reflexes in monkey during saturation dives in dry chamber. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1981:381–390.
8. Harris DJ, Bennett PB. Force and duration of muscle twitch contractions in humans at pressures up to 70 bar. J Appl Physiol: Respir Environ Exercise Physiol 1983;54:1209–1215.
9. Paillard J. Réflexes et régulations d'origine proprioceptive chez l'homme. Thèse Fac Sci Paris (Série A nr 2858–3729), Paris, Arnette, 1955;293P.
10. Bouaziz Z, Bouaziz M, Hugon M. Modulation of soleus electromyogram during electrical stimulation of medial gastrocnemius nerve in man. Electromyogr Clin Neurophysiol, 1975;15:31–42.
11. Lippold OCJ. Oscillation in the stretch reflex arc and the origin of the rhythmical 8–12 c/s component of physiological tremor. J Physiol 1970;206:359–382.
12. Eklund G, Hagbarth K-E, Hägglund JV, Wallin EU. Mechanical oscillations contributing to the segmentation of the reflex electromyogram response to stretching human muscles. J Physiol 1982;326:65–77.
13. Harris DJ, Bennett PB. Soleus H-reflex studies in man at 40–70 bar in He-$O_2$-$N_2$ (trimix). Undersea Biomed Res 1984;11:49–64.
14. Bennett PB, Coggin R, McLeod M. Effect of compression rate on use of trimix to ameliorate HPNS in man to 686 m (2250 ft). Undersea Biomed Res 1982;9:335–351.
15. Pozos RS, Iaizzo PA, Petry RW. Physiological action tremor of the ankle. J Appl Physiol: Respir Environ Exercise Physiol, 1982;52:226–230.
16. Joyce GC, Rack PMH. The effects of load and force on tremor at the human elbow joint. J Physiol 1974;240:375–396.
17. Cussons PD, Matthews PBC, Muir RB. Enhancement by agonist or antagonist muscle vibration of tremor at the elastically loaded human elbow. J. Physiol 1980;302:443–461.
18. Matthews PBC, Muir RB. Comparison of electromyogram spectra with force spectra during human elbow tremor. J Physiol 1980;302:427–441.

# INVITED REVIEW: BEHAVIOR AND PERFORMANCE IN DEEP EXPERIMENTAL DIVING WITH MAN—A REVIEW OF RECENT WORK

*Z. Török*

Deep diving is defined in this paper as below 300 msw. As will be seen, current research is focused on depths around 450 msw. Maximum pressure to which man has ever been subjected is 686 msw and was attained using 10% nitrogen added to heliox as the diving gas. The above three statements embody much of the constraints and endeavors defining the current status of deep diving research in the western world. Pioneering work in dry compression chambers targeted depths far in excess of tomorrow's practical commercial and military requirements. On the other hand, answers to tomorrow's problems have been formulated on the basis of very few experiments, leaving many important sidelobes to those problems unexplored.

The choice of the diving gas referred to above is one of the most important tasks and much of current research effort addresses it. In the past few years trimix enabled notable achievements, yet narcotic and psychiatric effects emerging recently mitigate against its practical use. Helium had been introduced to prevent nitrogen narcosis; more recently, a small percentage of nitrogen was added to the diving gas to deal with problems initially thought to be caused by helium. The high pressure neurological syndrome (HPNS) is more than "helium tremors." Here, it is defined as the sum of all ill effects caused by an overdose of compression or pressure, or both, i.e., in the process of compressing men too far or too fast, or both. It is treated phenomenologically; no symptom or sign is excluded should it not fit some assumed unifying causative mechanism (1). Indeed, it is likely that no such unifying mechanism exists, a possibility that may deny HPNS the status of being a *syndrome* in the accepted sense of the word.

A center-by-center approach was followed as the facts were marshalled; in each case the results, facts, and interpretation of them were emphasized by those responsible for obtaining them. Cross-references were used to discuss these interpretations. Inevitable selection was done only to omit the relatively unimportant, and an attempt was made to avoid bias. Previous reviews of the subject were written in 1974 (2) and 1981 (3).

## FRENCH HYPERBARIC STUDIES

Earlier experiments were aimed at exploring the limiting parameters of compression with heliox and trimix, and thereby elicited HPNS in man to study it (4–6). Then, with slow (261 h) compression profiles and heliox in two exceptionally deep chamber dives called *Physalis VI* (May 1972) and *Sagittaire IV* (June 1974), a first attempt was made to define the most important physiological problems awaiting man at 610 msw (7). From today's perspective this pioneering work constitutes pilot experiments to delineate problems for definitive study later.

Using hyperbaric EEG changes (*see* Török, Ref. 8, in this *Proceedings*) as indicators, especially increase in theta-band power and also performance tests, the *Coraz* series of four dives were performed in 1976. In identical 4-h compressions to 300 m, trimix with 9% $N_2$ (*Coraz I*) and with 4.5% $N_2$ (*Coraz II* and *III*) was compared to heliox (*Coraz IV* [9–11]). At 300 m the EEG changes were found to be greater in trimix than in heliox in most divers. Power in the theta band, for example, increased to over 10 times control values in trimix, remaining below 8 times normal in heliox. Furthermore, subjects (the same two in *Coraz I* and *II*) demonstrated more EEG changes in 9% $N_2$ than in 4.5% $N_2$. These changes were maximum 3 to 7 h after arrival at 300 m, decreasing to normality in 14 h (12).

Starting at 240 m and persisting for some hours, paroxysmal EEG activity was seen in both subjects frequently enough to interfere with power spectral analysis of the data (13). The same two subjects (*CB* and *AJ*) took part in earlier 300- to 360-m dives using rapid compression and heliox (*Physalis I–IV*) and also in a slow-compression heliox exposure (11 days to 610 m, *Sagittaire IV*) without producing paroxysmal activity, in spite of the provocation of the visual evoked response stimulator (7).

It is significant that paroxysmal transients have not been reported in other manned trimix exposures and that, in general, they are rarely seen (14,15).

To compare performance in heliox with that in trimix at two different nitrogen levels, a set of three cognitive and psychomotor tests were used. These were applied during *Sagittaire IV* at 400 msw after 4 days compression, and during *Coraz I* and *II*, both after 4-h compression to 300 m with 9% and 4.5% $N_2$, respectively (16). Individual variability was avoided by the use of the same two subjects. This fact, along with the order in which the experiments were performed, strengthens uniquely the deductions made. Rey's test of number ordering (10 min) scored higher cognitive function; a visual two-choice reaction

time test estimating vigilance and simple decision time gave the mean lag of 33 manual responses; and a 2-min pegboard test of manual dexterity indicated, above all, the effect of tremor on motor coordination.

The mean of all three tests for both subjects show performance in trimix containing 4.5% $N_2$ to be no worse at 300 m than for surface controls—even allowing for one erroneously high control score as suggested by the authors (15). The worst score was that of the number ordering test, which showed 8% decrement. The mean decrement at 400 msw in He-$O_2$ was 10%, the worst was vigilance at 15% decrement. The compression with 9% $N_2$ was associated with 20% decrements in both vigilance and higher cognitive function. Thus it is clear that on these test results efficiency was better at 300 m in trimix with 4.5% $N_2$ after fast compression than at 400 m in He-$O_2$ after very slow compression and that efficiency was much worse in trimix with 9% $N_2$ (16).

In the *Coraz* dives with nitrogen, less tremor and dysmetria was seen than in heliox. However, euphoria and other behavioral problems associated with nitrogen in this series, together with the comparative EEG and performance results, gave rise to the authors' preference for heliox (7,9,12,16). None of the combinations of compression rate and gas mixture used was satisfactory. This was illustrated by the statement that none of the teams was able to work for at least 4 h on arrival (12).

The next phase of French deep-diving research was characterized by an interactive deductive process linking hypothesis-testing work on the baboon with manned diving trials. A highly desirable way forward, in general, the like of which is to be found, within diving, only in decompression research. The project was spurred on, no doubt, by the potential significance of the paroxysmal EEG changes (possibly preconvulsive) found in the *Coraz* series in trimix and also by questions relating to some inconsistencies of results and economy of gas mixing. By 1980, 32 baboons had been used in 16 exposures testing various gas mixtures and compression profiles, with EEG changes providing a major criterion for decisions (17,18). Nitrogen was found to be effective for prevention of hyperbaric convulsions before 800 msw in the baboon, a condition never yet encountered in man. Nitrogen also decreased other electrophysiological cortical phenomena, such as EEG frequency changes, and on this basis the following compression profile was designed for the baboon (19,20). It comprised exponentially decreasing compression rates interrupted by stages of constant pressure for a 40-min period every 100-msw increment. Before each stage of constant pressure 0.55 bar $N_2$ was injected (21). Maximum depth attained was 1080 m, and 29 h bottom time were achieved at 1000 m (20,22). Interestingly, new EEG signs of a modified HPNS developed at 800 m and deeper, namely, focal seizures localized to the occipital cortex, clonic and spasmodic motor phenomena, and decreasing EEG amplitude (23).

One significant finding from the *Coraz* series (650 msw), borne out in the later *Cornelius* 1000-m exposures (both with baboons), was that for best results nitrogen is to be injected into the chamber atmosphere at the end of each 100-m step of compression, i.e., just before each stage of constant pressure is reached. This effect is very large, and no explanation was proposed (22,24).

In an early application to man of the above results in 1976, *Janus IV*, 8 divers were compressed to 400-m saturation depth in 25 h. Trimix with 4.8% $N_2$ and four short stages of constant pressure were used, the first at 180 m. Increases in theta band power of up to 18 times normal were found early in compression, with tremor and other motor signs of HPNS also quite significant (17,25,26). Some of the potential value of performance tests carried out (27) was lost by inadequate predive practice of the subjects and resulted in a tendency for performance to improve with time. Taking the highest of the two predive scores as control, investigators noted 10–12% decrements in all three tests performed on arrival at 400 msw. Four days later, still at 400 msw, scores in the tests of visual choice reaction time and number ordering returned to normal, and those in the pegboard test of visuomotor coordination changed little, as in the *Coraz* series (9,28–30).

Further work with the baboon followed, and after lengthening the stages of constant pressure, the compression to 450 msw in the dive *DRET 79/131*, using 4.8% $N_2$, lasted about 40 h. Stepwise decreasing rates were used from 0.5 msw/min for the first 100 m; then 0.4, 0.25, 0.20 for each subsequent 100-msw increment; and 0.14 msw/min for the final 50 msw. Stages 150-min long separated each 100-msw increment (19).

Though frontal theta power increased up to 10 times normal in the EEG, tremor stayed constant at about twice normal level (17) and performance scores were favorable (31). Decrements averaged for the 8 subjects were 10% on the pegboard test and visual choice reaction time and 2% on number ordering. All but the pegboard score returned to normal a day later.

A further significant conclusion from the above work with different gas mixtures concerns the tremor of hyperbaric exposures and the nature of HPNS. In the *Coraz* series trimix seemed effective in diminishing tremor and dysmetria when compared to equivalent He-$O_2$ exposures, yet EEG signs of HPNS were increased in trimix (9). Similarly in *Janus IV*, once tremor (strictly a 10% decrement in visuomotor coordination scores) was established at 400 msw, it remained stable while other test scores returned to normal in 4 days of exposure to constant pressure (27). In the dive *DRET 79/131* the same dissociation of the time course of tremor (constant) from both cognitive performance indices (improving) and EEG changes (diminishing) was found at 450 msw (17). These facts accord with experience of HPNS in Alverstoke, U.K. (32) and elsewhere (33), namely, that HPNS is of heterogenous origin. Similarly, the work with *Papio papio* and men concerning the large effect that the timing of nitrogen injection had produced—a fact probably incompatible with any theory of the nature of the critical volume hypothesis proposing direct pressure vs. narcotic gas antagonism.

On comparison of *DRET 79/131* with *Janus IV*, lack of somnolence, improved motor signs (tremor, dysmetria, myoclonus), and increased vigilance scores in *DRET 79/131* were associated with the introduction of the 2½-h constant-pressure stages and an almost 10-fold slowing of the initial compression rates (27,34). On comparison with data obtained in 1974 with a 50-h, nonstop heliox compression to 460 m in *Janus IIIA*, and a somewhat slower (50 h with

one stage to 395 m) heliox compression in *Janus IIIB*, use of trimix containing 4.8% nitrogen was followed by markedly better results on any criterion (17,25).

Dives in the *Janus* series were essentially open-sea dives, culminating in an excursion to 501 m; they used divers selected at lesser depths. Based on this experience, the French hyperbaric team predicted that upon further advances of technological and human factors nature, man will be able to work below 500 m in the sea (25,35). The *Janus* series served as a reminder of the ultimate objective of the complex and expensive scientific work that is taking place in the relative safety of compression chambers in research laboratories.

Next, the French group set the practical objective of identifying those parameters that change consistently in both shallow (180-m He-$O_2$) and deep (450-m He-$N_2$-$O_2$) dives in the same subject. If it existed, this criterion when measured at 180 m would predict the performance of a given diver at 450 m and thus differentiate between resistant and susceptible subjects (36). The results showed that there was no comprehensive answer.

Twenty-four divers were compressed in 15 min to 180 msw using He-$O_2$, with 105 min bottom time. Their EEG changes and, independently, their psychomotor and cognitive performance scores were sorted into high and low hyperbaric response groups. Then, 8 of these subjects across all the groups were compressed in 38 h to 450 msw in trimix containing 4.8% $N_2$, and stayed for 50 h. The EEG changes and performance scores of each subject at 450 msw were correlated with those at 180 msw and the following major conclusions emerged. The magnitude of EEG changes correlated very well at 180 msw and 450 msw for each man individually; the 180-msw changes thus predicted the 450-msw changes. Scatter of psychometric performance scores was large, and no predictive trend was seen. It was clear that there was no correlation between EEG changes and performance of the same diver. These two variables were shown to be independent of each other. With the number of subjects small, as it was in each group, the authors were forced to postulate effects of uncontrolled variables such as motivation. Their conclusions, however, ring true in the light of unquantified experience at Alverstoke and probably elsewhere.

There are no generally accepted theories of relevance here relating to the origin of theta activity in the EEG or why power in this particular frequency band from anterior regions of the head should increase in response to hyperbaric exposure. This accepted, the use made of the EEG signal is perceived as empirical, or an example of the *black box* approach. It should be noted that the preponderance of low-frequency power seen in many metabolic and nervous system diseases is dominant in the delta (below 4 Hz) band, whereas the theta frequencies (4–8 Hz) respond best to hyperbaric exposure. Further doubt is cast here by the fact that the EEG components responding to closure of the eyes (defined as alpha activity) may decrease in frequency by as much as 2 Hz in hyperbaric experiments and thus migrate out of the frequency band reserved for them (8–13 Hz) by normobaric practice. The possibility of such migration of peaks elsewhere in the spectrum interferes further with interpretation other than on an empirical basis.

Thus, it seems that EEG changes at 180 msw predict EEG changes at 450

msw, but neither correlate with performance scores at 450 msw, which is also independent of performance scores at 180 msw. Yet, the parameter of prime interest is efficiency at depth, unimpaired cognitive and motor faculties mirrored by minimal decrements on a wide spectrum of tasks, and tested in the unforgiving medium of the open sea. To date, in various French hyperbaric experiments between 300 and 610 msw 63 subjects took part to constitute the largest pool of experience available to any team working to solve the problems presented by these pressures.

Current French work is concentrating effort on a series of 450-msw dives on trimix, 4.8% $N_2$, with long bottom times. ENTEX V had a compression profile and nitrogen injection procedure as in the successful *DRET 79/131* dive (34) and bottom time and decompression lasting 12 days each. The results were consistent with recent findings obtained on the same compression profile, namely: cognitive efficiency originally showed 15% decrements, which improved to 5% a day later; manual dexterity decreased by 10% and improved only slightly in the 12 days; theta band power increased to 400% (37). The most recent dive in the series, ENTEX VIII, yielded very similar performance scores and results in general. A detailed analysis of the evaluation of HPNS during 12 days at 450 msw in these dives is made by Rostain et al. (*see* Ref. 38; in this *Proceedings*).

In an attempt to study causal relationships between nitrogen and some HPNS constituents at 450 msw, varying concentrations to a maximum of 10% $N_2$ were breathed from a mask for 2 h. The usual EEG, tremor, and performance parameters showed widely differing response patterns and, significantly, absence of ill effects of a kind that would prevent gas switches from being done in a working dive (*see* Rostain et al., Ref. 39; in this *Proceedings*). The wide variability seen points yet again to the practical importance of diver selection. If one allows for this, there seems to be very little doubt that useful work is possible by divers at 450 msw, provided they are supported by suitable equipment.

## AMTE(PL) (formerly RNPL), ALVERSTOKE, ENGLAND, STUDIES

The recently completed *Deep Dive Series* at Alverstoke started in 1975. Dives were shallow at first to define caging effects and improve experimental techniques; about two dives a year were performed since to progressively higher pressures (40). *Dives 5* and *6* (heliox) failed to reach 300 msw on the planned uninterrupted compression profile at the rate of 1 m/min: HPNS developed at around 250 msw. *Dives 7* and *8* were performed to 420 msw so that investigators could differentiate components of HPNS due to pressure per se from rate-of-compression effects (32). Pressure of 60 msw/day was added to the chamber pressure in 6 increments, separated by 2 h. With an unplanned 2 days' hold in addition, only minor signs of HPNS were seen in *Dive 7* after 9 days' compression to 420 m. *Dive 8* progressed symptom-free to 390 msw, then dur-

ing the last 20 msw of compression the classical picture of full-blown HPNS developed.

In *Dive 9B*, 540 msw was reached on a staged compression profile in 4 days, on heliox. On each subsequent day, 180 msw, 300 msw, 420 msw, and 540 msw was reached, with compression rates of 5 msw/min to 420 msw, 3 msw/min to 480 msw, and 1 msw/min to 540 msw. Tremor was prominent in one diver; apart from this no significant ill effects occurred until several hours passed at 540 m. Then marked symptomatic deterioration developed, only to improve some days later on decompression. Two detailed reports contain most of the scientific work performed in the first eight experiments of this deep dive program (32,40), and an overview of especially their interpretation was given by Török (41).

Questions of fundamental interest in the Alverstoke *Deep Dive Series* were related to the subjects' performance under hyperbaric conditions. Most of the work was carried out over the years by the MRC Applied Psychology Unit at Cambridge. In an attempt to determine the relative susceptibilities to hyperbaric effects of a wide range of cognitive performance factors, 10 tests were assembled to form a 2-h-long battery. On the basis of previous work with divers, namely, the Sindbad test procedure designed for the U.S. Navy by Reilly and Cameron (42), Baddeley's previous results (43), and also more general information on stress effects (44), it was expected to encompass the whole spread of significant factors likely to limit cognitive efficiency. The following paper-and-pencil tests were used: arithmetic adding, paired associative memory, grammatical reasoning, Stroop conflict and control test, visuospatial orientation, number similarities, semantic processing, digit recall, and visual search.

Results from five dry chamber dives, 300 to 540 msw in heliox, were presented by Lewis and Baddeley (45). To emphasize steady-state effects, investigators obtained performance scores about 24 h after maximum depth was reached. Scores measuring speed of performance averaged across all depths, 300 to 540 m, and all tests and all subjects, yielded 7% slowing when each subject's speed was compared to his own normal preexposure control, already well practiced. Error-rate scores measured by 6 of the tests increased to 12% at depth from the 8% predive average. Several important points must be borne in mind when interpreting these results. To amass individual scores of performance tests of very different kind requires many unjustifiable assumptions (uniform normal distribution, continuity, linearity, absence of ceiling effects, and the like). The error rates often represent few items, giving rise to quantization error. Although each subject was his own control, there was large intersubject variability, and the same was true of the sensitivity of the different tests.

The wide range of performance tests used permitted conclusions to be drawn about the selectivity of hyperbaric effects. For a performance decrement to be interpreted as resulting from hyperbaric heliox conditions, there must be a decrease of efficiency at depth when compared to predive controls and also an increase of efficiency late in decompression when compared to performance at depth. On these criteria (46) the associate memory test was shown not to re-

spond, a finding indicating that the heliox environment at depths 300–540 msw does not have a blanket effect on cognitive function.

Perceptual processing was significantly slowed as indicated by equal decrements in the Stroop conflict and Stroop control tests as well as in the visual search tasks. Short-term memory impairments were demonstrated by the digit recall and number similarities tasks.

The variability of the results and some inescapable features of experimental design were such that decreasing cognitive efficiency as a function of increasing pressure could not be demonstrated. Testing for interaction between the various scores and pressure, speed and accuracy of arithmetic, the Stroop test, and semantic processing yielded significant results. These tests therefore are the most likely ones to produce pressure-dependent scores in further experiments.

To ascribe the above decrements to pressure, one must exclude incidental variables. For accumulating sleep deficit this has been done by correlating the subject's own daily estimate of his sleep quality and mood with performance scores. The poor correlation found implied that although sleep was impaired in the chamber, this impairment did not explain the decrements of cognitive ability. Test scores in general increased in decompression, sleep quality remained the same.

Subsequently, in a study comparing three simulated dives, namely, trimix (10% $N_2$) with nitrox at the same nitrogen partial pressure profile and heliox at the same depths (*see* Török, Ref. 8; in this *Proceedings*) a subset of the original test battery was used (47,48). There were substantial decrements from 420 msw on heliox (e.g., the "Arithmetic correct" score was about 30% below normal a day after arrival at 540 msw). On trimix deeper than 300 msw, subjects were less efficient on all tests than on heliox at the same depth. On three occasions in the trimix dive, notably on arrival at 420 msw and 660 msw, the subjects were unable to perform the tests successfully. Because they were cooperative, and attempts were in fact made, zero scores were recorded.

On arrival at 420 msw in the trimix dive several features of the subject's mental state resembled nitrogen narcosis, e.g., euphoria, inability to concentrate, poor performance of complex or unfamiliar tasks, and the ensuing amnesia. Recovery a day later was dramatic, permitting further compression. There was a further deterioration a few hours after arrival at 660 msw, characterized by lassitude, somnolence, nausea, vomiting, myoclonic jerks, and dyspnea, as well as poor concentration and performance. The subjects remained fully cooperative and cheerful, complying with instructions as best they could. No improvement occurred until several days into decompression. This state of events was clearly not brought about by the 6.7-bar $N_2$ present, as demonstrated by the findings in a nitrox exposure on the same time profile. In one subject, decrements so produced were minimal; in the other, cognitive performance remained normal (7). Therefore, the term *HPNS* may be validly used to describe the ill effects seen, caused by pressure.

Török's study (*see* Ref. 8; in this *Proceedings*), concerning the same three chamber dives at Alverstoke, was essentially comparative. He used power-spectral

techniques to measure changes in tremor of the outstretched arm. To characterize EEG changes, Török used a novel parameter: the theta-to-alpha power ratio (49). The pattern shown by postural tremor and EEG were consistent, and the triangular experimental design (nitrogen only in nitrox, high hydrostatic pressure only in heliox, both in the trimix exposure) permitted the following conclusions. First, high hydrostatic pressure as present in two of the three simulated dives was accompanied by approximately equal increases in tremor, to about three times normal, and even greater EEG changes. Second, nitrogen on its own at partial pressures up to 5.6 bars did not cause significant changes in either tremor or EEG. Third, the conclusion emerges on comparison of EEG and tremor data obtained in trimix with those from the heliox exposure at the same depths and is equivalent to a minimal restatement of Baddeley's results.

It is clear that in the Alverstoke deep trimix dive nitrogen did not decrease the EEG and tremor changes or the hyperbaric decrements seen in cognitive efficiency. These results are also compatible with the assumption that nitrogen and high pressure interacted positively in producing some of the augmented effects causing a modified HPNS. The possibility of these two effects summating has been predicted (50,51) and seen in hyperbaric experiments with rodents (52,53).

## DUKE UNIVERSITY, DURHAM, NC, USA, STUDIES

In 1979 it seemed that He-$O_2$ as a diving gas was not going to provide the means of combating HPNS effectively. At any rate, trimix looked more promising, its potentials as yet insufficiently explored. From previous work with men there were some discrepancies to be explained, foremost among these the relationship between speed of compression and the optimal quantity of nitrogen to be added in the breathing mixture. Four simulated dives were planned at Duke University, USA, named *Atlantis*, initially to 460 m. The primary objective of these well-planned and well-reported chamber dives was to explore the effect of an interaction between two variables, nitrogen content and speed of compression, each at two values: a) nitrogen content at the 10% value, as predicted by the critical volume hypothesis of excitable cell membranes "to permit abolition of HPNS" (54); and b) half this concentration suggested by much of the earlier French work. Two compression rates were selected, the slower at 24 h, 40 min to 460 m, of the same order as *Janus IV* in 1976 (25 h to 400 m), the other optimistically twice as fast, i.e., 12 h, 20 min to 460 m. A third compression profile initially planned, namely, 6 h to 460 m (55), was never attempted. The rates of compression employed were a gradual slowing, e.g., 10 msw/min for the first 108 m, to 0.25 msw/min for 430–460 m (with 6 stages of constant pressure 5 min to 2 h in duration) for the faster profile. When investigators observed the good mental state of the subjects at 460 m in *Atlantis II*, the target depth of the series was extended to 650 msw (55) and in *Atlantis III* to 686 msw (2250 ft), the current maximal hyperbaric exposure of man (56).

In this sense it is clear that the dive series produced greater results, in excess of plans, at the outset.

Because the density of trimix containing 6.56 bars $N_2$ and 0.5 bar $O_2$ at 650 m is 17.3 g/L, about 15 times the density of normobaric air (and one and one-half times that of He-$O_2$ at 650 m), a secondary objective of the *Atlantis* series was to define the limitations this environmental factor, and possibly nitrogen narcosis, imposes on the performance of the respiratory and cardiovascular system at different rates of physical work. A complex and demanding experiment was designed for this purpose, involving cannulation of the radial artery in the chamber and requiring one working day per subject to complete (56). Its results and implications are outside the scope of the present review.

Criteria for assessing the relative merits of the varying compression profile and nitrogen dose were largely provided in the four *Atlantis* chamber dives by a battery of test and measurement procedures that lasted about 1 h and were performed separately, up to four times per day (57). Cognitive function was measured by the following five tests: arithmetic adding, grammatical reasoning (AB sentences), Stroop control and conflict, number similarities, and digit recall from short-term memory. Visuomotor coordination was assessed by a pegboard test, a handtool task, and the ball bearing test, the hyperbaric use of the latter dating back to Haldane (58). Most of these procedures have been used extensively in other hyperbaric research programs; however, direct comparison of performance scores are only valid if all the parameters were identical, notably the duration of each test.

The cognitive test group, especially digit recall and grammatical reasoning, were used in the *Atlantis* series to indicate the presence of nitrogen narcosis. Caution is required in this interpretation, in view of Baddeley's recent work (45) showing that cognitive test scores are also affected in He-$O_2$ dives, especially arithmetic adding, grammatical reasoning, and short-term memory. The use of the visuomotor decrements as indices of HPNS rests on firmer foundations, because nitrogen narcosis is most unlikely to cause tremor or ataxia. The time-honored ball bearing test is known to produce variable scores, presumably as a response to increase in the "inner tension," noted in the autonomic group of HPNS components (1).

Postural finger tremor was measured by an accelerometer; again, for direct comparison with results of other laboratories the position of the subjects and transducer parameters should be compatible. Spontaneous EEG activity was recorded from occipital v vertex electrode placements (57). Though increased theta activity during compression may appear earlier in posterior areas of the scalp (15), much less theta increase is shown at this location than anteriorly, the site favored in French hyperbaric research. Because increased theta band power is not caused by nitrogen at these doses, to infer HPNS from this change may be done with more confidence than to infer the presence of nitrogen narcosis from decrements in cognitive test scores. Assessment of the subjects was supported by a questionnaire relating to health and sleep, subjects' own diaries, and their subjective assessment of their own mood.

The *Atlantis* project got underway in April 1979, and for the first dive the

faster of the two compression rates (12 h, 20 min to 460 m) with the lesser of the two nitrogen concentrations (5%, premixed in helium) were selected (55,59,60). Initial results were disappointing. During the deeper parts of compression HPNS developed, characterized by nausea, with vomiting in one subject, fatigue, somnolence, and lapses of consciousness termed *microsleep* (59). The subjects marked their mood tending towards drowsy, lethargic, troubled, and depressed.

Decrements on cognitive tests exceeded 50% in the worst test, namely, arithmetic adding (mean of 3 subjects); generally, they were around 50% during the first day. Visuomotor coordination on the handtool test was just as poor (50% at its worst) but on the pegboard and ball bearing tests somewhat better (at 30%) for the first 2 days. Electroencephalographic theta band power was initially over 180% of control in occipital scalp regions and decreased to 120%, where it remained until decompression commenced. Intention tremor was large enough to render demanding tasks (taking venous blood samples or cannulating a radial artery) difficult but without frustrating the completion of these tasks. No increase of postural hand tremor was shown by the accelerometer data. Sleep quality was poor with vivid dreams and nightmares until decompression started. Thus, by any of the important assessment criteria, marked HPNS was present in all three subjects in the first day.

Most of the unpleasant symptoms and signs of HPNS improved by the third day and some by the second day; this improvement enabled the highly motivated subjects to complete the work program of the dive. Their cognitive test scores, however, remained 1.0–2.5% below normal control values until 340 msw was reached in decompression. Electroencephalographic theta band power remained at the 120–130% mark for a few days longer, until at 300 msw the subjects were transferred to a larger adjacent living chamber.

*Atlantis II* a year later had the same fast compression profile to 460 m as the first dive in the series, coupled this time with 10% $N_2$ as the new experimental variable. The result was marked improvement in the subjects' well being, mood, morale, sleep quality, and tremor. Unlike these mostly subjective markers, the performance test scores, both cognitive and visuomotor, were the same as in the first dive. A Student's *t* test showed no difference in the performance decrements whether 5% or 10% $N_2$ was used in the diving gas. Electroencephalographic recording was repeatedly attempted, but because of a series of technical problems data sufficient for analysis were not collected (59).

After the demanding respiratory experiments were completed as planned (56), and encouraged by the good state of the subjects, investigators resumed compression on *Day 5*, and 650 msw was reached 3 days later, using progressively slowing rates. Helium only was employed for this further compression in *Atlantis II*, hence the proportion of chamber nitrogen gradually fell on *Days 5–8* to reach 7.8% at 650 m, where the subjects stayed for 24 h. Decrements of both visuomotor and cognitive tests increased during this second period of compression, but did not exceed the values reached on the first day at 460 m.

Gas density at 12.3 g/L produced its own problems, such as mouth breathing, painful sneezing, slow eating, and fatiguing speech. The oldest of

the three subjects (40 years) found dyspnea on inspiration disturbing, this effect marred his whole stay at deeper than 600 msw. The other two subjects adapted to this additional environmental stress without undue difficulty.

An interesting finding is concerned with the nitrogen concentration during decompression. To offset some of the time penalty attendant on the use of nitrogen during decompression, investigators allowed its concentration to fall by performing lock maneuvers with helium only. In this slow manner the original nitrogen concentration of 7.8% at 650 m fell to just above 5% at 460 m, where it had been 10% on the first day in compression. From 600–550 msw in decompression myoclonus, bizarre dreams, and some intention tremor appeared to last until 180 m was reached (55). These three features do not add up to HPNS, but the phenomenon is worthy of detailed analysis.

At this point both dives so far completed used the 12 h, 20 min "fast" compression profile to 460 m. The target depth was increased to 650 msw without jeopardizing the comparability of data at 460 m. For the remaining two dives with "slow" compression a new profile was designed to reach 650 msw in 6 days, 8 h. The gradually slowing pressure time course advanced to 400 msw in the first day at rates 9 msw/min to 0.25 msw/min. Slower rates and decreasing daily increments were used and, finally, on days 6 and 7 only a 25-msw advance was made at 0.05 msw/min. Trimix containing 10% $N_2$ (premixed) and 0.5 bar $O_2$ was the diving gas. After 4 days, 15 h at 650 msw and a further 12-h compression, 686 msw was reached for a 24-h period.

The planned work program was completed, with the subjects fit and well most of the time. From the start the compression and bottom time of *Atlantis III* was punctuated by reports of symptoms and signs attributable to HPNS. Nausea, faintness, dizziness, fatigue at 50–200 msw, slight euphoria and poor sleep at 460 msw, mild early morning nausea, euphoria and disorientation at 570 msw impaired concentration, and sleep problems at 650 msw were mentioned (57). Visuomotor coordination and most of the cognitive tests resulted in mean decrements of around 20%; the arithmetic adding test yielded error rates worse than 30% most of the time and at 650 msw reached 50%. These decrements, some of them quite large, returned to normal at 27 msw in decompression. Postural tremor measurements showed 300% maximum increases separately in one subject, generally much better in the other two men. Electroencephalographic theta band power increased to 200–240% of normal in a different subject at 400–686 msw, again corresponding increases in the other two men were much lower.

*Atlantis IV*, the last of this series, was performed in October of 1982. Trimix containing 5% $N_2$ was combined with a slow compression profile that was essentially the same as the one used in the previous experiment. Early reports (60) indicate the absence of euphoria during most of compression and up to 570 msw fewer adverse symptoms noted by the subjects than in *Atlantis III*. In general, the results of the three tests of visuomotor coordination used indicated decrements above 10% but below 25% at all depths. A striking exception was the ball bearing score of −60% at 650 msw not seen before in the series, perhaps an indication of tremor. From 570 msw an event, possibly unique in

diving, dominated the whole experiment. One of the subjects reported sleep difficulties, lightheadedness, and a sense of detachment with frank initially unstructured visual and auditory hallucinations. Light sedation seemed to help at first, but upon further deterioration decompression was started after 2 days at 650 msw. Scientific reports, no doubt imminent, must be awaited for a complete description and analysis of these events and for details of performance scores and electrophysiological data recorded in *Atlantis IV*.

If one assumes that the course of events affecting one subject only from 570 m onwards are fortuitous in nature and indicate nothing more than the need to screen subjects for suitability, the indications are that the subjects' efficiency and well being may have been better than that seen in *Atlantis III* with the 10% $N_2$ content.

Apart from the now traditional means of assessing hyperbaric effects on man, namely, cognitive and visuomotor test scores, tremor measurements, and EEG changes, the state of the spinal cord reflexes has also been used in the last three *Atlantis* dives. It was previously shown that on one hand, in deep heliox dives spinal reflexes were enhanced (61,62) and on the other, hyperbaric nitrox exposure at 20–90 msw may be associated with depressed spinal reflex excitability (63,64). On the basis of the critical volume hypothesis, no change from normal control values may be expected in any compression on trimix that optimized compression rate and nitrogen content simultaneously. Using generally accepted standard methodology (65), investigators selected the recruitment ratio of the electrically elicited Hoffman reflex as the parameter most likely to provide the above information (66).

In *Atlantis II* the H recruitment ratio increased to 119%, and this increase was taken to indicate either insufficient ambient nitrogen content (10%) or too fast compression (12 h, 20 min to 460 m). *Atlantis III* had the same nitrogen concentration, but compression time was doubled. During compression an operationally unacceptable degree of narcosis developed. This narcosis was paralleled by an H recruitment ratio 5% below normal. Further work is needed to support the usefulness of this specialized parameter as an objective indicator of success, or otherwise, of a trimix compression. The above index of spinal reflex excitability is not the only one potentially useful in this context: other measures are described in Harris and Bennett (Ref. 67; in this *Proceedings*).

## NORWEGIAN HYPERBARIC EXPOSURES

The hyperbaric complex of the Norwegian Underwater Technology Center at Bergen (initially called Norwegian Underwater Institute) was set up specifically to solve problems of operational diving related to Norwegian and oil interests. Physiological problems constitute but a small part of this objective. The importance of studying the properties of trimix as a potential diving gas ranked so high that the new Institute was inaugurated by a pair of simulated dives to 300 m in 1980, one in heliox (0.4 bar $O_2$), the other trimix containing 10% $N_2$ (68). It was clear from the outset that the problems arising from the high gas

density and the 20% or so decompression time penalty would cause trimix to be abandoned during decompression in any case. For study of possible effects, the replacement in 10–20 h at depth of trimix with heliox was introduced to the already complex dive plans of *Deep Ex 80*.

The compression profile was staged, with gradually decreasing rates (6.0–1.5 msw/min) planned to reach 300 m in 4 h, 44 min in both dives. Severe HPNS was encountered in the heliox group in 2 out of the 3 subjects with nausea, dizziness, and tremor; compression was held for 8 h, 41 min at 250 msw (69). The trimix group also suffered from these ill effects but at much lower intensity. In addition, all three trimix subjects had some euphoria, one noted blurred vision, the last two symptoms persisting for the duration of the exposure to trimix. Postural hand tremor on the Klove-Matthews technique (69) increased to 400 and 260% in heliox and trimix, respectively. Bilateral occipital and parietal EEG records were obtained with the subjects' eyes closed. Low-frequency band power (2.0–7.0 Hz) increased to 300%, and alpha activity (8.0–13.0 Hz) decreased to 50% of controls at maximum depth in heliox, with large interhemispheric and intersubject variability. In the trimix group EEG remained within normal limits.

Five tests of cognitive function were performed (arithmetic adding, grammatical reasoning, short and long-term memory, number similarities; 10 min total test time). Maximum decrement in the heliox group was found at 250 msw, namely, 18.6%. At that depth mean decrement in trimix was 15.0%; later at 300 msw it increased to 30.4% below control scores. Memory, both long- (15 min) and short-term, was especially affected. Performance was still poor in trimix 26 h later and improved dramatically after the gas switch to heliox. The authors (69) interpreted these facts as marked HPNS in heliox and mild nitrogen narcosis for all the 26 h at 300 msw in trimix (70).

The next Norwegian experiment, *Deep Ex 81* a year later, was planned along similar lines to 500 msw (71). Three subjects were compressed on heliox in 26 h, 45 min to 500 msw; the profile included a 3-h hold at 216 msw, or a 21-h hold at 376 msw, and compression rates were 6.0–2.0 msw/min (72). In this event, compression on trimix (10% $N_2$) required 41 h, 20 min, comprised two shallow 10-min stages, five 2–9 h deep stages, and compression rates of 9.0–0.1 msw/min. Both groups suffered from ill effects: vertigo, nausea, vomiting attacks, visual disturbances, poor sleep, aversion to food, and difficulty in concentration. These symptoms occurred in both groups: vertigo and tremor characterized heliox; euphoria, vomiting, and somnolence were predominant in trimix. One subject in each group was almost unaffected, the worst subject was in the trimix group. Both compressions were longer than originally planned.

In both groups EEG changes occurred and below 300-msw were of about equal magnitude: 2 to 7-Hz band power at 400%, alpha activity at 50% of normal controls. Postural hand tremor was much worse in trimix—increases up to 20 times normal were recorded (73,74).

The efficiency of the subjects' motor systems was tested by an extended battery of six tests. At 500 msw the mean decrement on these tests was of the

same order, namely, 37.5% for the heliox group, 34.3% for trimix. Loss of cognitive efficiency was 11.3 and 26.0%, respectively (74).

The trimix group was subjected to a comparatively rapid change of diving gas. Starting on *Day 4*, in 24 h or so the nitrogen content of the chamber gas was lowered from 10 to 2% (75). Within a few hours after nitrogen was discarded, most of the large decrements in cognitive efficiency seen only in the trimix group improved significantly. This fact and the comparative scores of the two groups support powerfully the conclusion of the Norwegian team, namely, that poor performance and cerebration in trimix were caused by nitrogen, a narcotic agent. All measures of performance used except for tremor indicated superior efficiency of the heliox group (76).

This procedure of change of diving gas was apparently responsible for increased tremor, nausea, and some psychological changes. Predominant among these was the occurrence at night of severe visual and auditory hallucinations simultaneously in two out of three subjects (76). When increased tremor and myoclonus was observed previously in association with lowering the dose (partial pressure) of nitrogen at 300 m (55), return of HPNS was favored as explanation. In light of recent evidence from *Atlantis IV*, the Alverstoke 660-msw *Dive 12b* as well as *Deep Ex 81*, this view, probably based on the critical volume hypothesis of direct pressure-narcotic agent antagonism, may well have to be revised.

With practical problems of the working diver in mind, one of the objectives of *Deep Ex 81* was to explore the interaction of three stressors: cold, fatigue, and HPNS. The performance of a group of divers breathing trimix was compared to scores of the heliox group (*see* Vaernes et al., Ref. 77; in this *Proceedings*).

The main conclusion of the Norwegian team from both *Deep Ex 80* and *81* was that heliox rather than trimix is the gas of choice for deep diving because of the absence of narcosis, its lesser density, and faster saturation decompression. To 500 msw, compression on heliox may be as rapid as on trimix. For divers to work at such pressure, both selection procedures for HPNS resistance and long-term postdive neuropsychological follow-up were recommended on the basis of experience gained in *Deep Ex 81* (78).

Current Norwegian work is concentrating on feasibility studies with deep open-sea working dives in mind. In the immediate future NUTEC at Bergen is likely to be one of only two hyperbaric centers in the western world likely to continue productive work at depths below 300 msw.

## DISCUSSION

There is no simple answer as to the boundaries of "too fast and too far" compression. Large individual differences in man's susceptibility to HPNS is emphasized by all research workers. *Cryptogenic* would be a better term for these differences; the term would emphasize that causes may as yet be hidden rather than essentially individual in nature. Empirically, the following concepts

emerge. First, there seems to be a *threshold* around 150 msw in that fast compression rates of 15–30 msw/min are possible below this target depth without encountering HPNS. Second, slow, continuous compression rates (1 msw/min or 10 msw every 2 h) do not represent complete solutions. Contrastingly, staged compression profiles have provided encouraging results. There is some evidence from Alverstoke and, more recently, from the 350-m *Seaway*-NUTEC dive, that if the period of constant pressure (*stage*) is long enough to allow for adaptation over about a day, then the rates of compression do not have to follow some inverse function of pressure.

It would be a mistake to regard HPNS as a linear phenomenon subject to laws of proportionality, linked causally to either rate of compression or depth. Its determinant function belongs rather to the realm of catastrophe theory. It is the body's *desperation response*, produced when the scope of some homeostatic mechanism is exceeded. Between the trigger and the response, HPNS, there seems to be a lag of a few hours that develops even if no further stimulus is added in the form of further compression during this lag. Once manifest, HPNS may not subside without decompression. It is difficult to interpret results of Alverstoke *Dives 8*, *9b*, and *12b*, and the U.S. Navy's 1800-ft dive at Panama City in any other way. In this U.S. Navy wet chamber dive in November 1979, heliox and 12 days staged compression were used to the target depth of 548 msw (79,80). A distressing range of symptoms and signs developed during compression and failed to improve in the 5 days bottom time. Dyspnea, marked fatigability, reduced efficiency of cerebration, myoclonus, disturbed sleep, nausea, and gastrointestinal problems with average weight loss exceeding 10% body weight were the most significant ill effects. It was encouraging that on long-term follow-up no lasting ill effects were reported (*see* Becker, Ref. 81; in this *Proceedings*).

From the above ideas, especially from those concerning the lag between stimulus and affect, an interesting possibility emerges. It is necessary to assume, however, that the amount of decompression that can be carried out in the few hours concerned is capable of preventing manifestations of HPNS almost instantaneously. In January 1981, Buhlmann compressed his subjects to 500 m in a day, and they were said to have experienced nausea. After a few hours they decompressed to 450 m as if from an excursion and stayed overnight. The next morning the volunteers were well, performing their tasks successfully; indeed, they were fit for further compression. Analysis must be left to those in full possession of the relevant facts; however, is this simulated dive at Zurich a model for future successful ways of getting to depth—illustrating concepts like "over fast" compression followed by excursion decompression to target pressure?

The high pressure neurological syndrome is almost certainly not a homologous single entity; its varied manifestations and their differing time courses in man make this quite improbable. Historically, research effort has always been concentrated to separate (and define) the role of two causative factors: ill effects caused by excessive rate of increase in pressure and ill effects caused by increased gas pressure per se.

Traditionally, the amount of nitrogen in trimix is expressed as a percentage of the chamber atmosphere and not in partial pressure terms. The only time this makes a difference is when pressure is changing. Thus, during compression with premixed inert gases the percentage of nitrogen may stay the same (ignoring chamber oxygen and water vapor) but nitrogen partial pressure rises linearly with pressure. For the physiologist the important parameter a priori is nitrogen partial pressure, representing the dose as a drug of this narcotic gas, the concentration in tissue fluids to which neurones in the central nervous system will be exposed at a pharmacokinetic delay of some minutes. The very fact of emphasis being placed in HPNS/compression research on percentage as opposed to dosage quantities highlights depth-related ill effects of HPNS.

There is empirical evidence that at least the worst effects of significant HPNS in the deeper exposures develop to be manifest or increase to be maximal at a lag that may be about 2 h. During compression with premixed He-$N_2$ gas some hours later these ill effects will thus be treated (in the sense of therapy) with a dose of a narcotic pharmacological agent higher than it was when a chain of biological events was set in motion, probably at the point of some important homeostatic mechanism being overcome (its dynamic range exceeded) by the environment. Several points of consequence follow from this concept, assuming as it does that nitrogen is an effective therapeutic medicine of at least some important HPNS constituents having nothing to do positively or negatively with their causation.

It would, for example, offer a possible explanation for the French findings, namely, that a nitrogen injection into the chamber gas is to be done at the end of a 100-m increment of compression at the start of a stage of constant pressure. The above view is also compatible with the finding of significant deterioration of the subjects' physiological state at constant pressure, such as was the case at 660 m in the Alverstoke *Dive 12b* using trimix with 10% (or 6.6-bar partial pressure) nitrogen.

A third layer of complexity in the possible interrelationship between findings would become accessible by the assumption that not only is nitrogen an effective therapeutic medicine, but also it has its own undesirable effects. When prescribing a given dose (partial pressure), one would aim at an acceptable balance between the two effects. Again, the fundamental assumption of preventing HPNS or titrating it with nitrogen did not have to be invoked. One would set performance decrements caused by HPNS against the sum of therapeutic minus narcotic effects of nitrogen and measure the results regarding it as a function of nitrogen dose. This view would, however, also fail to explain the findings in experiments of triangular design, like the Alverstoke *Dives 12b* with trimix, *Dive 13* in heliox and *Dive 14* in nitrox, when no subtractive effects of nitrogen were seen (*see* Török, Ref. 8; in this *Proceedings*). Why should the same two subjects show gross inefficiency and equally gross euphoria on arrival at 420 m in trimix (4.2-bar nitrogen partial pressure) when on nitrox mixture at 37 msw (same nitrogen dose, same time course to reach it) there was but a mere trace of these effects seen, if that (48)?

Enduring hypotheses purporting to offer quantitative predictions better than

to a first approximation in the biological sciences are rarely linear. Lags, directional differences of response and other nonlinearities, hierarchical cascade of systems, adaptation, and even abruptly changing response modes are often encountered. Is the critical volume hypothesis, assuming that it is to be applied to manned deep-diving experiments in the future, ready for modification to encompass recent evidence?

On a more pragmatic level: a cursory glance at the evidence presented here may lead to the view that in deep diving, even after optimizing the compression procedure and gas composition, a residual decrement of cognitive test performance, say 10%, is inevitable. If so, a choice is to be made between two approaches. The first assumes that the underwater task must be carried out. The alternative starting point is the diver, and the question is asked whether or not he is fit to go out, given a certain magnitude of decrement. The fluctuating mental state and diminished reliability associated with trimix is of particular concern in this instance. Similar decision points were reached in aviation about hypoxia and cabin pressurization, in road traffic control with permissible concentrations of alcohol in the blood, and in compressed air diving, limiting it to 45–50 msw. In aviation, where an eminently avoidable environmental stressor (hypoxia) is coupled to potentially disastrous consequences of small pilot errors, the option of no acceptable decrements was taken (82). Different relevant factors in deep diving may well lead to a different conclusion. Is the time right for us to prepare for a deliberate decision process of this nature about human performance in the field of deep diving?

## References

1. Török Z. The symptomatology of HPNS—a proposed classification. In: Elliott DH, ed. Proceedings of the eighth annual congress of the European Undersea Biomedical Society. Lubeck: EUBS, 1982:104–118.
2. Hunter WL, Bennett PB. The causes, mechanisms, and prevention of HPNS. Undersea Biomed Res 1974;1:1–28.
3. Bennett PB. The HPNS in man. In: Bennett PB, Elliott DH, eds. The physiology and medicine of diving. 3rd ed. London: Baillière Tindall, 1982:262–296.
4. Brauer RW. Seeking man's depth level. Ocean Ind 1968;3:28–33.
5. Fructus XR, Brauer RW, Naquet R. Physiological effects observed in the course of simulated deep chamber dives to a maximum of 36.5 atm in He-$O_2$. In: Lambertsen CJ, ed. Underwater physiology IV. Proceedings of the fourth symposium on underwater physiology. New York: Academic Press, 1971:545–550.
6. Fructus P. Deep diving. In: Society of Underwater Technology, Proceedings Conference Oceanology International. Brighton, UK: SUT, 1969.
7. Rostain JC, Naquet R. Human neurophysiological data obtained from two simulated He-$O_2$ dives to 610 msw. In: Shilling CW, Beckett MW, eds. Underwater physiology VI. Proceedings of the sixth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1978:9–19.
8. Török Z. Deep diving: Trimix and heliox compared by EEG and tremor criteria. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Bethesda: Undersea Medical Society, Inc., 1984:697–705.
9. Rostain JC, Gardette-Chauffour MC, Naquet R. HPNS during rapid compressions of men breathing He-$O_2$ and He-$N_2$-$O_2$ at 300 m and 180 m. Undersea Biomed Res 1980; 7:77–94.
10. Charpy JP, Murphy E, Lemaire C. Performances psychometriques apres compressions rapides a 300 metres (Coraz). Rev Med Subaq Hyperbare 1976;59:192–195.

11. Rostain JC. Le tremblement au cours de compressions rapides: influence de l'azote dans le melange respiratoire. Med Subaq Hyperbare 1976;15:267–270.
12. Rostain JC, Naquet R, Fructus X. Study of effects of trimix and heliox mixtures during rapid compressions. Undersea Biomed Res 1976;3(Suppl):A13–14.
13. Naquet R, Rostain JC, Fructus X. HPNS: clinical and electrophysiological studies in man. In: Halsey MJ, Settle W, Smith EB, eds. The strategy for future diving to depths greater than 1000 ft. Eighth UMS Workshop. Bethesda: Undersea Medical Society, Inc., 1975. (WS:6-15-75)
14. Rostain JC, Charpy JP. Effects upon the EEG of psychometric performance during deep dives in He-$O_2$ atmosphere. EEG Clin Neurophysiol 1976;40:571–584.
15. Fructus X, Agarate C, Naquet R, Rostain JC. Postponing the HPNS to 1640 feet and beyond. In: Lambertsen CJ, ed. Underwater physiology V. Proceedings of the fifth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology. 1976:21–33.
16. Lemaire C, Murphy EL. Longitudinal study of performance after deep compressions with heliox and He-$N_2$-$O_2$. Undersea Biomed Res 1976;3:205–216.
17. Naquet R, Rostain JC. HPNS in man: an account of French experiments, p48–53. In: Smith EB, Halsey MJ, Daniels S, eds. Techniques for diving deeper than 1500 feet. Proc 23rd Workshop, Undersea Medical Society, 1980. (Publ. WS[DD] 6-30-80)
18. Rostain JC. Le syndrome nerveux des hautes pression chez l'homme et le singe *Papio papio*. Univ Marseille, These docteur-es-sciences naturelles, 1980.
19. Rostain JC, Gardette B, Gardette-Chauffour MC, Naquet R. HPNS in man during 38 hours compression to 450 m with $N_2$ injections. Abstracts of 7th Symp Underwater Physiology. Undersea Medical Society, 1980:16.
20. Rostain JC, Imbert JP, Gardette B, Lemaire C, Dumas JC, Naquet R. Compression methods II. Study of the effects of profiles and $N_2$ injections on HPNS of the baboon *Papio papio*. Undersea Biomed Res 1978;5(Suppl):45–46.
21. Rostain JC, Gardette B, Gardette, MC, Dumas JC. HPNS of baboon *Papio papio* during slow exponential compression with $N_2$ injection to 1000 msw and beyond. Undersea Biomed Res 1979;6(Suppl):45.
22. Rostain JC, Dumas JC, Gardette B, Imbert JP, Lemaire C, Naquet R. The HPNS of the baboon *Papio papio*: Effects of nitrogen and the compression curve. Proc Internat Union Physiol Sci (Doin, Paris) 1977;13:639.
23. Rostain JC, Gardette-Chauffour MC, Doucet J, Gardette B. Problems posed by compression at depths greater than 800 m in the monkey *Papio papio*. Undersea Biomed Res 1981;8(Suppl):15–16.
24. Rostain JC, Dumas JC, Gardette B, Imbert JP, Lemaire C, Naquet R. Compression au melange helium-azote-oxygene de singes *Papio papio* a 1000 metres. Med Aero Spat et Med Sub Hyp 1977;16:266–270.
25. Fructus X. Limits for both open water and chamber diving. In: Smith EB, Halsey MJ, Daniels S, eds. Techniques for diving deeper than 1500 feet. Proc 23rd Workshop, Undersea Medical Society, 1980:27–33. (Publ. 40 WS [DD] 6-30-80)
26. Rostain JC, Naquet R, Lagarde JM. Le SNHP au cours d'une compression en 24 heures a 400 metres. Med Aero Spat et Med Sub Hyp 1977;16:271–275.
27. Lemaire C, Charpy JP. Efficacite sensorimotrice et intellectuelle apres une compression a 400 m en 24 h (Janus IV). Med Aero Spat et Med Sub Hyp 1977;16:296–298.
28. Lemaire C. Capacite de travail psychsensoriel en ambiance hyperbare. Le Travail Humain 1979;42:13–28.
29. Rostain JC. Le tremblement, au cours de compressions rapides: influence de l'azote dans le melange respiratoire. Med Subaq Hyperbare 1976;15:267–270.
30. Charpy JP, Murphy E, Lemaire C. Performances psychometriques apres compressions rapides a 300 metres. Revue de Med Subaq Hyperbare 1976;15:192–195.
31. Lemaire C. Evaluation de la performance psychomotrice entre 10 et 450 metres en ambiance helium-oxygene-azote. Med Aero Spat et Med Sub Hyp 1980;19:211–214.
32. Hempleman HV et al. Human physiological studies at 43 bar. Orpington, Kent: Defence Research Information Centre, 1980. (AMTE(E) Rep R80-402)
33. Fructus X, Rostain JC. HPNS—A clinical study of 30 cases. In: Shilling CW, Beckett MW, eds. Underwater physiology VI. Proceedings of the sixth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology 1978:3–8.
34. Rostain JC, Gardette B, Gardette-Chauffour MC, Naquet R. HPNS in humans during a 38 hour compression

to 450 msw with $N_2$ injections. Abstracts of VIIth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1980:16.
35. Buckman D. French divers work 10 hrs at depth of 460 m (1508 ft). Ocean Ind 1977;12:41–44.
36. Rostain JC, Lemaire C, Gardette-Chauffour MC, Doucet J, Naquet R. Criteria analysis of selection for deep diving: EEG and performance. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society Inc., 1981: 435–443.
37. Rostain JC, Lemaire C, Naquet R. HPNS in man during a 12 day stay at 450 m in He-$N_2$-$O_2$. Undersea Biomed Res 1982;9(Suppl):22–23.
38. Rostain JC, Lemaire C, Gardette-Chauffour MC, Naquet R. Evolution of HPNS in 16 divers during long stays at 45 bars breathing He-$N_2$-$O_2$. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:665–672.
39. Rostain JC, Lemaire C. Effects of different He-$N_2$-$O_2$ breathing mixtures on HPNS at 45 bars. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc. 1984:641–647.
40. Hempleman HV et al. Observations on men at pressures of up to 31 bar. Orpington, Kent: Defence Research Information Centre, 1978. (AMTE[E] Rep R78–401)
41. Török Z. Deep diving—the UK experience. In: Smith EB, Halsey MJ, eds. Techniques for diving deeper than 1500 ft. Proc 23rd Workshop. Bethesda: Undersea Medical Society, Inc., 1980:54–61. (Publ 40 WS(DD) 6–30–80)
42. Reilly RE, Cameron BJ. An integrated measurement system for the study of human performance in the underwater environment. 1968. (ONR Rep NR 196-074)
43. Baddeley AD, Figueredo JW, Hawkswell Curtis JW, Williams AN. Nitrogen narcosis and performance underwater. Ergonomics 1968;11(2):157–164.
44. Wilkinson RT. Some factors influencing the effect of environmental stressors upon performance. Psychol Bull 1969;72:260–272.
45. Lewis VJ, Baddeley AD. Cognitive performance, sleep quality and mood during deep oxy-helium diving. Ergonomics 1981;24:773–793.
46. Lewis V, Andrews B, Baddeley A, Herbert M. Psychological investigations in dives 7 and 8 to 420 msw. In: Hempleman HV et al, Human physiological studies at 43 bar. 1981. (AMTE[E] Rep R80-402)
47. Logie RH, Baddeley AD. A trimix saturation dive to 660 m: Studies of cognitive performance, mood and sleep quality. Medical Research Council (UK), 1982. (MRC Rep RNP 2/82)
48. Logie RH, Baddeley AD. Simulated deep sea diving in trimix, oxy-helium and oxy-nitrogen: Studies of cognitive performance, sleep quality and mood. In: Coombes K, ed. Proc Ergonomics Soc Conference 1983:177–180.
49. Török Z. Power spectral analysis of EEG in experimental diving: The use of the theta to alpha ratio. EEG Clin Neurophysiol 1983;55:40.
50. Roth SM. Anaesthesia and pressure: antagonism and enhancement. In: Fink BR, ed. Molecular mechanisms of anaesthesia. Progress in anaesthesiology. New York: Raven Press, 1975;1:405–420.
51. Hsia JC, Boggs JM. Protein perturbation hypothesis of anaesthesia. In: Fink BR, ed. Molecular mechanisms of anaesthesia. Progress in anaesthesiology. New York: Raven Press, 1975;1:405–420.
52. Thomas JR, Burch LS. Inert gas narcosis: Avoidance behaviour in rats breathing elevated pressures of $N_2$ and He. Physiol Psychol 1975;1:411–416.
53. Thomas JR. Reversal of $N_2$ narcosis in rats by He pressure. Undersea Biomed Res 1976;3:249–259.
54. Simon S, Katz Y, Bennett PB. Calculation of the percentage of a narcotic gas to permit abolition of the HPNS. Undersea Biomed Res 1975;2:299–303.
55. Bennett PB, Coggin R, Roby J, Miller JN. Prevention of HPNS in man by rapid compression with trimix to 2132 ft (650 m). In: Bachrach AJ, Matzen MM, eds. Underwater physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1982: 345–355.
56. Salzano JV, Stolp BW, Moon RE, Camporesi EM. Exercise at 47 and 66 ATA. In: Bachrach A, Matzen MM, eds. Underwater Physiology VII. Proceedings of the seventh symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1982:181–196.

57. Bennett PB, Coggin R, McLeod M. Effect of compression rate on use of trimix to ameliorate HPNS in man to 686 m (2250 ft). Undersea Biomed Res 1982;9:335–351.
58. Case EM, Haldane JBS. Human physiology under high pressure: Effects of $N_2$, $CO_2$, and cold. J Hyg Camb 1941;41:225–249.
59. Bennett PB, Coggin R, Roby J. Control of HPNS in humans during rapid compression with trimix to 650 m (2132 ft). Undersea Biomed Res 1981;8:85–100.
60. Bennett PB. Probing the limits of human deep diving. Phil Trans (Lond) 1984 (in press).
61. Roll JP, Lacour M, Hugon M, Bonnet M. Spinal reflex activity in man under heliox conditions at 31 and 62 ATA. In: Shilling CW, Beckett MW, eds. Underwater physiology VI. Proceedings of the sixth symposium on underwater physiology. Bethesda: Federation of American Societies for Experimental Biology, 1978:21–27.
62. Harris DJ. Hyperbaric hyperreflexia: tendon jerk and Hoffman reflexes in man at 43 bar. EEG Clin Neurophysiol 1979;47:680–692.
63. Bishop B, Hoffmann H, Wallis I, Shindell D. Effects of increased ambient pressure and nitrogen on man's monosynaptic reflexes. J Appl Physiol 1975;38:86–90.
64. Harris DJ, Towse EJ. Depression of myotatic reflex responsiveness in compressed air. Orpington, Kent: Defence Research Information Centre, 1980. (AMTE[E] Rep R79-409)
65. Hugon M. Proprioceptive reflexes and the H reflex: methodology of the Hoffman reflex in man. In: Desmedt JE, ed. New developments in electromyography and clinical neurophysiology. Basel: Karger, 1973;3:277–293.
66. Harris DJ. Soleus H reflex studies in man at 40–70 bar in trimix. Undersea Biomed Res 1984;11:00–00.
67. Harris DJ, Bennett PB. Alteration of soleus electromyogram silent period in man at pressures up to 70 bars. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:731–738.
68. Peterson RE, Tønjum S, Martinsen O, Kahrs BW, Svendsen EW. Deep Ex 80, general project description. Bergen: Norwegian Underwater Institute, 1980. (NUI Rep 53/80)
69. Vaernes R, Bennett PB, Hammerborg D, Ellertsen B, Peterson RE, Tønjum S. Central nervous system reactions during heliox and trimix dives to 31 ATA. Undersea Biomed Res 1982;9:1–14.
70. Vaernes R. Deep Ex 80: Effects on the CNS during deep dives. Neuropsychological testing. Bergen: Norwegian Underwater Institute, 1980. (NUI Rep 51/80)
71. Peterson RE, Bennett PB, Vaernes R, Dick AP, Tønjum S. Testing of compression strategies for diving to 500 msw. Undersea Biomed Res 1982;9(Suppl):22–23.
72. Vaernes R, Hammerborg D, Ellertsen B, Peterson R, Tønjum S. Saturation at 51 ATA on heliox and trimix with gas change from trimix to heliox—an analysis of recovery from HPNS and narcosis at depth. In: Elliott DH, ed. Proc Eighth Annual Congress of European Undersea Biomed Society, Lübeck 1982:213–248.
73. Vaernes R, Hammerborg D, Ellertsen B, Peterson R, Tønjum S. Central nervous system reactions during heliox and trimix dives to 51 ATA, Deep Ex 81. Undersea Biomed Res 1982;9(Suppl):22–23.
74. Vaernes R, Hammerborg D, Ellertsen B, Peterson R, Tønjum S. Central nervous system reactions during heliox and trimix dives to 500 msw, Deep Ex 81. Undersea Biomed Res 1984; 10:169–192.
75. Vaernes R. Central nervous reactions during Deep Ex 81. Bergen: Norwegian Underwater Technology Centre, 1982. (NUTEC Report No. 7)
76. Peterson RE, Tønjum S, Vaernes R. Evaluation of compression strategies for deep diving. In: Elliott DH, ed. Proc Eighth Annual Congress of European Undersea Biomed Society, Lübeck 1982:452–458.
77. Vaernes R, Pasche A, Tønjum S, R. Peterson. Working in water at 500 msw breathing heliox: An analysis of diver performance as a function of HPNS and body temperature. In: Bachrach AJ, Matzen MM, eds. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:683–696.
78. Tønjum S et al. Deep Ex 81, a simulated dive to 500 msw. In: Elliott DH, ed. Proc Eighth Annual Congress of European Undersea Biomed Society, Lübeck 1982:173–181.
79. Thalmann ED, Piantadosi CA, Spaur WH. U.S. Navy 1800 ft dive. Pressure (Undersea Med Soc) 1980;9:1–5.
80. Spaur WH. Recent U.S. Navy experience in saturation diving. In: Smith EB, Halsey MJ, Daniels S, eds. Techniques for diving deeper than 1500 ft. Proc 23rd Workshop, Undersea Med Society, 1980:62–66. (Publ. 40 WS(DD) 6-30-80)

81. Becker B. Neuropsychologic sequelae of a deep-saturation dive: A three-year follow-up. In: Bachrach AJ, Matzen MM. Underwater physiology VIII. Proceedings of the eighth symposium on underwater physiology. Bethesda: Undersea Medical Society, Inc., 1984:707–712.
82. Denison DM, Ledwith F. Complex reaction times at a simulated cabin altitude of 8000 ft. Flying Personnel Research Committee (UK) London: MOD/HMSO, 1975. (Rep No 1235)

# AUTHOR INDEX

# SUBJECT INDEX

by

C. W. Shilling

I



L



M