UNDERWATER PHYSIOLOGY

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Underwater Physiology

PROCEEDINGS OF THE FOURTH
SYMPOSIUM ON UNDERWATER PHYSIOLOGY

Sponsored by

Institute for Environmental Medicine
The University of Pennsylvania Medical Center

Physiology Branch, The Office of Naval Research

The Undersea Medical Society

Edited by C. J. LAMBERTSEN

1971

ACADEMIC PRESS NEW YORK and LONDON

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
Berkeley Square House, London W1X 6BA

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 72-137621

PRINTED IN THE UNITED STATES OF AMERICA

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PREFACE

Of all of the endeavors undertaken in this decade, the successful accomplishment of manned lunar landing and the beginning of detailed lunar exploration have most excited the imaginations of earth's population. While the cost was extreme the step was inevitable, and it was possible to watch the drama unfold over the quarter million miles of distance from earth to the lunar surface. The expeditions, feats of extreme technical complexity, were accomplished through the meticulously coordinated activities of many thousands of individuals over a period of preparation encompassing almost this entire decade. Their admirable success depended heavily upon the precision of advanced electronic vectoring, ranging, control, computation, and communication. While man himself was a vital force, compensating for equipment limitations and applying his intelligence, senses, and hands toward the success of the mission at all stages, he also was almost completely protected from the stresses of the otherwise intolerable space environment by his spacecraft and suit.

During this same decade a much less heralded but equally hazardous extension of man's capabilities has occurred in manned undersea activity in sealed submersibles, resembling their spacecraft counterparts, and by divers unprotected from the cold, dense, high pressure undersea environment. Individuals and small groups have made major advances—extending diving durations to months, diving depths to the limits of our continental shelves, and penetrating in capsules to the deepest part of the sea. These advances have occurred in spite of the severe handicaps imposed by the submarine environment.

The circumstances of manned undersea exploration have resembled the space probes only in small part. While direct visual and acoustic monitoring has been possible over the distances between earth and lunar explorer, darkness and turbidity often limit undersea vision or video monitoring to a range of a few feet, and, at the greater depths, transfer of human intelligence by vocal communication is disturbed by the necessity for substituting helium for a denser and more narcotic inert gas.

In diving, every gas respired is a potentially incapacitating or even lethal toxin as the pressure of the gas increases with diving depth. With the dramatic extensions of open sea diving and laboratory pressure chamber studies, pressures equivalent to at least 1700 feet of seawater have now been reached. The increasing work of moving gas through the pulmonary airways, work which is negligible in the astronaut, is approaching the limits of tolerance for the deep diver who must spend many days at great pressure to make his undersea activity truly useful. However, it is the pressure associated with depth itself and not decompression from diving that should be considered the ultimate limitation. Inevitably a depth will be

reached in which the work of breathing performed by the respiratory musculature, even in the diver's resting state, will be so severe that the respiratory exhaustion which must occur will lead to "respiratory decompensation," and risk of death will exist during the multiday decompression from extreme excursions. Even with the expected shortening of decompression requirements, it will continue to take more days to return by decompression from extended direct exploration of the continental shelves than to return to earth after the same period of time spent in exploration of a lunar "sea."

This volume represents one more step in the continuing effort to discover and understand the physiologically limiting effects of undersea, high pressure exposures. The scope of the presentations and discussions ranges from the most fundamental of biological reactions through the integration of physiological stresses to the description of limits actually encountered in diving operations. This volume represents one of a series of compendia of correlated questions, data, and conceptual interpretations, providing information and insight both to the new student and the advanced investigator of unusual environmental conditions. As has continued to be the case, the results of the physiological explorations in this relatively specialized field of undersea medicine bear directly and importantly upon many other aspects of the biomedical sciences. Thus the studies of narcotic influences of inert gases interest the clinical anesthesiologist, psychologist, psychiatrist, and neurologist. Mechanical limitations of ventilation at great depth provide the pulmonary physiologist with information and understanding concerning the asthmatic or emphysematous patient. The mechanisms of oxygen intoxication and methods for protection against it pertain both to the most basic sciences of biology and to the therapeutic use of oxygen at normal atmospheric pressure and in hyperbaric states. These detailed studies of tolerance to environmental extremes, therefore, have scientific and practical pertinence extending far beyond the primary goal of preparing for extension of man's activity beneath the sea. They stand as exemplary and farsighted at this time of final awakening of man to his ignorant destruction of his natural atmosphere and environment.

C. J. LAMBERTSEN

Previously published symposia in this series (sponsored by the University of Pennsylvania and the Office of Naval Research): First Symposium on Underwater Physiology, National Academy of Sciences-National Research Council, Publication 377, Washington, D.C., 1955; Second Symposium on Underwater Physiology, National Academy of Sciences-National Research Council, Publication 1181, Washington, D.C., 1963; Third Symposium on Underwater Physiology, Williams and Wilkins, Baltimore, Maryland, 1967.

ACKNOWLEDGMENTS

The sponsors of this fourth step in a planned documentation of progress and purpose in undersea physiology acknowledge their heavy indebtedness to the many individuals whose work assured the successful completion of these proceedings. Special mention can be made of only a few.

Dedicated and exceptional contributions were made by Miss Nancy Struble, continuously from planning through the intricacies of her role as assistant editor in the publication of the proceedings. Dr. Leonard Libber and Miss Suzanne Kronheim of the Physiology Branch, Office of Naval Research, provided the continuity of purpose and support that has permitted this series to proceed without interruption over now many years. Dr. Edward L. Beckman, Mrs. Elizabeth Smith, and Mrs. Mary Sieber contributed the meticulous and extensive technical editorial assistance essential to integration of international contributions to a scientific document. Finally, the graphic skills of Mrs. Betty Hanley provided many of the diagrams and illustrations upon which ready understanding of these chapters depends. The extensive and tireless service of these and other contributors is greatly appreciated by all of the participants and especially by the editor who was so directly supported by their efforts.

Part I. OXYGEN. MECHANISMS OF TOXICITY

THE SCOPE OF CHEMICAL OXYGEN POISONING

Niels Haugaard

The work of Paul Bert (3) in the last century established without doubt that breathing oxygen at pressures above a certain value is incompatible with normal life. He observed that increased pressures of oxygen produced a characteristic syndrome in mammals that he called oxygen poisoning. The most marked and dramatic manifestation of oxygen toxicity was the opisthotonus and clonic–tonic convulsions induced in mammals exposed to hyperbaric oxygen. Paul Bert also understood and demonstrated that oxygen poisoning was not a phenomenon confined to animals with a central nervous system, but could be elicited in lower animal organisms and plants as well. At a time when the existence of enzymes had not been firmly established, he recognized that "ferments," substances that were capable of degradative reactions such as digestion of meat, could be inactivated by oxygen.

The history of the discovery of O_2 poisoning and the early work in this field has been treated in several review articles (1, 5, 6, 8, 14) and will not be discussed in detail here.

It should be emphasized that, although O_2 is necessary for the production of energy and survival of all aerobic cells, it is also a universal cellular poison. It is only because cells in the course of evolution have developed special defense mechanisms against the toxic effects of O_2 that life as we know it has been able to flourish. In a sense, the study of O_2 toxicity is the study of the ways in which organisms manage to protect themselves against the oxidizing potential of molecular O_2. Viewed in this way, O_2 poisoning is not a special phenomenon seen only under unusual circumstances such as in deep-sea diving or when animals or men are exposed to elevated pressures of oxygen in high-pressure chambers. Toxicity of O_2 is a fact that all organisms on earth have to contend with. The difference between the biological effects of O_2 at 0.2 atm and at 1, 2, 3, or 10 atm is only one of degree. Physiological or biochemical studies of animals or man exposed to elevated pressures of O_2 can therefore also be expected to lead to important information about the role of O_2 in regulating cellular activity at the tension of O_2 normally present at sea level.

Another important consideration in this connection is that the concentration of O_2 that produces a toxic effect on cellular metabolism is not necessarily that present in the gaseous atmosphere surrounding the organism. In a mammalian cell the tension of O_2 is a function of the blood supply, the diffusion of O_2 from the blood vessel to the cell, and finally the rate of O_2 uptake per unit weight of tissue. In rapidly respiring tissues, such as brain cells, the O_2

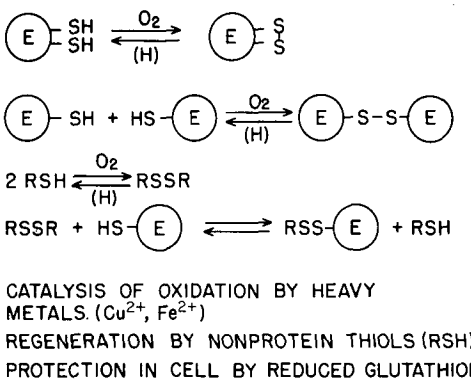

FIG. 1. Possible mechanisms involved in the inactivation of SH enzymes by O_2.

tension is therefore far less than that of the arterial blood (11). Therefore, when convulsions occur in animals or man exposed, for example, to inspired O_2 tensions of 3 atm, the P_{O_2} that produces the toxic effects in the CNS ranges from nearly 3 atm at some enzymic locations to considerably below 1 atm in other intracellular sites (12).

The mechanism by which O_2 exerts its toxic effect on cells has intrigued many investigators and is a problem that is still unsolved. One reason for this is that there is not one but probably a great many sites at which O_2 exerts effects on metabolic reactions or on specific cellular functions.

Studies with isolated enzymes and tissue preparations *in vitro* have shown that many enzymic reactions are resistant to prolonged exposure to high pressures of oxygen (HPO), but also that many enzymes are quite easily inactivated by O_2 (1, 5, 8, 14). Among these are the so-called sulfhydryl (SH) enzymes, which contain an SH group which is necessary for their activity. Among these are several hydrolytic enzymes, many dehydrogenases (including a key enzyme in glycolysis), glyceraldehyde phosphate dehydrogenase (GAPD), and certain enzymes concerned with the respiratory chain and oxidative phosphorylation.

Inhibition of SH enzymes by O_2 theoretically can occur by one of several mechanisms. These are illustrated in Fig. 1. If two SH groups are present close to each other on the surface of the protein enzyme, oxidation can occur within the molecule to form a disulfide (S-S) linkage. This may be the mechanism of O_2 effect upon GAPD, which has two SH groups in cysteine moieties separated by only three amino acids. If an enzyme protein contains only one SH group or SH groups widely separated, oxidation may occur between two enzyme molecules. Such a bimolecular reaction between enzymes is not likely to occur to any great extent and inactivation of an SH enzyme will probably take place mostly by mechanism 3 illustrated in Fig. 1. This mechanism represents a prior oxidation of a nonprotein, cellular SH compound such as glutathione followed by mixed disulfide formation. The last reaction is reversible and is also the reaction by which oxidized SH enzymes can be reactivated by a substance such as reduced glutathione (GSH).

The schema demonstrates the dual role that gluthathione, a universal constituent of cells, can play. By being oxidized it can react with SH groups of enzymes to inactivate them. In its reduced state it can regenerate enzyme thiol groups and in this way reverse the toxic effects of O_2.

TABLE I

MOLECULAR MECHANISMS OF OXYGEN TOXICITY[a]

1. Direct oxidation by molecular O_2
   $2RSH + \frac{1}{2} O_2 \rightarrow RSSR + H_2O$
2. Oxidation by free radical formation
   $RSH + O_2 \rightarrow RS\cdot + HO_2\cdot$
   $RSH + HO_2\cdot \rightarrow RS\cdot + H_2O_2$
   $2RS\cdot \longrightarrow RSSR$
   $H_2O_2 \xrightarrow{\text{catalase}} H_2O + \frac{1}{2} O_2$

   Sum: $2RSH + \frac{1}{2} O_2 \rightarrow RSSR + H_2O$

[a] For a discussion of free radical mechanisms in O_2 toxicity, see Gilbert (7).

Considerable thought has been given to the manner in which the oxidation of SH groups by O_2 is brought about. Two possibilities are illustrated in Table I. In one, an increased concentration of O_2 may by mass action drive a reaction such as the oxidation of GSH toward the right ($2GSH + \frac{1}{2}O_2 \rightarrow GSSG + H_2O$). On the other hand, free radicals such as $RS\cdot$ and $HO_2\cdot$ could be formed during hyperbaric oxygenation. $HO_2\cdot$ subsequently can oxidize a second thiol group to produce the free radical $RS\cdot$ and hydrogen peroxide. Two moieties of $RS\cdot$ can then combine to form a disulfide. The hydrogen peroxide can be expected to be degraded by the ubiquitous enzyme catalase. Note that the net result of the two processes is the same. Which mechanism is the predominant one is not known; however, the fact that certain trace metals, such as cupric and ferrous ions, are essential for the oxidation of SH groups by O_2 favors the view that free radicals are involved in this mechanism of O_2 toxicity.

Among the possible biochemical sites of O_2 toxicity are: (1) SH enzymes; (2) thiol-containing coenzymes, lipoic acid, coenzyme A, and GSH; (3) flavoprotein enzymes, particularly those containing nonheme iron in addition to SH groups; (4) enzymes requiring pyridoxal phosphate as a coenzyme—of particular interest here is glutamic acid decarboxylase (GAD), the enzyme responsible for the formation of γ-aminobutyric acid (GABA) in the nervous system; and finally, (5) lipid peroxidation should be considered. Lipids containing double bonds are essential constituents of cell membranes and many enzyme aggregates. These lipid substances form peroxides in the presence of a high concentration of O_2 and can be destroyed during this process. Lipid peroxidation is catalyzed by several trace metals, especially iron, and may play an important role in the development of O_2 toxicity.

Figure 2 illustrates the steps in carbohydrate metabolism that are susceptible to the toxic action of O_2. In glycolysis the enzyme GAPD is quite easily inactivated (10). This has been demonstrated with several *in vitro* systems. After inactivation by O_2, the enzyme can be reactivated by incubation with an SH reagent. The next step in glucose oxidation that has been found to be inactivated by O_2 is the oxidation of pyruvate and this may involve the oxidation of either lipoic acid or coenzyme A to the oxidized form. In the TCA cycle itself several dehydrogenases contain SH groups and have been demonstrated to be inactivated in *in vitro* systems by O_2. In the respiratory chain there are a number of flavoprotein enzymes that are

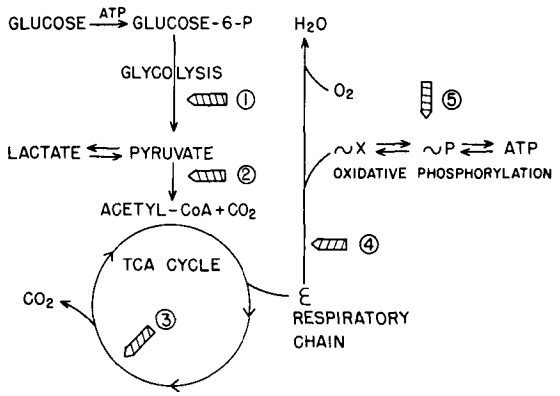

FIG. 2. Enzyme reactions in carbohydrate metabolism and energy production susceptible to O_2 toxicity.

exceptionally vulnerable to O_2 toxicity. Inactivation of one of these may be involved in the inhibition of reverse electron flow (the reduction of NAD by succinate) demonstrated by Chance and his collaborators (4). Finally, we come to oxidative phosphorylation, the formation of ATP linked to the reactions of the respiratory chain. These processes have recently been found to depend on the presence of free SH groups since agents that react with SH groups, such as organic mercurials and various disulfides, can completely inhibit oxidative phosphorylation by mitochondria (9).

In the search for a biochemical site of O_2 toxicity, too little emphasis has probably been placed on localization of particular physiological functions that may be interfered with, inhibited, or possibly stimulated in the organism exposed to an elevated pressure of O_2. The characteristic convulsions exhibited by mammals at 3 atm O_2 or greater certainly indicate that neuronal pathways in the CNS do not operate normally. The disturbance of the metabolism of glutamate and GABA as a possible cause of the neuronal dysfunction will be discussed later by Dr. Wood.

Important though they are, derangements of CNS function are not the only signs of O_2 toxicity and many other cellular functions are interfered with during exposure of an animal to an elevated tension of O_2. Many physiological processes involve the transfer of molecules or ions across the cell membrane; the transport of some of these such as sodium or glucose constitutes what has been called "active transport"—movement across a membrane of a substance by a process that requires the expenditure of energy by the cell. The sites of such reactions should be considered as potential sites at which O_2 could exert a toxic effect.

Other possible sites of O_2 toxicity are synapses in the nervous system (autonomic or central), sites at which neurohumors are released, exert an action on the postsynaptic membrane, and are finally destroyed. It is conceivable that one or more of the reactions of synaptic transmission are influenced by an elevated pressure of O_2.

A third possible site of O_2 toxicity involves the mitochondria. These organelles are involved not only in the production of energy by oxidative phosphorylation but also in the uptake and release of Ca and other ions in the cell. Free SH groups have already been mentioned as essential for oxidative phosphorylation. Oxidation of such groups by O_2 can be expected to interfere

TABLE II

THE ACTION OF TRACE METALS ON OXYGEN TOXICITY IN RAT BRAIN HOMOGENATES[a,b]

Trace metal added	Δ Glucose (μmoles/ml/40 min)		ATP net synthesis (μmoles/ml/40 min)	
	10% O_2	100% O_2	10% O_2	100% O_2
None	3.76	3.31	1.03	0.64
Fe^{2+}	2.82	1.91	0.37	0.15
Co^{2+}	3.60	3.77	1.19	1.04
Fe^{2+} + Co^{2+}	3.55	3.46	1.16	0.62

[a] The homogenates were incubated at 37° C in the presence of KCl, $MgCl_2$, phosphate buffer, glucose, and AMP as phosphate acceptor. The results are the mean values from 10 or 12 separate incubations.

[b] Results are from Ph.D. thesis of C. Williams, University of Pennsylvania, 1969 (15).

with normal cell function. Since O_2 at 1 atm or less has been found to inhibit cell division in tissue cultures, the cell nucleus should also be considered as a possible site of O_2 toxicity.

Bean and Bohr (2) and Riggs (13) showed that O_2 at 7 atm caused an immediate and dramatic decrease in tension of a smooth muscle preparation. This effect was completely reversed by returning the preparation to O_2 at 1 atm. These experiments raise the possibility that HPO may affect the contractile proteins themselves or possibly the release or uptake of Ca by cellular elements.

Finally, in a list which is far from complete, is included the possibility that an increased tension of O_2 might interfere with the normal synthesis and release of acetylcholine or norepinephrine at nerve terminals. Thus far, no evidence for or against such a hypothesis has been presented.

Certain of our own experiments concern the effects of O_2 on systems studied *in vitro*. Such studies may not reveal what happens in the intact organism exposed to O_2, but they do illustrate some of the specific actions that O_2 can exert on metabolic reactions. Williams, in our laboratory, has shown that O_2 at 1 atm, as expected from experiments with SH inhibitors, interferes with oxidative phosphorylation and Ca uptake by rat liver mitochondria (15). However, the damage produced was greater than that observed after addition of most SH reagents and it is possible that the effect of O_2 involved not only oxidation of SH groups but other reactions (possibly lipid peroxidations) as well.

Experiments were also carried out with rat brain homogenates (15) which were incubated with α-oxyglutarate (α-OG) as substrate and AMP as phosphate acceptor. Substrate oxidation and net ATP formation were measured. In every experiment the formation of ATP was inhibited to a greater extent and, what is perhaps more important, at an earlier time than the oxidation of the substrate α-OG. The experiments illustrate the great vulnerability of oxidative phosphorylation to O_2 toxicity, at least in *in vitro* systems.

The experiments reported in Table II are concerned with the effect of trace metals on O_2 toxicity on brain metabolism *in vitro*. In the absence of added trace metals, glucose utilization was inhibited to a smaller extent in 100% O_2 than it was in 10% O_2. However, ATP accumulation in the brain preparation is markedly depressed. Ferrous ions inhibited both glucose

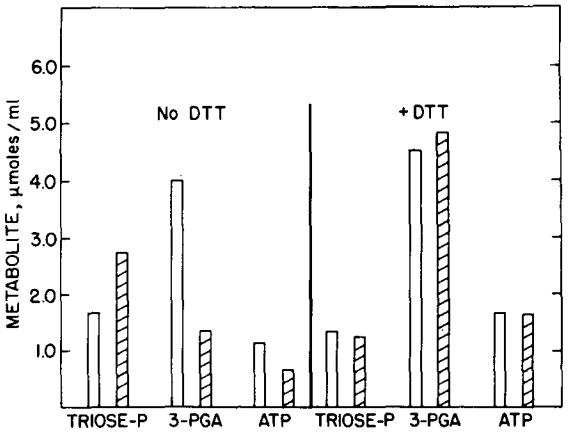

FIG. 3. Oxygen toxicity in heart homogenates. Heart homogenates were incubated for 15 min at 37° C with fructose diphosphate as substrate. Glycolytic intermediates were determined at the end of the experiment. For details, see Horn *et al.* (10). (□) 10% O_2; (▨) 100% O_2.

utilization and net ATP formation and accentuated O_2 toxicity. Cobalt ions had quite the opposite effect. They completely prevented O_2 toxicity and overcame the effect of ferrous ions so that in the presence of both ferrous and cobalt ions the system behaved as if no trace metal ions had been added. The experiments illustrate the remarkable effect that trace metals have on O_2 toxicity. Some metal ions accentuate O_2 toxicity and others are capable of protecting enzymes against inactivation by O_2. The role of metal ions in influencing O_2 poisoning deserves further study.

Figure 3 shows the results of experiments with heart homogenates (10). In this system, in contrast to brain homogenates, O_2 at 1 atm produces an inhibition of GAPD leading to a marked decrease in the rate of glycolysis. With fructose diphosphate as substrate there is a definite crossover point at the phosphoglyceraldehyde dehydrogenase reaction leading to an accumulation of metabolites, such as triose phosphates, above the metabolic block and a decrease in concentrations of metabolites, such as 3-PGA, below the block. In this system ATP formation from glycolysis is also inhibited by 100% O_2 in comparison with 10% O_2. In the presence of the thiol compound dithiothreitol (DTT) all effects of O_2 are abolished.

The *in vitro* experiments reported here demonstrate the vulnerability of several metabolic reactions to inactivation by O_2 and the effects that trace metals have on the toxic action of O_2 on metabolism. The experiments illustrate what *may*, but not necessarily what *does*, happen in the intact organism when it is exposed to an elevated pressure of O_2.

It is evident from all the foregoing that the "scope of oxygen toxicity" is broad both in mechanism and effect. I would like to emphasize what appears to me to be the most important characteristics of O_2 toxicity.

1. It can manifest itself in a great variety of ways. Most, if not all, cells are susceptible to O_2 toxicity. Toxic effects of O_2 have been demonstrated in bacteria, plants, cell cultures, amphibians, and mammals. Effects vary from inhibition of cellular movement and division of cells to impairment of highly specialized functions such as those performed by the lung, retina, or CNS.

2. Oxygen poisoning must involve many fundamental biochemical reactions such as those concerned with energy production, transport of substances across membranes, and the oxidation and synthesis of vital tissue constituents.

3. Oxygen toxicity is most likely associated with the oxidation of certain easily oxidizable chemical entities such as SH groups. Other processes may also be involved, particularly peroxidation of lipids.

4. Oxygen toxicity is not a unique and esoteric phenomenon seen only when organisms are exposed to extreme conditions of high pressures of O_2. It occurs in man in the form of interference with pulmonary function after hours of breathing pure O_2. On exposure to a pressure of O_2 above 1 atm, there undoubtedly occur a great number of cellular changes, most involving metabolic alterations, long before the overt signs of gross O_2 poisoning appear. A study of such early changes is of the utmost importance for the eventual understanding of the mechanism of O_2 toxicity and of the way in which O_2 tension can influence cell metabolism and function.

5. Finally, the progress and severity of O_2 poisoning can be influenced by a number of agents. This subject will be discussed later in this session. Let me just say here that many substances and conditions have been shown to influence O_2 toxicity under different experimental conditions: trace metals, chelating agents, SH compounds and disulfides, hormones, body temperature, and diet. It is very likely that studies of the influence of exogenously administered substances on O_2 toxicity in animals will lead to the discovery of agents that will offer man significant protection against O_2 toxicity when he is exposed to pressures of O_2 greater than that found in air at sea level.

It is clear to all who have become familiar with the subject that O_2 toxicity is not an obscure area of physiology, but one of the most important and interesting problems in the whole field of biology.

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OXYGEN TOXICITY IN NEURONAL ELEMENTS

J. D. Wood

It would be surprising if O_2 seizures should prove to be caused solely by one chemical mechanism. Since numerous systems and compounds in the body tissues are sensitive to HPO, it seems more likely that O_2 poisoning is due to a variety of factors—some related to one another, some unrelated, and some more important than others. Effects of O_2 upon γ-aminobutyric acid metabolism is in the last category, and it is with this subject that this presentation will deal.

γ-Aminobutyric acid (GABA: $NH_2CH_2CH_2CH_2COOH$) is a simple short-chain acid con-

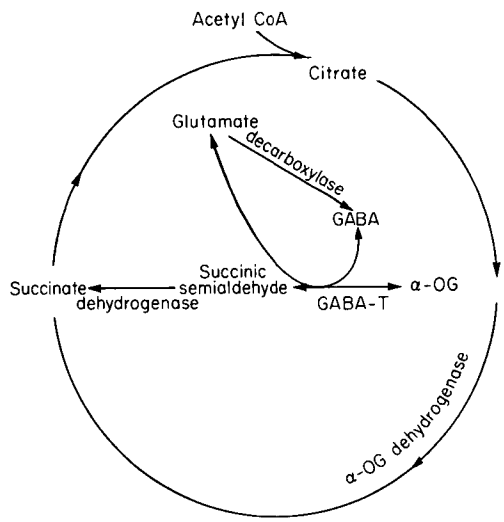

FIG 1. Metabolic reactions involving GABA and their relationship to the reactions of the TCA cycle. Only the cycle intermediates pertinent to the present discussion are shown.

taining an amino group at one end and a carboxyl group at the other. It is found in vertebrates in significant amounts only in the CNS where it probably plays two roles—that of a modulator or inhibitor of nerve transmission (3), and that of an intermediate in oxidative metabolism (8) (Fig. 1).

The pathway from α-oxoglutarate (α-OG) to succinate involving GABA is known as the *GABA shunt*, and radioisotope studies (7) indicate that about 17% of oxidative metabolism goes via the shunt in comparison with 83% that goes via the full metabolic cycle. GABA may therefore be a vital link between nerve transmission and oxidative metabolism in the CNS. The effect of HPO on GABA metabolism has seemed worthy of investigation, and the results of such a study follow.

Effect of HPO on GABA Metabolism

As Fig. 2 illustrates, HPO was shown in an earlier investigation (13) to cause a significant decrease in brain GABA levels in rats. Of prime importance was the finding that decreased levels were also observed in those animals that had not convulsed. The decrease in GABA could not therefore be attributed to the seizures per se. The GABA levels were also determined 1 hour after the end of the exposure. They had reverted to almost normal levels by that time, indicating that the changes were reversible. These findings are comparable to those of

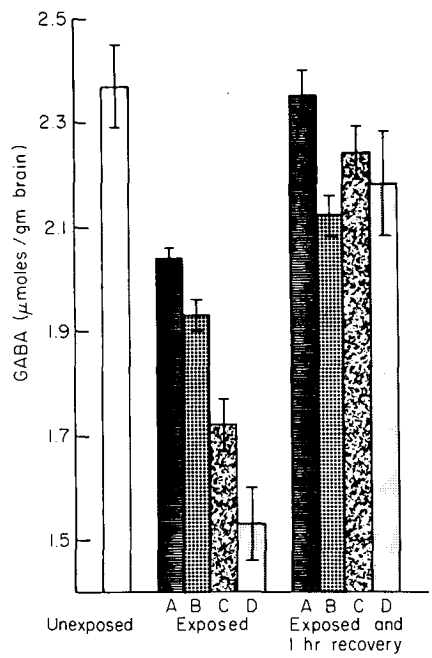

FIG. 2. Brain GABA levels in rats exposed to HPO. (A) 2-min compression to 75 psig O_2 followed immediately by 5-min decompression to ambient pressure. (B, C, and D) 33-min exposure to 75 psig O_2 with the occurrence, respectively, of no convulsions, mild convulsions, and severe convulsions (13).

TABLE I

Concentration of Total α-Amino Acids in Brains of Rats Exposed to 75 psig O_2 for 33 Min

Convulsive state	Total α-amino acids (μmoles/gm brain)
(Unexposed)	32.2 ± 0.5[a]
Nonconvulsed	31.4 ± 0.5
Mildly convulsed	31.7 ± 0.5
Severely convulsed	32.9 ± 0.5

[a] All values are the mean ± S.E. for five groups, each containing three brains (13).

Lambertsen (5), who has described the reversible nature of O_2 poisoning. Moreover, the effect of HPO was specific for GABA since no change in the concentration of total α-amino acids was observed (Table I).

It has been recognized for many years that there is a considerable difference among animal species in susceptibility to HPO convulsions (1), as illustrated in Fig. 3. In the present investigation, the susceptibility to seizures was determined quantitatively by measuring the HPO exposure required to cause convulsions in 50% of the animals tested (expressed as convulsion threshold for 50% of the animals, CT_{50}). This measurement was compared with the decrease in brain GABA for different species exposed to HPO. It is obvious from Fig. 4 that a correlation existed between the CT_{50} value and the decrease in GABA.

The time to onset of HPO seizures can, of course, be altered by varying the pressure of the O_2. Therefore, using pressure as the variable and mice as the experimental animals, the CT_{50}

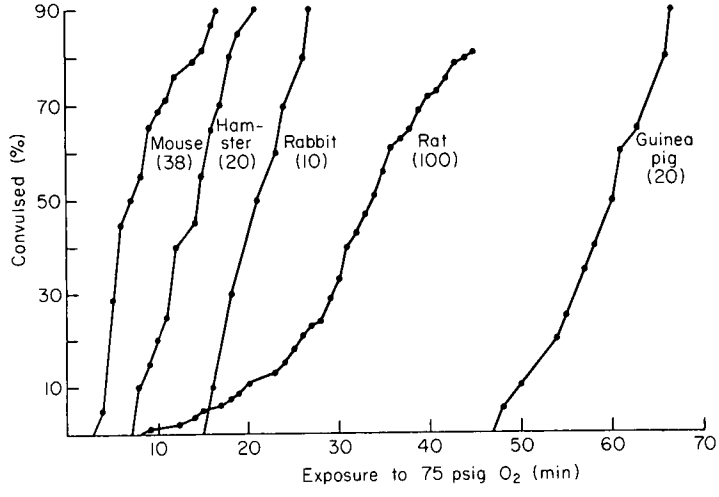

FIG. 3. Susceptibility of mammalian species to O_2 poisoning. The number of animals per group indicated in parentheses. From Wood *et al.* (17), by permission of the publishers.

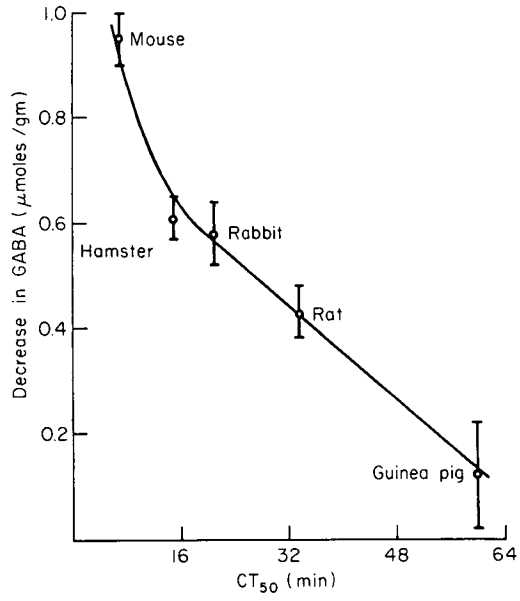

FIG. 4. Decrease in brain GABA level relative to susceptibility to O_2 poisoning. CT_{50} indicates the 75 psig O_2 exposure required to convulse 50% of the animals. Decrease in GABA was measured using a 25-min exposure to 75 psig O_2. From Wood *et al.* (17), by permission of the publishers.

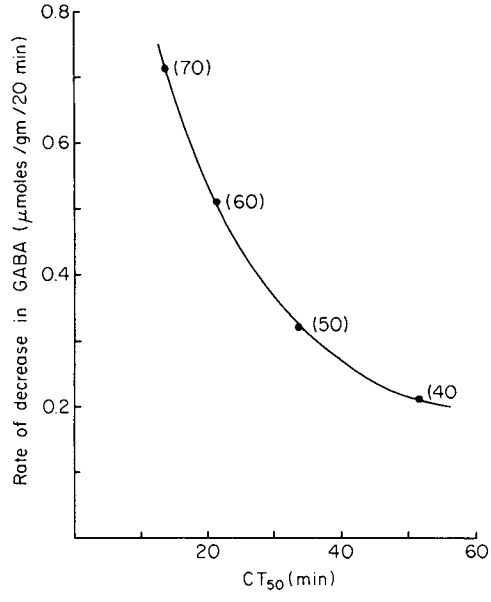

FIG. 5. Correlation between rate of decrease in brain GABA concentrations and susceptibility to HPO seizures in mice at different pressures. CT_{50} indicates the exposure required to convulse 50% of the mice. Numbers in parentheses indicate psig O_2 (18).

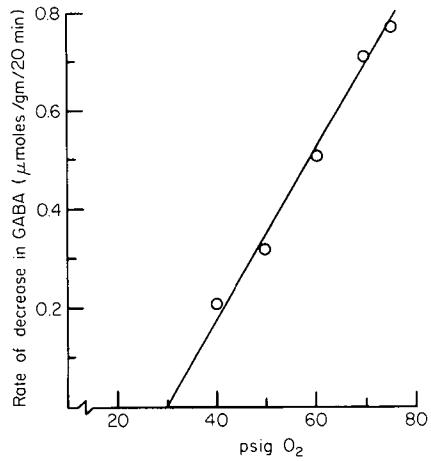

FIG. 6. Rate of decrease in brain GABA concentration in mice as a function of O_2 pressure. From Wood *et al.* (18), by permission of the publishers.

values were compared with the rate of decrease in GABA. Once again, the correlation was good (Fig. 5). When the rate of decrease in GABA was plotted against the O_2 pressure, a most interesting correlation emerged (Fig. 6). A linear relationship between these factors was observed, but, more importantly, the critical pressure causing brain GABA to decrease was 30 psig (3 atm abs). This is the same pressure that has previously been recognized as the one inducing HPO seizures in animals and in man (5, 11).

Another well-known factor influencing O_2 toxicity is the amount of CO_2 in the breathing mixture (1, 2, 12). As earlier workers such as Marshall and Lambertsen (6) have observed, 0.5% or 1.0% CO_2 in the mixture hastens the onset of seizures in mice breathing O_2 at 60 psig, whereas 5% CO_2 prevents the seizures (Table II). Most interestingly, a 0.5% or 1.0% CO_2 level accelerated the rate of decrease in brain GABA caused by HPO, whereas a 5% CO_2 level almost completely prevented any decrease in GABA. These results are therefore in keeping with the correlation previously observed between susceptibility to HPO seizures and decrease in brain GABA concentrations.

TABLE II

Effect of CO_2 on HPO-Induced Convulsions (60 psig O_2 for 20 Min) and Changes in GABA Levels in Brains of Mice (18)

CO_2 in breathing mixture (%)	Decrease in GABA (μmoles/gm/20 min)	CT_{50} (min)
—	0.51	21.3
0.5	0.74	14.0
1.0	0.91	5.7
5.0	0.04	—

TABLE III

Protective Action of Different Dosages of GABA against Oxygen Poisoning (45-Min Exposure at 75 psig) in Rats (15)

GABA injected intraperitoneally 15 min prior to exposure (20 animals each group) (mmoles/kg body wt)	Rats with generalized seizures (%)	Deaths (%)
None	70	45
5	35	35
10	35	20
20	20	10
30	15	15

The correlation between CT_{50} values and decrease in GABA under all the conditions described above is illustrated in Fig. 7, in which all points lie on or close to the curve of best fit.

If a deranged GABA metabolism is involved in the etiology of HPO-induced seizures, then the administration of GABA prior to exposure to HPO might prevent or delay convulsions. That this protection does indeed exist is demonstrated in the results shown in Table III.

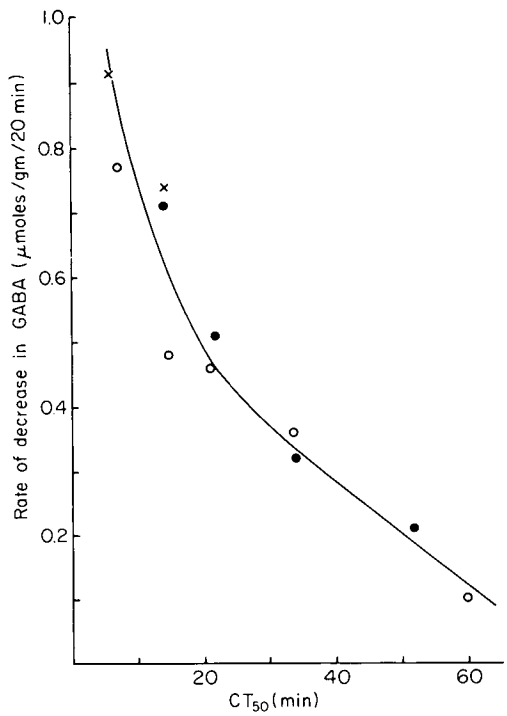

FIG. 7. Correlation between susceptibility to HPO seizures and decrease in brain GABA. CT_{50} same as Fig. 5. (○) Various species, 75 psig O_2; (●) mice, various pressures; (×) mice, 60 psig O_2 + CO_2. From Wood *et al.* (18), by permission of the publishers.

The evidence we have gathered concerning the involvement of GABA metabolism in the production of HPO seizures may be thus summarized:

1. HPO causes a decrease in GABA levels.
2. The GABA decrease occurs prior to convulsions.
3. The GABA decrease is reversible.
4. The decrease is specific for GABA among the amino acids.
5. Susceptibility to seizures correlates with the rate of decrease in GABA levels for (*a*) different animal species, (*b*) different pressures, and (*c*) different CO_2 concentrations.
6. The same O_2 pressure that induces convulsions also brings about decreases in GABA levels.
7. GABA administered intraperitoneally protects an animal against HPO seizures.

Causes and Effects of Decreased GABA Levels

The possible causes and effects of low GABA levels are shown in the schematic diagram of a nerve synapse in Fig. 8. The GABA-forming enzyme, glutamic acid decarboxylase (GAD), is found primarily in the nerve endings (9) and is probably associated with the synaptic vesicles (4, 9). In contrast, the GABA-degrading enzyme system, GABA-α-oxoglutarate transaminase (GABA-T), is found only in the mitochondria (9). Roberts and Eidelberg (8) found that brain GABA levels are normally determined by the GAD activity rather than by the GABA-T activity, despite the much greater potential activity of the latter enzyme (17).

This finding suggests that a large portion of GABA in the brain tissues does not have ready access to the catabolizing enzyme. In other words, membrane permeability probably plays a major role in the control of GABA levels. Salganicoff and DeRobertis (9) suggest that GABA formed in nerve endings is released into the synaptic cleft either directly or via the release of synaptic vesicles into the cleft. In either event, it appears that this extracellular GABA is the one involved in the inhibition or modulation of nerve transmission (3).

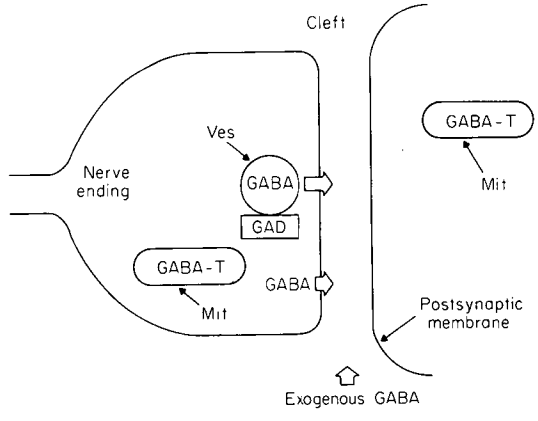

FIG. 8. Structural and biochemical organization of a synaptic complex. Mit = mitochondria; Ves = synaptic vesicles.

The HPO-induced reductions in brain GABA could therefore be brought about by any one, or a combination, of three mechanisms: (*1*) inhibition of GAD, (*2*) activation of GABA-T, or (*3*) increased membrane permeability, which would allow GABA more rapid access to GABA-T. There is good evidence from both *in vitro* and *in vivo* studies that GAD is inhibited by HPO (10, 14, 16). This inhibition does not, however, seem to be the sole cause of low GABA levels, in view of recent work (17) indicating that an increased catabolism by GABA-T may also occur. There is not yet evidence indicating whether this increased catabolism is due to an actual activation of GABA-T or to the greater permeability of GABA, but I suspect the latter.

If low GABA levels induce HPO seizures, the cause is probably a low extracellular concentration of the amino acid. If this is so, total brain GABA levels are useful in assessing the role of GABA in HPO seizures only if they accurately reflect the extracellular concentration. We have perhaps been lucky in maintaining a normal intracellular–extracellular ratio throughout our experiments; it is conceivable that certain medical treatments or physiological conditions might very well alter this ratio of GABA in the tissues. Until a technique is available for the accurate determination of extracellular GABA concentrations, the problem of measurement will remain a major obstacle in the complete evaluation of the role of GABA in HPO seizures.

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THE INTRACELLULAR OXIDATION-REDUCTION STATE AT HIGH AND LOW OXYGEN CONCENTRATIONS

Britton Chance

The necessity of O_2 for intracellular bioenergetic reactions, on the one hand, and the damage to sensitive enzymes by higher pressures of O_2, on the other, suggest the need to elaborate in some detail the quantitative nature of dangerously low and dangerously high O_2 tensions. The cells of mammalian organs—liver, kidney, heart, and brain—afford experimental data that are relevant to human responses to HPO. Our attention will be focused mainly upon primary rather than secondary responses, i.e., those occurring in the first few minutes of anoxia or hyperbaric conditions. Convulsions are the first external sign of hyperbaric toxicity in small animals and man; the primary consequences occurring at the cellular level in the same time range of a few minutes are the topic of this paper. The more involved effects which occur in a quarter of an hour or several hours will not be discussed here, nor will the somewhat different responses of the organs of amphibia, for example, the toad bladder (J. Allen and H. Rasmussen, communication at this symposium).

In Fig. 1 I have attempted to present in a single diagram the range of O_2 concentrations approaching tissue anoxia at the left-hand end of the scale, and tissue damage at the right-hand end, for the different cellular oxidase systems (4, 5). The abscissa represents the logarithm of the O_2 concentration in micromolar units, which are approximately equal to P_{O_2} in mmHg at these levels. The biochemical responses at the two ends of the scale are evaluated by direct recording of the fluorescence of intracellular reduced pyridine nucleotide (PN). At the left, this component is too far reduced for appropriate energy metabolism, while on the right it has become too highly oxidized as a consequence of primary events in hyperbaric toxicity affecting mitochondrial reactions.

The various cellular oxidase systems which must be considered in the total picture of O_2 metabolism show different affinities for O_2 (6). Thus, the ordinates of Fig. 1 differ for the various curves. The curve labeled "mitochondria" illustrates the increasing activity of the mitochondrial respiratory chain as the P_{O_2} increases from left to right, and its effectiveness in causing increasing oxidation of reduced PN in the mitochondria. Intracellular O_2 concentrations of as little as 0.1 μM satisfy the needs of the mitochondrial system, and provide satisfactory oxidation of the pool of reduced PN and activation of the energy resources of the mitochondria. Tissue gradients may cause the capillary O_2 tension to be somewhat higher than 0.1 μM; our

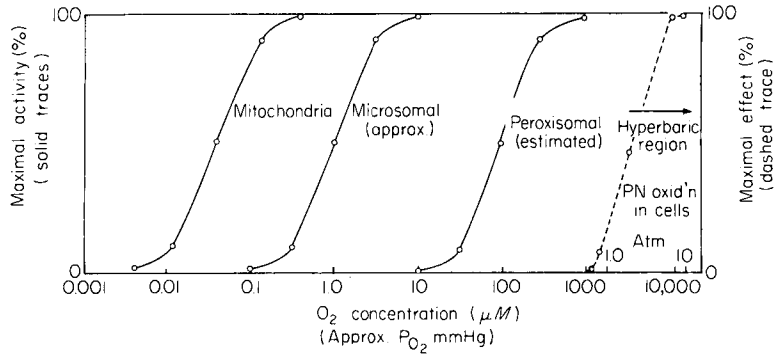

FIG. 1. Schematic diagram of O_2 affinity of respiratory systems.

experiments suggest that small mammals breathing 4 to 5% O_2 have reached the critical intracellular P_{O_2} as indicated by direct measurement of tissue fluorescence (5). On the other hand, when the O_2 pressure reaches approximately 5 atm, as shown at the right-hand side of the diagram, the PN oxidation proceeds to a much higher level and the toxic effects of HPO become evident.

The microsomal system which is responsible for the detoxification reactions and steroid metabolism of the liver, and the peroxisomal system which may operate in the pathway of glyoxylate metabolism in certain tissues, have considerably higher O_2 requirements than do the mitochondria (R. W. Estabrook, C. De Duve, personal communication). It is probable that temporary anoxias which inactivate these systems by reducing the intracellular O_2 tension to 0.1 μM do not have any immediate biochemical or physiological repercussions, because this O_2 tension is not low enough to impair the operation of the cytochrome system of the mitochondria.

Since the mitochondrial response to hyperbaric O_2 represents one of its most rapid and sensitively localized biochemical effects, it seems essential to emphasize some of its aspects in detail. The methodology by which the fluorescence of reduced PN may be used to indicate the intracellular oxidation–reduction state in mitochondria (1) and in intact tissues of anesthetized animals (2), and its application to hyperbaric conditions (3–5) have been described elsewhere.

Two effects were predominant in our studies: first, an increased oxidation of intracellular PN, and second, an increase of the ATP/ADP ratio (4). Both these responses are of considerable interest, since they are found in liver, kidney, and brain cortex and are thus not organ-specific. In general, an increased ATP/ADP ratio would be considered to be beneficial for cell metabolism, since the metabolic transition is in the direction of increased phosphate potential. However, the observation that these increases in the ATP/ADP ratio were accompanied by increases in the oxidation state of PN well beyond the level which is characteristic of optimal metabolism led us to suspect that the phenomenon was a consequence of an interruption of a pathway of ATP utilization rather than of an increased biosynthesis of ATP (4).

Since the mitochondria are the principal site of the cellular systems which synthesize ATP, results obtained from intact tissues could be compared with studies on the isolated organelles. Mitochondria obtained from the same animals as were exposed to HPO were evaluated for

their response to hyperbaric O_2. These studies demonstrated a convincing correlation of the pressure and time profiles for the oxidation of PN in the isolated mitochondria and in the parent tissue, suggesting that the mitochondrial redox state is a primary target of HPO. Although, indeed, there may be other targets as well, this has proved to be the most readily verified in an isolated cell fraction and is of such a nature as to be consistent with the increase in the ATP/ADP ratio.

Our explanation ran along the following lines: NADH is maintained reduced in mitochondria *in vivo* not only by the NAD-linked dehydrogenases of the citric acid cycle but also by a pathway of electron transport which leads from high potential substrates such as succinate, α-glycerol phosphate, and, more recently, palmitoyl-L-carnitine, through the energy-coupling site I, to the NAD dehydrogenase of the respiratory chain. Energy conserved in the entire respiratory chain can be focused upon site I to cause electrons to flow against the normal thermodynamic gradient between the succinate/fumarate couple at approximately 0 mV to the NAD/NADH couple at -320 mV. Essentially, the mechanism requires that the oxidation–reduction state of a low-potential component at -320 mV be raised to a potential of approximately 0 mV, or vice versa. The pathway of electron flow and the pathway of energy coupling involve mersalyl-sensitive components with reactive -SH groups, which recent work (7) has identified more precisely with the rotenone-sensitive site and possibly with energy coupling at site I as well. Hyperbaric O_2 appears to inhibit the flow of electrons against a thermodynamic gradient, possibly at the mersalyl-sensitive site, thus decreasing the energy utilization in the pathway of reversed electron transfer and consequently increasing the ATP/ADP ratio. This also decreases the level of reduction of mitochondrial NADH, and it may well be that many reactions which depend upon a highly reduced state of NADH in the mitochondrial space are inhibited as this component becomes more oxidized. Therefore the decreased utilization of ATP has a multiplicity of causes rather than a single cause.

High Pressure Oxygen as a Tool in the Study of Metabolic Control Phenomena

Of the many rapid perturbations that can be imposed upon metabolizing tissues, one of the most rapid and effectively reversible is that provided by HPO. In order to identify the sites of decreased ATP utilization—the main manifestation of hyperbaric O_2 toxicity at the cellular level—a detailed analysis of profiles of metabolite intermediates is desirable. Although the cortex of the brain, the organ most sensitive to variations in O_2 tension, might seem to be the most appropriate material for such studies, the many pathways of metabolism in the brain, together with the heterogeneity of cell types, make this a difficult subject for incisive studies of metabolic control. Studies of other mammalian tissues such as kidney or liver, and of amphibian tissue (J. Allen and H. Rasmussen, presented at this symposium) as well, may also shed light on the detailed mechanism of metabolic control phenomena under conditions of hyperbaric O_2.

The increased oxidation level of intracellular pyridine nucleotide and the increased ATP/ADP ratio discussed here are by no means the only effects of hyperbaric toxicity; as emphasized above, there are a variety of consequences which follow at different times and in different organs. While the response of mitochondrial PN can be complete in 5 min, 50 min are required to inhibit α-ketoglutarate oxidation. Similar differences of response time can be observed for hyberbaric toxicity of the lungs as compared with other tissues. Thus, in order to achieve a

generalized and effective correlation of the available experimental results, attempts should be made to establish a common time and pressure scale for all those working in this area.

The application of *in vivo* readout of metabolic states from the cortex of anesthetized animals is well in hand. It is now possible that suitable light pipes and implanted cannulae may permit a continuous monitoring of the brain cortex of the conscious animal under normoxic and hyperbaric conditions, an approach which would be of advantage in correlating the threshold for convulsive responses of the conscious animal with biochemical readout of oxidation–reduction states in the brain cortex. As further technological advances are made, other incisive comparisons of the intracellular redox state with gross physiological manifestations of hyperbaric toxicity may become possible as well.

ACKNOWLEDGMENT

This research was supported by PHS grant GM 12202.

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NATURAL RESISTANCE TO OXYGEN POISONING

Brian G. D'Aoust

The varied and sometimes contradictory results of research on O_2 poisoning (3, 8) suggest that, despite our recognizing the importance of a general inhibition of energy metabolism (9, 14, 15, 24, 34, 45, 49, 50) and membrane lipid peroxidation (51) (and the latter's probable significance in mitochondrial (28) and nerve synaptic function (51)), we are considerably further from a general understanding of the phenomenon than we would wish—perhaps as far as a cell homogenate is from an intact cell. The uncertainties in relating *in vitro* results to the whole animal are balanced by the limited chemical information that experiments on the intact animal have provided—although such experimentation does reveal the actual time course of O_2-induced convulsions. The *in vitro* and *in vivo* approaches, which are clearly complementary, begin at two quite different investigative points and are moving toward one another.

By analogy, it is important to distinguish between two quite different questions regarding the response of organisms to O_2: what makes them O_2-sensitive, and what renders them O_2-resistant? The reason for thus separating the two sides of the same coin lies in the somewhat different assumptions implied in each question; any hypothesis that can explain the mechanism of O_2 poisoning in a particular system must also be consistent with the mechanism of O_2 resistance in the same system. The purpose of this presentation is to emphasize the importance of a comparative approach to the problem of O_2 poisoning.

Oxygen-Resistant Systems

The widespread destructive effects of O_2 in many organisms (4, 22) have made it important to identify any natural means by which an organism can protect itself. The most direct approach, then, is to look for organisms that are intrinsically insensitive to elevated (>0.21 atm abs) O_2 pressures. Research on such organisms should reveal the essential characteristics of O_2-resistant cells, and should, furthermore [as Haugaard (24) has suggested], become a useful tool in investigations into fundamental cellular properties.

Two examples of intrinsically O_2-resistant systems will be discussed. One is the gas-concentrating mechanism in the swimbladder of fish, which functions under HPO (5); the other is that part of the photosynthetic apparatus of plants which produces O_2 photochemically. Other examples are organisms living in shallow estuaries, lakes, and ponds in which the dis-

solved O_2 may approach 0.6 atm abs during certain periods. Though certainly of interest, these organisms will not be considered here.

Gas Transport in the Swimbladder

Interest in the physiology of the swimbladder has been prompted chiefly by the question of gas transport into the swimbladder against an O_2 pressure gradient that in deep-sea fish is extremely steep (38, 39). Below a depth of approximately 100 m, O_2 comprises 85–95% of the gas in the swimbladder, and this percentage is apparently maintained in species living at great depths (17). Thus the P_{O_2} in the swimbladder of marine fish is approximately equal to 0.09 times the depth of water in meters. Pressures of 100 or 200 atm are common to a number of species, and recently a specimen with a functional swimbladder, *Bassogigas*, was taken at 7160 m (35). At such a depth this fish would contain a P_{O_2} of approximately 640 atm in its swimbladder.

Unfortunately, the availability of such specimens is an inverse function of its physiological interest; investigators have therefore had to be content with less spectacular species. However, there now exists a satisfactory theory concerning the mechanism of gas secretion in the swimbladder that is fairly well supported experimentally (18, 30, 38, 39, 42–44), and two recent reviews of it are available (1, 21).

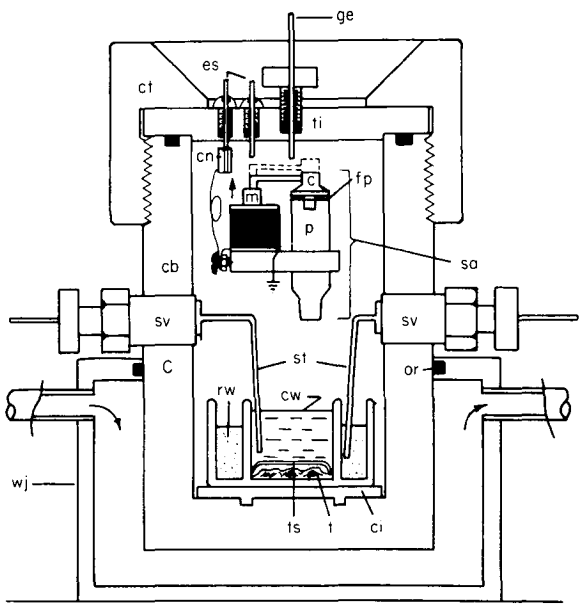

Fig. 1. Pressure chamber used for studies of metabolism in the gas gland. C, Chamber; cb, chamber body; ct, chamber top; cn, connector; ci, chamber insert (bottom); c, cap; cw, center well of glass tissue vessel; es, electrode seals; fp, filter paper seal of solenoid-operated pipette; ge, gas exhaust; m, magnetic core of solenoid; or, O-ring seal; p, pipette; rw, ring well; sa, solenoid assembly; st, sample tubes; sv, sample valves; t, tissue; ts, Teflon screen; wj, water jacket. From D'Aoust (11), by permission of the publishers.

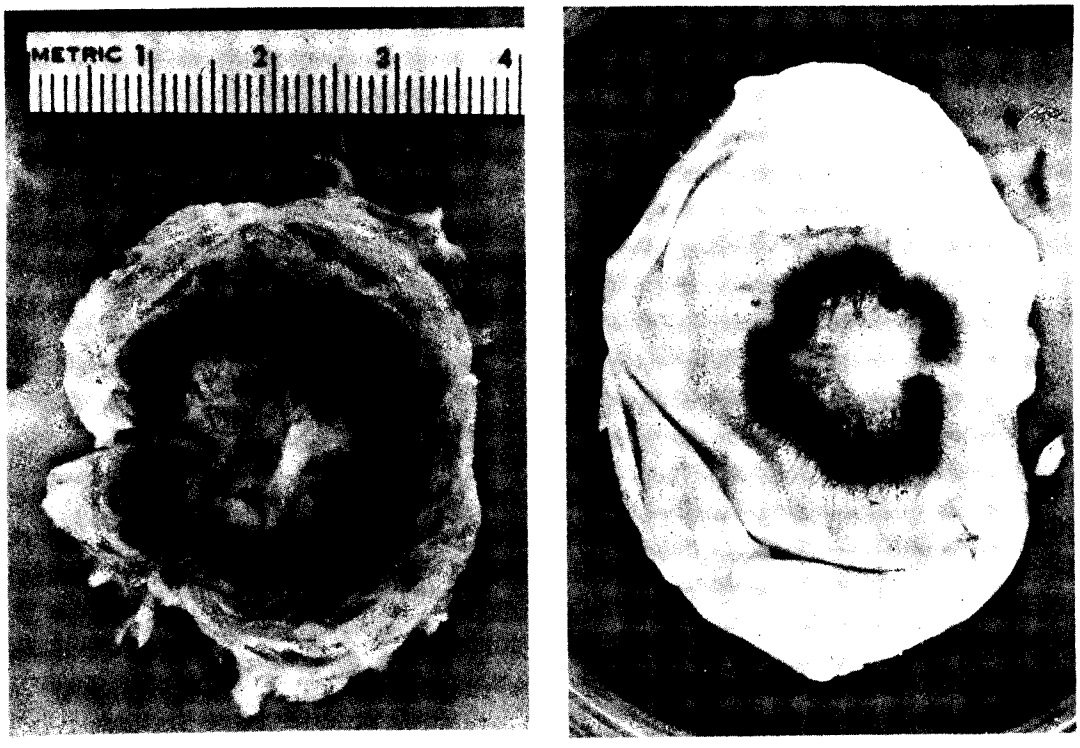

FIG. 2. Dissected swimbladder of *S. miniatus* opened dorsally showing (right) gas gland consisting of rete mirabile and epithelial tissue on ventral wall; and (left) gas gland after separation from swimbladder wall, as used in hyperbaric experiments. From D'Aoust (13), by permission of the publishers.

Of interest here is the fact that the gas gland tissue is located inside the swimbladder and must be assumed to be equilibrated with the O_2 tensions therein. Moreover, this tissue apparently functions glycolytically to produce sufficient lactic acid (2, 13) for the process of gas secretion (18, 30, 38, 43). Much of the work reported here was directed toward answering the question: Does the gas gland tissue produce lactic acid under HPO? If this is so, it would seem that the "Pasteur effect" is not operative in this tissue, which in turn suggests that aerobic energy metabolism is a minor catabolic pathway, or is under some form of constant repression.

As indicated earlier, compromises were inevitable in choosing a fish that lives at a sufficient depth to be interesting but that also is in relatively abundant supply. The species used was the vermillion rockfish, *Sebastodes miniatus*—a common spectator around SeaLab II—which has been caught commercially at a depth of 200 m. Most specimens used in this investigation were taken at depths of 30–60 m, the bladder containing 80–95% O_2 (as measured with the Scholander ½-ml analyzer) (37).

The methods used have been described in greater detail elsewhere (11, 13). Figure 1 shows the pressure chamber and related apparatus as they were used in studies of glycolysis in N_2 and O_2 atmospheres. The gas gland tissue shown in Fig. 2 was dissected virtually intact with very little damage. It was washed in buffered saline, which simulated the blood with respect

TABLE I

GLYCOLYTIC RATES (μM LACTATE/MG DRY WT/HR) UNDER O_2 AND N_2 BY GAS GLAND TISSUE OF *Sebastodes miniatus*

Pressure (atm abs)	O_2	N_2
6	0.02–0.19	0.03–0.28
10	0.13–0.30	0.16–0.62
30	0.05–0.13	—

to pH (P_{CO_2} = 0.01 atm), osmolarity, and the major ions present. It was then transferred to 5 ml of the same buffered medium in a glass vessel specially designed to fit the chamber (Fig. 1). The pH level of the medium was maintained constant by means of an external CO_2 buffer adjusted so that it was in equilibrium with 0.01 atm of CO_2 at 15°C. After pressurization samples were taken at 10- to 30-min intervals and were analyzed for total lactate, glucose, and/or radioactivity if labeled substrates were used. Production of $^{14}CO_2$ was monitored by sampling the CO_2 buffer (which contained 98% of the CO_2 in the system) and counting in a 1-L ion chamber in air at atmospheric pressure. It was hoped in this way to monitor indirectly the extent of O_2 consumption.

The results of experiments on intact fresh gas gland tissue have been previously reported (13), but are reviewed here since they are of interest in the problem of O_2 toxicity. Considerable variation in glycolytic rate occurred under all conditions. The variability was probably the result of using an intact organ, which no doubt reflected different metabolic states of the gland prior to dissection.

Table I shows glycolytic rates produced by a range of O_2 and N_2 pressures. These are rather low rates of lactic acid production. The glycolysis observed could be inhibited by both fluoride and oxamic acid. Under the experimental conditions, a comparison of O_2 and N_2 at different pressures revealed no significant difference in glycolytic rate. It must be emphasized, however, that probably only a resting metabolism was observed, and subtle differences would therefore not be detected at these rates. A calculation of the lactate production rates necessary to support gas secretion, based on the countercurrent multiplier theory (18, 30), suggests that a greatly accelerated rate of glycolysis must occur during gas secretion. Under the conditions of these experiments, of course, the tissue is not perfused and glucose and lactate exchange must therefore take an abnormal route.

Although it is unlikely that these results indicate the normal glycolytic activity of the tissue during gas secretion, they do indicate the capacity of the tissue to produce lactic acid under O_2 pressures known to be toxic to the intact fish (12, 29, 36). These results further indicate that the glycolytic pathway need not be considered inherently O_2-sensitive (45). It is quite likely, however, that when glyceraldehyde-3-phosphate dehydrogenase activity is assayed in this tissue, it will prove to be O_2-sensitive—as was shown by Horn and Haugaard (26) in the enzyme of rat heart homogenates under considerably lower pressures than were used here.

The possibility that oxidation of pyruvate to CO_2 and water was occurring at the same time as the production of lactate is unlikely because of the similar rates of lactate production under both N_2 and O_2 pressure, as shown in Table I. This was further checked by use of uniformly

TABLE II

PERCENTAGE CONVERSION OF GLUCOSE TO LACTATE AND CO_2 UNDER O_2 AND N_2

	Lactate		CO_2	
	$\bar{X}$	S	$\bar{X}$	S
O_2: 6–30 atm abs	7.4	5.2	0.066	0.022
N_2: 6–10 atm abs	11.5	6.7	0.15	0.14

labeled glucose as a substrate. Table II indicates the percentage conversion of glucose-^{14}C both to $^{14}CO_2$ and ^{14}C-lactate. Again, there was considerable variability, but it is clear that $^{14}CO_2$ was produced at about 100th the rate of lactate under N_2 and O_2 pressure. Other experiments (23), in which 3-4-labeled glucose was used as a substrate and in which LDH was inhibited with 0.01 *M* oxamic acid, failed to increase the amount of $^{14}CO_2$ produced, thus supporting the conclusion that most of the CO_2 originated through the pentose–phosphate pathway under both O_2 and N_2 (13).

These last results support Fänge's suggestion (21) that systems for converting pyruvate to CO_2 are weakly represented in the gas gland tissue, which is consistent with the known sensitivity of aerobic glucose catabolism to O_2 (15, 24, 45). Nevertheless, an appreciable uptake of O_2 has been demonstrated in the gas gland tissue of the scup, *Stenotomus* (2). Fänge (20) himself, furthermore, demonstrated succinic dehydrogenase activity, by the Thunberg procedure, in the gas gland tissue of the cod, *Gadus*, which lives in approximately the same depth range that the rockfish does. Dorn (16) has extensively studied this same tissue in the eel with an electron microscope, and although the mitochondria of the gas gland are small, they are fairly numerous. There does not as yet appear to be sufficient evidence to rule out the citric acid cycle in this tissue.

It is, however, of interest that homogenates of freeze-dried gas gland tissue of *S. miniatus* (in which glycolytic activity was preserved) produced a cytochrome spectrum that was apparently devoid of cytochrome A. A similar check on freeze-dried tissue from a species living at 1000 m (O_2 at 100 atm), *Coryphenoides acrolepsis*, showed only a small γ peak and no α absorption at four times the concentrations of a homogenate of *S. miniatus*. Absence of cytochrome A would seem to be the logical inference for a tissue that cannot absorb O_2. It is tempting to speculate that this lack may be an adaptive characteristic of the gas gland tissue in deep-sea fish.

Although such speculation awaits experimental support, it seems reasonable that the metabolic machinery most sensitive to O_2 poisoning—namely that associated with O_2 consumption and aerobic production of ATP—is apparently weak in the gas gland tissue. On the other hand, the very great extremes of O_2 pressure to which this tissue is exposed at least suggest that active protective mechanisms against O_2 toxicity (22, 33, 51) can be ruled out. What, therefore, we might term "passive" adaptations relative to the structure and conformation of subcellular systems probably account for most instances of O_2 resistance. This view is consistent with the other example of O_2 resistance to be discussed here—namely, the photochemical production of O_2.

Photochemical Production of Oxygen

A P_{O_2} of 1 atm abs has long been known to inhibit photosynthesis in *Chlorella* (10, 47), and even the atmospheric P_{O_2} (0.21 atm abs) has recently been shown (6) to be inhibitory to CO_2 fixation in higher plants (P_{CO_2} = 0.0003 atm abs). Thus one might question whether there can be any plant system associated with photosynthesis that is intrinsically O_2-insensitive. It is clear, however, that if a plant cell is equilibrated with O_2 at 0.21 atm abs (as it must be at the compensation point of photosynthesis), any O_2 production will raise the intracellular P_{O_2}. One might calculate, by extrapolating on a steady-state gradient, just what the intracellular or intragranal P_{O_2} must be to support an observed rate of O_2 evolution.

Such calculations are rather complex, however, depending as they obviously do upon both diffusion and convection inside and outside the cells. Moreover, it is difficult to picture the shape of the O_2 gradient, since chloroplasts are distributed throughout the cell and are in various degrees of motion at all times. It nevertheless seems reasonable, in view of inhibitory effects of O_2 on photosynthesis, that this gradient must be extremely steep in the vicinity of the photosynthetic unit. This gradient is hypothesized in Fig. 3, and is shown to be essentially 90% complete within a distance of 500 Å. Any greater distance would presumably result in photosynthetic inhibition. The diagram is not meant to be complete with respect to current concepts regarding chemical and physical mechanisms in photosynthesis; it is meant only to indicate the probable limits over which an O_2-tension gradient must occur.

In short, one need only assume that the primary photochemical conversion of water to O_2 is the reaction least sensitive to elevated O_2 pressure. It is known that photosynthetic O_2 production is not inhibited by hydrostatic pressures up to 1000 atm (46). Water pre-equili-

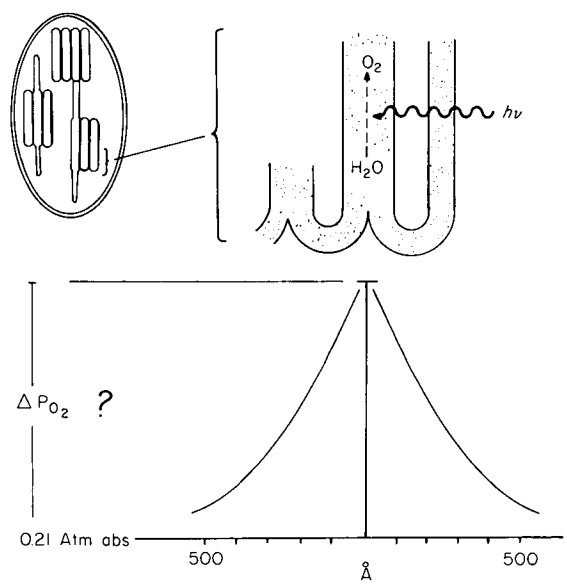

Fig. 3. Hypothetical P_{O_2} gradient in the vicinity of the photochemical apparatus. Pictured is a segment of a granal partition (48), of which the photoact is considered a subunit, with the relative P_{O_2} above 0.21 atm abs plotted against approximate distance in angstroms from the O_2-producing center.

brated with air at these pressures will have a P_{O_2} of 0.8 atm (19), so that this pressure of O_2 can be assumed to have no effect on the Hill reaction.

Recently, it has been shown that light induces a transport of hydrogen ions [H^+] into chloroplasts or particulate thylakoid preparations thereof (27). This system is presently being extensively studied not only by those interested in photosynthesis, but also by investigators of transport processes in general, and of membrane structure and function in particular (25, 27, 31, 32, 40, 41). To follow light-induced "proton pumping," all that is necessary is to use a pH electrode immersed in an unbuffered or weakly buffered suspension of ruptured chloroplasts, supplemented with suitable electron carriers (27).

Because of our complementary interests in photosynthesis and pH measurement under hyperbaric conditions, Dr. Robert Williams of the American National Red Cross Laboratory and I collaborated in a study of this system. Measurements of pH were made with a Corning combination electrode, the center (pH) chamber of which had been vented and cannulated to eliminate pressure gradients and to allow replacement of internal solutions (see Fig. 4). The top insert fits in the chamber shown in Fig. 1. Internal solutions were replaced following each

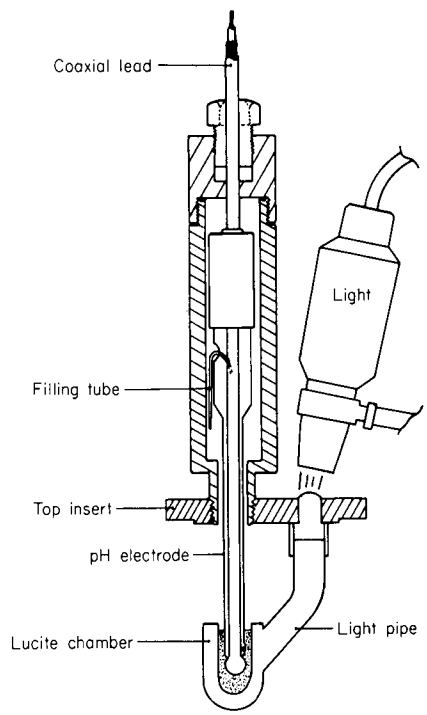

FIG. 4. Adaptation of pH electrode for use in pressure chamber shown in Fig. 1. Pressure sealing was accomplished by the packing nut at the chamber's top, which squeezes a silicone rubber washer around the coaxial lead. Light was provided by a Nicholas illuminator with a 23-W bulb. The Lucite light pipe was cemented to the Lucite cell in which the light-induced pH change was monitored. The light pipe was partially immersed in water in the chamber to facilitate temperature equilibration. It was held to the Lucite plug in the top insert by means of a Tygon sleeve.

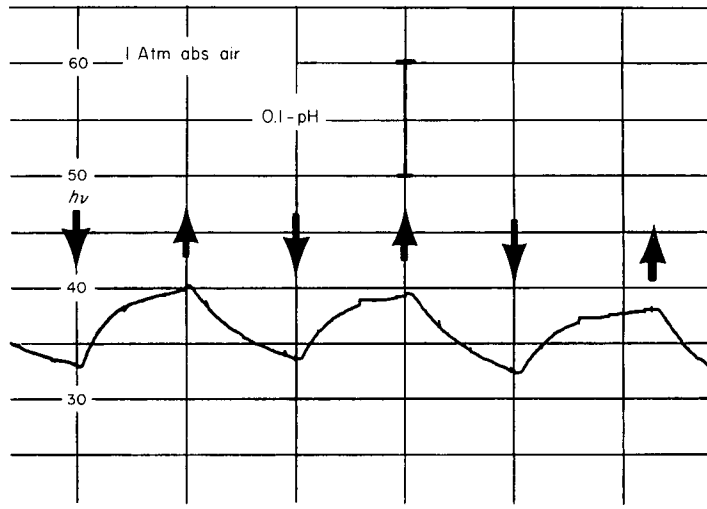

FIG. 5. Recording of light-induced pH increase taken in the chamber shown in Fig. 4 at 1 atm abs of air. In this and in Figs. 6 and 7, arrows pointing downward indicate light on; pointing upward, light off. Illumination was the same in all recordings; heavy vertical lines represent 1 min.

decompression. Checks on the effect of pressure on the reading of a standard buffer (pH 7.00) revealed a maximum change of 0.05 units in apparent pH upon pressurization to 100 atm abs.

Spinach leaves were homogenized in a blender in 0.5 osmolar NaCl (containing Tris, 50 mM; ascorbate, 10 mM; cysteine, 3.5 mM; $MgCl_2$, 0.5 mM). The homogenate was filtered, centrifuged at 5900 g for 10 min, resuspended in the NaCl solution diluted with 0.5 mM $MgCl_2$

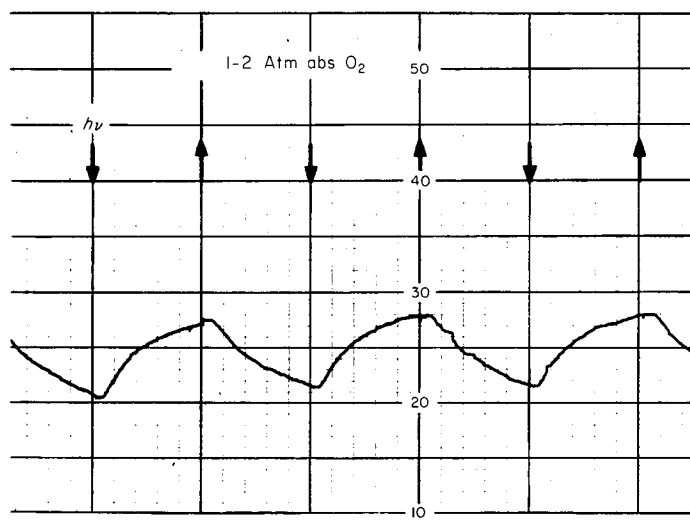

FIG. 6. Light-induced pH increase under a calculated P_{O_2} of 1–2 atm abs.

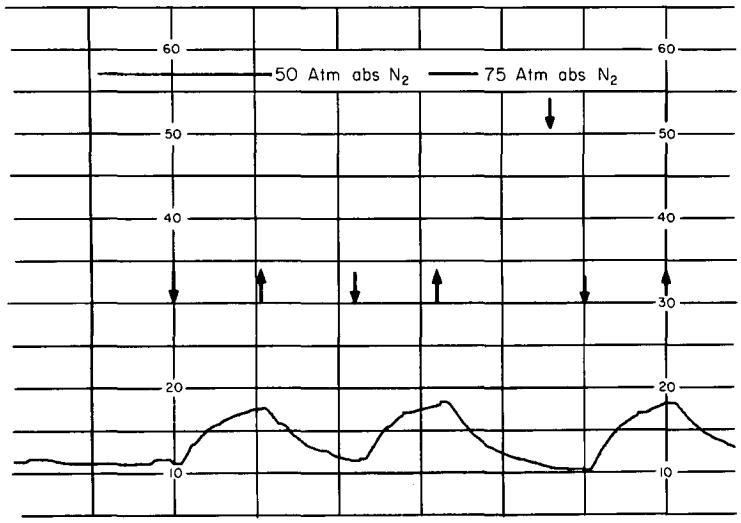

FIG. 7. Light-induced pH increase under 50 atm abs of N_2, changing to 75 atm abs of N_2, showing no detectable pH change associated with the pressure change during 15 sec.

(osmotic potential, 42 mOsmoles) and drawn several times rapidly through a hypodermic syringe, rupturing the chloroplasts. The grana were then centrifuged at 48000*g* and the pellet resuspended in the same solution. The solution was mixed 1:1 with a solution of 20 m*M* NaCl, 0.5 m*M* $MgCl_2$, and 0.08 m*M* phenazine methosulfonate (PMS), and then transferred to the Lucite cell.

A typical light-induced series of reversible pH changes occurring at 3°C is shown in Figs. 5, 6, and 7. The recording shown in Fig. 5 was obtained while the chloroplast solution was in the chamber in air at 1 atm abs. Figure 6 shows a similar recording obtained from the solution when it was pressurized in the chamber at 50 atm abs in 100% O_2. Oxygen equilibration depended chiefly on diffusion, and it was calculated that at this pressure equilibration to between 1 and 2 atm abs would have occurred within the span of the 90-min experiment. It therefore appears that O_2 at 1–2 atm abs is not inhibitory to this system. No drift whatsoever in the recording occurred over the last 30 min of the O_2 exposure. If O_2 has an effect on this system at this pressure, one might have expected the record to vary with the changing P_{O_2}. Essentially the same response (with the same preparation) was observed at 50, 75, and 100 atm abs of N_2 and persisted following slow decompression at the end of the experiment (Fig. 7). A slow change in pressure had no obvious effect on the pH increase.

The precise importance of these results relative to current concepts of photosynthesis (27, 40, 48) need not concern us here; in any event, further experimentation is needed. What is concluded is that the light-induced proton pump of grana is apparently insensitive to at least 1 atm of O_2, a result to be expected of the cellular system responsible for O_2 production. It is hoped that future experiments will determine the P_{O_2} that is limiting to this process. It seems reasonable to assume at present that the inhibitory action of O_2 on photosynthesis (6) is due to its effect on the so-called dark reactions of photosynthesis and not to inhibition of the photo chemical apparatus per se (10).

Conclusions

The examples of O_2 resistance cited here represent a somewhat complementary pair of systems for the study of O_2 toxicity. The tissue of the swimbladder, for example—which must carry out all the normal cellular functions of replication and protein and lipid synthesis by using energy derived from potentially O_2-sensitive catabolic processes—is excellent for use in the study of many cellular aspects of high O_2 pressure. The light-induced proton pump, on the other hand, constitutes a subcellular membrane system that necessarily can operate under hyperoxic conditions and that, in certain ways, resembles the mitochondrial systems, especially with respect to the energy levels between which they operate—that is, the H_2O–O_2 electrode (31). The dark synthetic reactions of photosynthesis, which are probably not intimately associated with the granal partitions, are apparently O_2-sensitive. Likewise, a number of citric acid cycle enzymes, which are assumed to be located intracristally, are also apparently O_2-sensitive. Redox reactions involving electron transport by components intimately associated with, or part of, the mitochondrial or granal membranes are perhaps inherently less O_2-sensitive than are those involving components less intimately associated with the functional membrane units. The light-induced proton pump is apparently insensitive to 1 atm abs of O_2, and is an integral part of the photoact itself. The studies of Chance (8, 9) have similarly shown that the cytochrome chain remains reduced during hyperbaric oxygenation, whereas *reduced* pyridine nucleotides or flavoproteins become oxidized. The fate of such cofactors in the gas gland tissue remains open to question.

It seems reasonable to assume that adaptive characteristics do exist in the gas gland tissue. These might involve: absence of the terminal cytochrome oxidase; a low capacity for oxidative phosphorylation; a lower steady-state ratio of reduced to oxidized pyridine and flavin nucleotides; a higher proportion of enzyme-bound cofactors, which might protect the latter against oxidation; and perhaps a higher proportion of saturated fatty acids in all parts of the tissue, especially the membranes and its subunits. Although the functional significance of the high fat content of the swimbladder of deep-sea fish (7) is not yet clear, mono- and diunsaturated fatty acids are nonetheless found in this tissue (R. Phleger, personal communication).

The low temperature (<5°C) at which deep-sea fish exist undoubtedly protects the swimbladder tissue somewhat against the toxic effects of O_2. Moreover, although the synergistic effects of hydrostatic pressure (52) must be considered, it is possible that pressure itself could protect against any oxidations in which water is an end product, if only by virtue of the considerably higher water activity at such depths. Such a mass action mechanism possibly reduces the secondary formation of free radicals and lipid peroxides that may be a link in the chain of events leading to O_2 poisoning (51).

These points will be investigated in future work, which will extend the above experiments as more deep-sea material becomes available. In any case it is clear that a comparative study of O_2-resistant tissues can contribute significantly to an understanding of the pathology of O_2 poisoning.

Acknowledgments

It is a pleasure to thank Capt. E. M. Neptune for his encouragement and interest in this work; Dr. Lutz Kiesow for providing cytochrome spectra; Dr. Robert Williams, with whom studies on photosynthesis were carried out, for use of recording equipment, and for much

fruitful discussion; Dr. Peter T. McDavid, Naval Dental School, for drilling the pH electrode; and Mr. H. Stup for construction of the chamber extension.

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CHEMICAL PROTECTION AGAINST OXYGEN TOXICITY

Aaron P. Sanders and William D. Currie

The use of chemical agents to protect against O_2 toxicity has, in general, been based upon three premises: (*1*) SH compounds provide (as they do in radiation protection) a reducing environment that lowers the effect of high O_2 tensions; (*2*) acid–base buffers balance possible pH changes resulting from elevated CO_2 concentrations; and (*3*) metabolic agents stimulate ATP production to fulfill the energy requirements of body tissues. Various investigators have reported the use of SH compounds (3, 10), acid–base buffers (1, 4, 5), and metabolic agents (6–9, 12, 13) in O_2 toxicity studies.

A measure of protection against O_2 toxicity has been achieved with this variety of agents at pressures that are usually below 6.0 atm abs of O_2. As is typical in research, investigators used different species of animals, different O_2 pressures, and various chemical compounds in widely differing dosages. Thus it has not been possible to make direct comparison of the effectiveness of the various chemical agents studied.

The work reported in this paper is the result of our efforts to compare the efficacy of several chemical compounds in counteracting O_2 toxicity, and to determine the O_2 pressure above which no protection is afforded by a specific chemical agent.

Methods

Male Sprague-Dawley rats, weighing between 150 and 200 gm, were fasted from 16 to 18 hr and were given intraperitoneal injections of a selected compound (or compounds) 50 min prior to being exposed to HPO. The compounds listed in the following tabulation were used:

Compounds (0.4 *M*, pH 6.4)	Dosage (mmoles/kg)
Glucose	10
Malate	10
Succinate	10 or 12
GABA	10 or 12
Glutamate	10 or 12
GSH	4, 10, or 12
α-GP	12
Cysteine	4
Cysteine + succinate	4 + 10, or 4 + 12
Tris	10 or 12

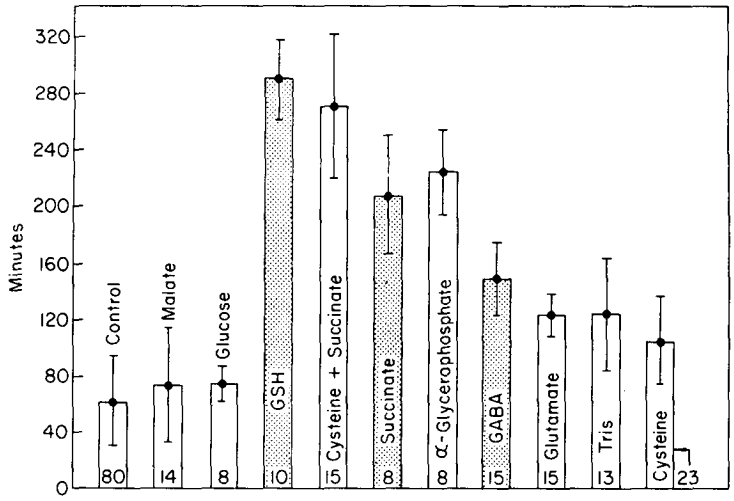

FIG. 1. Time to convulsions in control rats and in those injected with different compounds prior to exposure to 100% O_2 at 5 atm abs. (Compounds, and number of animals injected with each, indicated on vertical bars.)

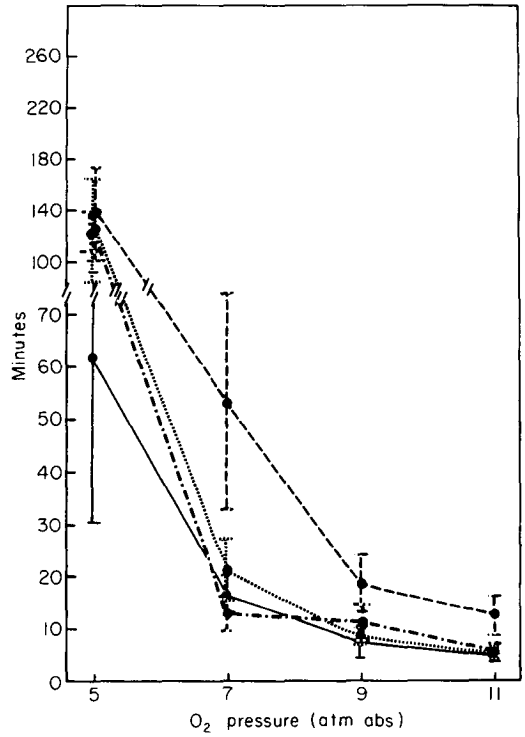

FIG. 2. Time to convulsions in control rats (—) and in those injected with 10 mmoles/kg glutamate (-·-), Tris (···), and succinate (– – –) prior to exposure to 100% O_2 at 5, 7, 9, and 11 atm abs.

After the 50-min incubation period, the animals were placed in an HPO chamber and exposed to 100% O_2 at 5, 7, 9, or 11 atm abs. The bottom of the chamber contained soda lime for absorbing CO_2. After the animals were placed in the chamber, it was flushed with O_2 to eliminate the N_2. The chamber was then closed, and pressure was increased at a rate of 1 atm abs/min until the desired pressure was obtained. Temperature in the chamber was maintained at 21° ± 0.5°C, and O_2 flow at 1 L/min per rat. Twelve rats, individually caged, were exposed during each experiment and were viewed continuously during the HPO exposure. Time zero was recorded when the desired chamber pressure was reached, and the interval prior to the onset of grand mal seizures was recorded for each animal.

Results

The results of the exposures to O_2 at 5 atm abs are shown in Fig. 1. The dosage of all compounds except cysteine, glucose, and malate was 12 mmoles/kg. Studies on the effects of glucose and malate were made at a dosage of 10 mmoles/kg and not extended to 12 mmoles/kg due to their ineffectiveness. Cysteine was administered at 4 mmoles/kg, since a 10- or 12-mmoles/kg dose would have been lethal. The list of compounds in the decreasing order of their effectiveness in delaying the onset of convulsions is this: GSH, cysteine + succinate, α-glycerophosphate (α-GP), succinate, GABA, glutamate, Tris, and cysteine. Glucose and malate offered no protection whatever against O_2 toxicity.

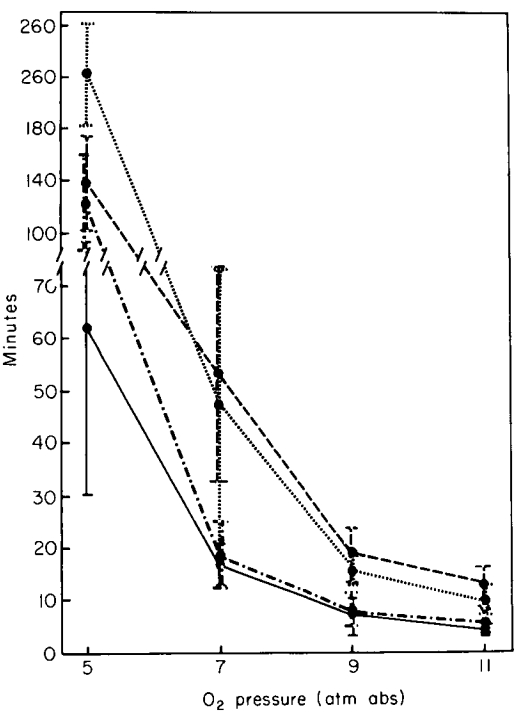

FIG. 3. Time to convulsions in control rats (—) and in those injected with 10 mmoles/kg GSH (• • •), succinate (– – –), and GABA (–•–) prior to exposure to 100% O_2 at 5, 7, 9, and 11 atm abs.

TABLE I

PROTECTION PROVIDED BY 4 MMOLES/KG DOSES OF GSH AND CYSTEINE DURING EXPOSURE TO 100% O_2 AT 5, 7, 9, AND 11 ATM ABS

4 mmoles/kg	5 atm abs	7 atm abs	9 atm abs	11 atm abs
GSH	198.7 ± 45.6 (min)	22.3 ± 6.8	10.5 ± 3.8	5.7 ± 2.3
Cysteine	106.9 ± 31.4	23.6 ± 6.8	10.6 ± 1.8	6.1 ± 1.3

The effects of the various compounds at the four pressure levels selected are shown in Figs. 2, 3, and 4. The response of rats exposed to Tris, glutamate, and GABA was not significantly different from that of the control rats at 7, 9, and 11 atm abs of O_2 pressure. Cysteine at 4 mmoles/kg provided minimal protection. GSH at 4 mmoles/kg produced a protection curve identical to the 4 mmoles/kg cysteine curve at 7, 9, and 11 atm abs of O_2 pressure (Table I). At 10 mmoles/kg, however, GSH provided slightly less protection than succinate did at these pressures. Cysteine + succinate provided the greatest protection of all substrates at 7, 9, and 11 atm abs of O_2.

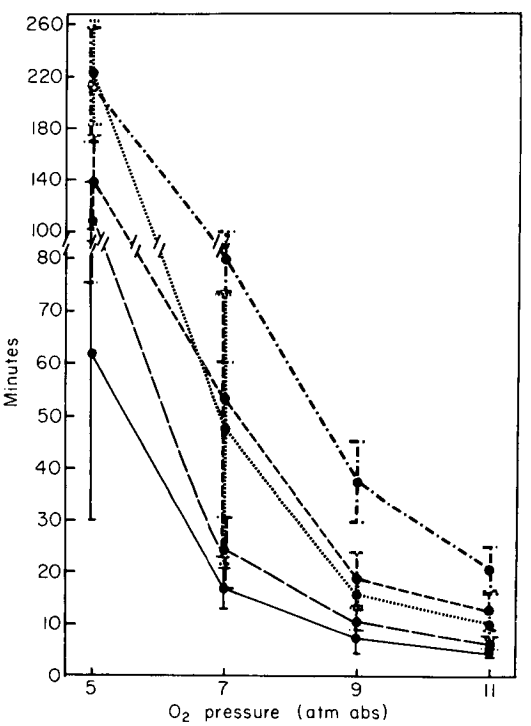

FIG. 4. Time to convulsions in control rats (—) and in those injected with 10 mmoles/kg GSH (•••), 4 mmoles/kg cysteine (— —), 10 mmoles/kg succinate (– – –), and 4 mmoles/kg cysteine plus 10 mmoles/kg succinate (–•–) prior to exposure to 100% O_2 at 5, 7, 9, and 11 atm abs.

Discussion

At 5 atm abs, GSH proved to be the best single protective agent against O_2 toxicity. However, a mixture of cysteine and succinate (4 and 12 mmoles/kg, respectively; data not shown) offered protection equal to that of GSH. A 4-mmoles/kg dose of GSH produced an average convulsion time of 198.7 ± 45.6 min (8 animals) (Table I). The 4-mmoles/kg cysteine dosage should be equal to GSH in affording protection to the SH group, yet the average time to convulsions was only 106.9 ± 31.4 min (23 animals). We have recently proposed (6) that at 5 atm abs of O_2, GSH offers SH-group protection plus metabolic substrate protection because the glutamyl component of GSH goes to succinate via the glutamate → GABA → succinic semialdehyde → succinate pathway. If the ultimate compound providing protection is succinate when GSH, glutamate, and GABA are utilized, then the protection offered by glutamate and GABA alone should disappear when increasingly higher pressures are used. This is because the time required to convert glutamate to GABA via glutamic decarboxylase, and GABA to succinic semialdehyde via GABA-T is longer than the time required to offset the effects of the rapidly building O_2 tensions (see Figs. 2, 3, and 4).

GABA and glutamate did not provide protection against O_2 toxicity at 7, 9, and 11 atm abs of O_2 pressures. At these pressures, GSH protection (4 mmoles/kg) was identical to that of cysteine of the same dose. At 5 atm abs, however, GSH protection greatly exceeded that of cysteine in the 4-mmoles/kg dosage. We therefore concluded that at 5 atm abs there is both SH-group and substrate protection (via conversion to succinate). However, at 7, 9, and 11 atm abs, there appears to be only the SH-group protection, since in equal doses (4 mmoles/kg) the GSH and cysteine protection was identical—perhaps because there is insufficient time for enzyme action to convert GSH to succinate at these pressures. At 5 atm abs, cysteine + succinate and GSH (10 mmoles/kg) provided the same protection. At greater O_2 tensions—7, 9, and 11 atm abs—GSH protection did not equal that of cysteine + succinate.

We have shown (6) that succinate can markedly stimulate ATP production in the brain tissues of the mouse, rat, guinea pig, rabbit, cat, and dog. We attributed the protective action of succinate against HPO to this ability to stimulate ATP production to keep up with the ATP demands of the cells. We have also demonstrated (7, 9) in O_2 toxicity that ATP concentration in the tissues decreases, and believe that this decrease is due to an interference with the cell's ability to maintain normal respiration and oxidative phosphorylation. Exogenous succinate was therefore used in an effort to sustain normal ATP levels. We showed that normal ATP levels, as well as other biochemical functions, were maintained with exogenous succinate throughout 90 min of exposure to 5 atm abs O_2 pressure. Control animals in earlier experimentation (7) showed markedly reduced ATP levels and altered biochemical functions. Glucose and malate did not provide similar protection, as evidenced by the time to convulsions at 5, 7, 9, and 11 atm abs of O_2.

ATP production from succinate (a FAD-linked substrate) is considerably faster than that from other Krebs cycle substrates that yield ATP via NAD. The NAD link to the electron transport chain, along with associated enzyme systems, is adversely affected by hyperbaric oxygenation (2, 11). Other substrates contributing electrons to the electron transport chain via a FAD molecule should offer protection similar to that of succinate. α-GP is one such compound and, as seen in Fig. 1, provided protection against O_2 toxicity at the 12-mmoles/kg dose in the 5 atm abs exposures. At 7, 9, and 11 atm abs, however, α-GP gave slightly less protection than succinate did.

The GSH protection (10 mmoles/kg) at 7, 9, and 11 atm abs is attributed to the SH-group protection only, and is less than that of succinate. Protection by Tris buffer was considerably less than that of either the metabolic substrate (succinate) or the SH group. The combination of SH and substrate protective substances—cysteine + succinate at 5, 7, 9, or 11 atm abs, or GSH (10 mmoles/kg) at 5 atm abs—offers the best protection of all against O_2 toxicity.

Succinate is rapidly used up. We therefore expected that succinate would be the best single protective agent against acute O_2 toxicity. However, in circumstances of long-term HPO exposure, succinate cannot be expected to provide continuous protection since the tissues will use it up preferentially as it becomes available. In contrast, GSH can be expected to provide better protection than succinate will in circumstances of long-term exposure to O_2 at lower pressures. GSH is used slowly until decreased ATP levels or stress phenomena lead to its hydrolysis, providing substrate protection via glutamate → GABA → succinic semialdehyde → succinate, in addition to SH-group protection from its (GSH) cysteinyl component (6). We are presently evaluating this action of GSH.

ACKNOWLEDGMENT

This investigation was supported in part by Public Health Service Research Grant GM-14226-03 from the National Institute of General Medical Sciences; and by Contract N00014-67-A-0251-0002 between the Office of Naval Research, Department of the Navy, and Duke University.

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EFFECTS OF OXYGEN ON BLOOD FORMATION AND DESTRUCTION

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Although the deleterious effects of increased O_2 pressures have been recognized since the classic work of Paul Bert (1), it has been only during the last several decades that the practical importance of O_2 toxicity has come to be generally recognized. The existence of a toxic influence of O_2 upon the formed elements of the blood has only recently been reported (2) and has led to detailed investigations of the effects of increased P_{O_2} upon erythrocytes (3, 10, 15, 16). Interest in these and other forms of O_2 toxicity stems from man's increasing dependence on the environmental control systems required in explorations of the ocean depths and the void of outer space (2, 6, 12, 13, 19). Growing interest in therapeutic use of O_2 at pressures greater than atmospheric has also stimulated study of the hematologic implications of O_2 toxicity (4).

Basic Considerations

Blood is normally exposed to a P_{O_2} of 100 mmHg at the alveolar capillary level (20), resulting in O_2 saturation of approximately 98% of the available hemoglobin. In an average man whose plasma volume and hemoglobin values are within normal ranges, the O_2 capacity of the arterialized blood is approximately 20 vol %. Of this value, approximately 19.75 vol % is carried in chemical combination with hemoglobin, whereas only 0.25 vol % is transported in physical solution within the plasma. Since hemoglobin is essentially saturated at normal ambient P_{O_2}, increasing the O_2 tension can only augment the blood's oxygen-carrying capacity by increasing the amount of O_2 that is physically dissolved.

The relationship between the amount of gas dissolved in plasma and the partial pressure of the gas is a linear function and is described by Henry's law of solubility of gases in liquids. The critical point to be made here is that, under hyperoxic conditions, the only O_2 increase to which the formed elements of the blood are exposed is the O_2 physically dissolved in the water of blood plasma and cells (i.e., the unbound O_2 and therefore the chemically active component).

Proposed Mechanisms of Oxygen Toxicity

Under conditions of hyperoxia, physically dissolved oxygen has been shown to damage RBC by: (*1*) Direct inhibition of glycolytic enzymes containing active SH groups (5, 17, 18),

and (2) formation of lipid peroxides from lipid moieties of the RBC membrane lipoproteins (3, 5, 9, 14, 16). Recently a third and more indirect mechanism of RBC damage has been postulated. Houlihan and his co-workers (7) have found that under hyperoxic conditions the products of catecholamine oxidation have a deleterious effect on RBC *in vitro*.

Although throughout this discussion O_2 is regarded as a causative agent in RBC changes observed during space missions and prolonged exposures to elevated P_{O_2} in ocean dives, it must be remembered that several permissive factors may be involved. These include diet, levels of physical activity and possibly adrenal medullary hormone levels (4, 5, 11).

Experimental Data and Hypothesis

In connection with the Gemini and Apollo space flight programs and the SeaLab III and Tektite diving programs, we have conducted a series of blood studies including measurements of RBC mass. Crew members and test subjects in the several projects were exposed to very different breathing atmospheres, ranging from pure O_2 in the space flights to mixtures of O_2 with He or N_2 in the diving situations. The atmosphere exposure profiles and changes in RBC mass which resulted are summarized in Table I. These operational programs differed significantly from one another in such respects as diet, ambient pressure, the presence or absence of gravitational stress, and the degree of exercise performed. However, in all of the studies based

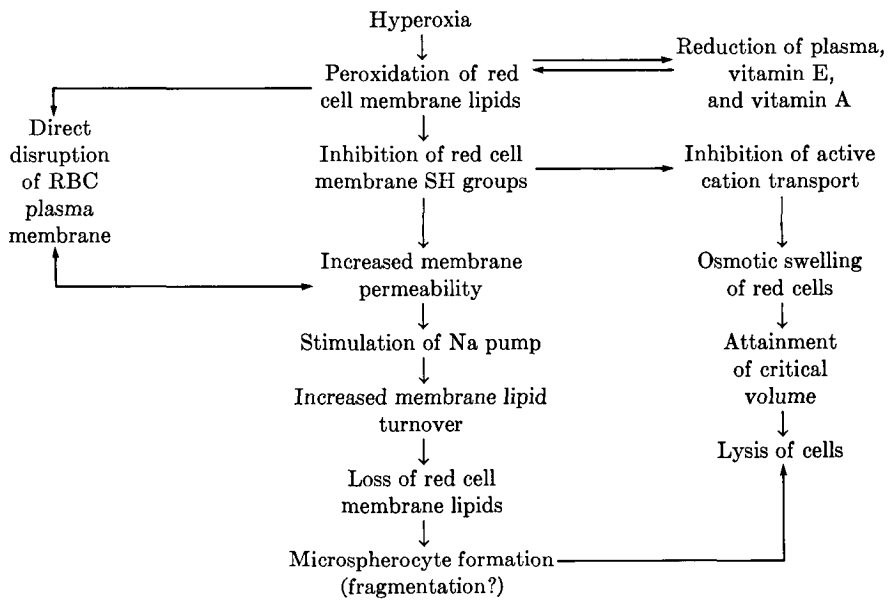
TABLE I

^{51}Cr Red Cell Mass Data

Study or mission	Atmosphere profile Minimum–maximum (mmHg)		No. of subjects	Exposure (days)	RBC mass change (%) Range	Mean
Tektite	160	O_2	4	60	+8.0	−2.0
	876	N_2			−7.4	
Apollo 8	237–304	O_2	3	7	+2.0	−2.0
	21–456	N_2			−4.0	
Apollo 7	237–304	O_2	3	11	−2.0	−3.0
	21–456	N_2			−9.0	
2TV-1	237–304	O_2	3	11	−1.6	−3.0
	21–456	N_2			−7.3	
Brooks AFB chamber study	236	O_2	4	21	+6.0	−3.0
	23	N_2			−10.0	
SeaLab III chamber study	209	O_2	3	12	+4.0	−5.0
	836	N_2			−9.0	
	12887	He				
Apollo 9	258–304	O_2	3	10	−4.0	−7.0
	0–456	N_2			−10.0	
Gemini 4	288	O_2	2	4	−12.0	−13.0
					−13.0	
Gemini 7	258	O_2	2	14	−8.0	−14.0
					−19.0	
Gemini 5	258	O_2	2	8	−20.0	−21.0
					−22.0	

TABLE II

"BEST-FIT" HYPOTHESIS CONCERNING SEQUENCE OF EVENTS IN RED CELL HEMOLYSIS BY HYPEROXIA



on atmospheres with greater than ambient P_{O_2} and the absence of diluent gas, a definite and significant reduction in RBC mass was produced.

GEMINI PROJECT

Hematology data obtained on the Gemini astronauts suggest that the flight-related reduction in RBC mass is characterized by the following:

1. Shortening of the ^{51}Cr half-life extrapolated over the flight interval.
2. An increase in the mean corpuscular volume (MCV).
3. An increase in the osmotic fragility of the RBC population, as measured immediately postflight.
4. Abnormal RBC morphology characterized by acanthrocytes, schistocytes, and microspherocytes.
5. Postflight reticulocytosis.
6. Inhibition of RBC phosphofructokinase and GA-3-PD.
7. Reduction in plasma vitamin E and possibly vitamin A.
8. Quantitative and qualitative alterations in RBC membrane lipids.

On the basis of these findings and information previously published by others, we devised the "best-fit" hypothesis outlined in Table II, which draws heavily upon Jacob's schema for the pathogenesis of hereditary spherocytosis (8). An experimental protocol was then designed to provide the hematological information necessary to appropriately modify this hypothesis.

Apollo Project

Data gathered during the Apollo missions provided us with our first basis for comparison of the hematologic data derived from the Gemini missions. Unlike the crew members of the Gemini flights, the crew of the first two manned Apollo flights (missions 7 and 8) displayed no significant reductions in RBC mass. Evaluation of these two Apollo missions and the Gemini mission profiles revealed that the only significant difference between the spacecraft environments was in the breathing mixtures. (The Gemini spacecraft had a 100% O_2 atmosphere.) Prior to Apollo 7 and 8 a change in Apollo launch procedures was made, requiring a spacecraft atmosphere of 304 mmHg O_2–456 mmHg N_2 at the time of liftoff. Although the in-flight atmosphere leak was replenished with 100% O_2, the spacecraft's P_{N_2} never got below 21 mmHg by the time of deorbit.

The Apollo 9 flight provided a unique opportunity to restudy the pure O_2 atmosphere used in the Gemini flights. This was occasioned by a programmed extravehicular activity (EVA) requiring spacecraft recompression with 100% O_2 at 260 mmHg after complete evacuation of the capsule. The EVA took place on the third day of the 9-day mission; the crew therefore had approximately 6 days of pure O_2 exposure, during at least 4 of which they were denitrogenated.

The Apollo 9 postflight hematological examination revealed a modest but significant reduction in mean RBC mass. Because the only essential difference between the Apollo 7 and 8 missions, on the one hand, and the Apollo 9 mission, on the other, was the presence or absence of N_2 in the spacecraft atmosphere, we took a closer look at our data from other pure O_2 and mixed-gas exposures. Comparison of these RBC mass changes (see Table I) revealed that in all exposures utilizing a mixed-gas atmosphere, no significant loss in RBC mass was detected. (A change of 5% is considered significant and accounts for technical and physiological variations.) Conversely, when a pure O_2 atmosphere was breathed for longer than 3 days, significant decreases in circulating RBC mass occurred.

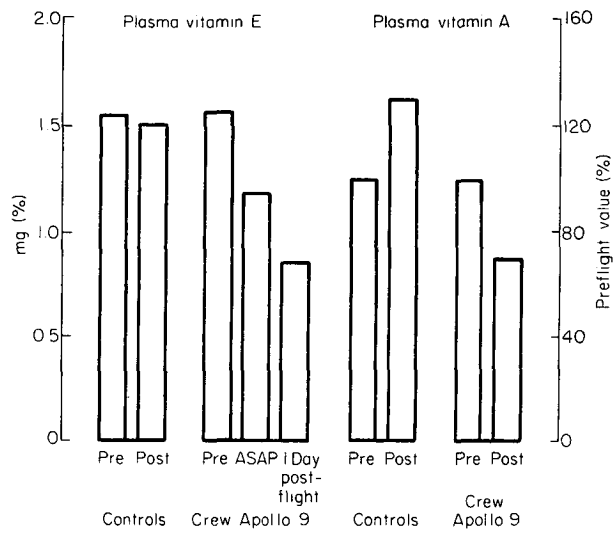


Fig. 1. Postflight changes in levels of plasma vitamin E and vitamin A in Apollo 9 crew.

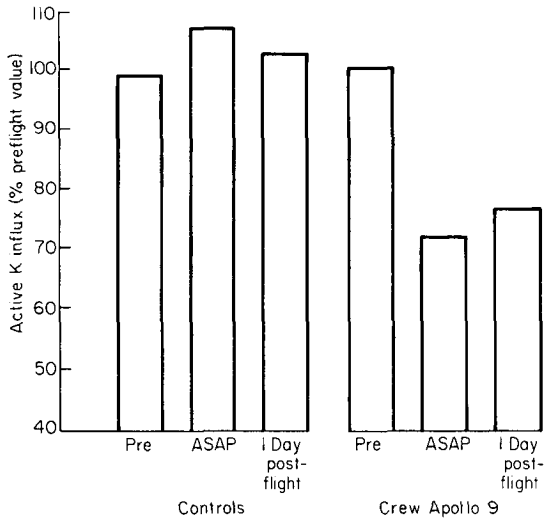


FIG. 2. Postflight changes in the active component of potassium (K) influx in erythrocytes of Apollo 9 crew.

The reduction in RBC mass observed following Apollo 9 occurred in conjunction with other significant biochemical changes. Specifically, these included: (*1*) Reduction in plasma levels of vitamins E and A (Fig. 1); (*2*) decreased activity of PFK and other enzymes containing active SH groups; (*3*) alterations in the transmembrane cation flux (Fig. 2); (*4*) reduction in total RBC membrane lipids, particularly the phospholipid fraction (measured as lecithin) (Fig. 3); and (*5*) abnormal RBC morphology characterized by acanthrocytoid cells, spherocytes, and schistocytes. Correlation of these data with our hypothesis concerning the effects of O_2 on the RBC shows a good "fit."

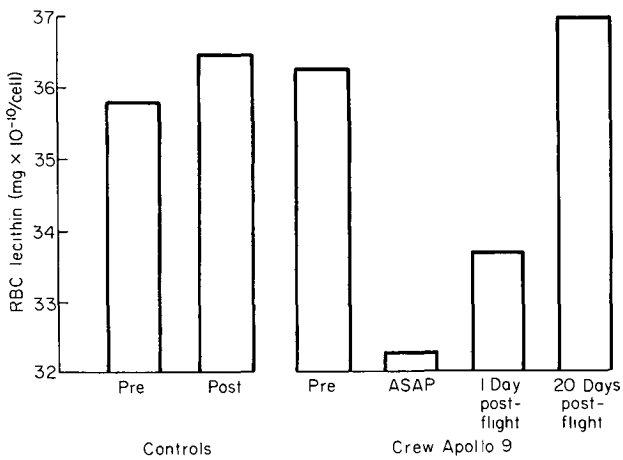


FIG. 3. Postflight changes in RBC lecithin in Apollo 9 crew.

Data obtained from the Apollo 10 circumlunar mission, throughout which a mixed N_2–O_2 gas atmosphere was maintained, indicate that no significant changes occurred in the parameters listed above. It appears that, as in Apollo 7 and 8, the presence of a diluent gas may be an important factor in determining the toxic effect of O_2 on the formed elements of the blood. Although these data strongly suggest that N_2 may play a moderating or protective role in the effects of hyperoxia on the blood, only further intensive study will reveal the actual effects of each gas.

Another factor that can cause a decrease in RBC mass is hyperoxic repression of erythropoiesis. In our experience to date, decreased erythropoietic activity has not been significant in the reductions in RBC mass observed. Our observations agree with those of Zalusky *et al.* (21), who studied the physiological effects of pure O_2 and of various mixtures of N_2 and O_2 at 258 mmHg. It should be pointed out that even complete inhibition of erythropoiesis would result in an effective reduction in RBC mass of less than 1% a day. We have measured losses much greater than this, although sufficient data are still lacking with respect to the significance of inhibited erythrocyte production in the observed reductions of circulating RBC mass.

As exposures to unnatural atmospheres continue to occur and with increasing durations, the influence of O_2 upon the blood will become increasingly important. In such situations it is now considered that:

1. The increase in physically dissolved O_2 contributes more to the chemical toxicity of hyperoxia than that which is chemically bound. Therefore, any elevation in O_2 tension above 160 mmHg may precipitate the death of susceptible cells.

2. The deleterious effect of O_2 on RBC leads to a decrease in the circulating RBC mass. This reduction results either directly from the inactivation of essential glycolytic enzymes by oxidation of SH groups, or indirectly by the formation of lipid peroxides from the lipoproteins of the RBC membrane and the subsequent inactivation of SH-bearing enzymes.

3. The addition of N_2 or He to a hyperoxic breathing mixture may significantly reduce or inhibit altogether the deleterious effects of hyperoxia. The protective effect of these gases seems to be disproportionate to their concentrations in the atmosphere. As more data are gathered, we may find that gases once referred to as "inert" do, in fact, have an active protective effect in a hyperoxic atmosphere, rather than having the passive role usually attributed to them.

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PART I. OXYGEN. MECHANISMS OF TOXICITY*

DISCUSSION

R. E. Davies, Chairman

Chairman Davies: I will call first upon Mr. Allen for comment on oxygen effects on active transport mechanisms.

Mr. Allen: We have been using as a model system for study the isolated toad bladder preparation, which actively transports sodium from its mucosal (or urinary) side to its serosal side. It is a very simple tissue, with one layer of cells on the urinary side of this tissue being responsible for sodium transport.

Sodium enters on the mucosal side, passes down its diffusion gradient, across the cell to the sodium pump where it is actively extruded on the serosal side, against the concentration gradient and with the concomitant utilization of ATP.

We chose this system for study of O_2 effects because its environment could easily be altered *in vitro*. We have used exposure to 100% O_2 at 145 psig pressure. The onset of O_2 poisoning as the inhibition of sodium transport occurs within 10 or 12 min at this pressure and within about 60 min at 5 atm pressure. This phenomenon is reversible, as is seen in mammalian convulsions. The poisoning is potentiated by aldosterone. When aldosterone is administered, the increase in sodium transport normally seen with this hormone in this tissue occurs; when hyperbaric O_2 is administered, there is a rapid falloff in transport.

We have measured metabolites of this tissue under hyperoxic conditions and found an increase of ATP levels after 15 min of HPO, at which time poisoning is just beginning, and also after 75 min, when transport is down to a level of about 50% of its baseline level. We have interpreted this as indicating a direct inhibition of the sodium pump, since at the time sodium transport is inhibited, ATP-substrate levels for this enzyme are increased.

We have also studied decarboxylation of pyruvate under hyperoxic conditions, and have found that metabolism is not decreased at times when poisoning is normally seen, but is in fact increased. The pattern is consistent with increased ATP levels and a decreased NADH level.

We therefore feel the first incident occurring under HPO in this tissue is a formation of lipid hydroperoxides. The evidence for this is that if lipid hydroperoxides are formed *in vitro* these will completely inhibit the sodium pump. These peroxides would also be expected to localize mainly in the lipid phase of serosal and other membranes.

The utilization of reducing equivalents is probably through glutathione. Our preliminary data indicate that NADPH levels are decreased, as are reduced glutathione levels. This would explain the increased glucose consumption (the decreased NADPH levels would bring about an increased glucose and pyruvate consumption).

Dr. Seemann: Have any human experiments been done with substances protective against oxygen poisoning?

Dr. Sanders: Not in our institution. The main reason is that we are using straight sodium succinate, which involves a large sodium load. You would produce severe alkalosis problems.

Dr. Lambertsen: There seems to be no human use of protective substances except for phenobarbital or other central depressants to delay onset of convulsions. Most of the protective substances, as in protection against radiation damage, are themselves toxic.

Dr. H. C. Davies: I wish to speak about the role of GABA in protection against convulsions. In high O_2

* *Panelists:* N. Haugaard, J. D. Wood, B. Chance, B. G. D'Aoust, A. P. Sanders, C. L. Fischer, H. C. Davies, J. E. Allen.

experiments on mice, glutamic decarboxylase levels fall and presumably the GABA level falls too. This is because glutamic decarboxylase is O_2-sensitive and the glutamic relationship is thus suppressed to GABA. If lipid peroxidation has occurred on membrane surfaces due to high O_2, damage to the transport mechanism, such as is seen in the sodium pump too, could cause potassium in the brain to leak from the mitochondria, pass through the cell membranes and be at the synapse. Krebs has shown that, in the presence of glucose, brain cortex slices absorb potassium together with glutamic acid. The ability of glutamic acid to aid in the transport of potassium ions across cell membranes is highly specific. Other amino acids do not replace glutamic acid in this function and glutamine does not replace it either.

Salganicoff and DeRobertis have shown that while GABA, glutamine, and α-ketoglutarate penetrate rather freely in isotonic conditions, glutamate cannot cross the barriers offered by nerve endings and mitochondrial membranes. It is possible that the effect of GABA is to penetrate the membranes where it is transaminated with α-ketoglutarate to succinic semialdehyde and glutamate. The glutamate is now able to hold the potassium from being transported into the synaptic cleft to some extent. The GABA thus protects against potassium leaking into the cleft.

The very high CO_2 tensions that have been shown by Marshall and Lambertsen to be anticonvulsant may also be related at this step to a metabolic reversal, with GABA and CO_2 forming glutamate, again making glutamate able to hold the potassium.

Chairman Davies: This theory was only invented ten hours ago, and thus it is able to be exposed before an expert audience rather more quickly than usual.

Dr. Chance: My question is a little bit more general, but might lay the scene for more sharply pointed questions on the hypothesis that Dr. Davies and, I think, Mr. Allen share a little bit in common. We use the convulsive seizure of an animal (usually the mouse) as our criterion of a hyperoxic lesion. We should agree to agree that this is a proper criterion; nevertheless, there are some discrepancies. The ATP rise that we observed in O_2 poisoning seems to be still elevated at the time when we observe the animal to exhibit convulsions. Since the derangement of ion balances is, I think, accepted as the key to convulsions, we want to be sure that this physiological evidence does jibe closely with is biochemical.

Dr. Sanders: Our reason for suspecting ATP, actually the whole reason we began hyperbaric work initially, was based upon the idea that, in severe hypoxia and in hyperoxia, animals pass through convulsions prior to death. Then we learned from studies of Dickens that succinate was one of the systems least affected.

We had done work in some hypoxia situations, and had noted that succinate was the last of many enzyme systems to be damaged under hypoxia; it is one of the most resistant systems. Under hypoxic conditions the α-keto pathway and others will be inactivated before the succinate-ATP production or ATP-respiration oxidative phosphorylation is inactivated.

Of course, evidence has never been presented that a drop in energy could set the stage for convulsions. We have considered that, preceding convulsions, a drop in the energy level of the tissue occurs. This could result in a hypopolarization (for example, by the sodium pump becoming less efficient), leading to an excitability state. At this stage an incident stimulus could very well trip the animal into convulsions.

This is just theory at this point, but as we got involved in hyperbaric oxygenation and used convulsions as a criterion of toxicity, we did follow the effects at 5 atm through 30, 60, and 90 min and saw that at 60 min there was still an elevated brain ATP in rats, yet at 90 min even the animals that had not convulsed had a decreased ATP.

To pursue this, but not with hyperbaric O_2, we decided to study convulsions in general using, first of all, hypoxia and, second, a convulsive agent to look at changes in ATP in animals prior to and after convulsion to get an idea of the energy level as related to the onset of convulsion.

We exposed animals to 0.4% O_2, which was as low an O_2 concentration as we could get and measure ATP levels. The average time for development of convulsions was about 23 sec, and there is no question that there was a drop in ATP preceding the convulsion.

Similar studies were done with 0.8% O_2 and 2% O_2. A longer time was required for development of a convulsion, but again the convulsion seizure set in at about 60 to 70% of the normal level of ATP. The animals appeared to by this change have developed a state of hyperexcitability. If these animals were picked up prior to the average time for convulsion, they convulsed in hand. It appeared that there was an excitability state—that if they received a massive stimulus they would convulse.

With hydroxylamine, a convulsive agent, exactly the same type of pattern occurs even though, in other words, we do not have a hypoxic state.

What we are saying is that we do believe that convulsions are a very good indicator of O_2 toxicity, based upon our concept of a decrease in ATP, which I believe is a direct result of the block that Chance has described. Chance has provided the best proof that NAD does go to the oxidized state and stays there under high O_2 conditions. We have seen an elevated ATP prior to the decrease which occurs as the animal goes into convulsions.

Corresponding with this decrease in ATP, we have seen marked increases in the free lysosomal enzymes leaking into the cell. In that critical period between 60 and 90 min of O_2 exposure, at the time the animal goes through convulsions, we have seen marked increases in the acid-soluble N_2, indicating lysosomal enzyme action with respect to the structural protein and, of course, other drastic changes with respect to key enzyme systems.

If we employ succinate protection during that same time interval, we maintain normal ATP levels, normal free lysosomal enzymes, normal acid-soluble nitrogen levels, and the normal enzyme activities associated with it.

Chairman Davies: Dr. Chance wishes to make a comment. Before Dr. Chance makes his comment I would ask: Did you measure phosphorocarotene in these brains?

Dr. Sanders: No, we did not.

Dr. Chance: These data are striking and to the point. We have done similar things on the time for de-energization of mitochondrial systems. It is true that in 23 sec one can observe the convulsions. The energy stores are still quite predominant but they are down some. Decapitation experiments, for example, follow this line as well, and I think there are data showing that GABA changes with decapitation.

The evidence is suggestive but not completely convincing that ATP is the only thing that changes. Of course, I would be happy if it were, but we have to realize that sometimes the correlation of convulsions with biochemistry is fortuitous, and maybe the basic structural lesion, which has been referred to by Mr. Allen and Dr. Davies, is something that one might pinpoint a little bit better than the response of the whole organism.

Dr. Sanders: I do not believe ATP is the sole effect. I do believe under the conditions of acute O_2 toxicity that it is the primary factor responsible for precipitating convulsions so rapidly. I think in the chronic state we might find a totally different set of circumstances where we do not see the same pattern of enzyme changes as in acute conditions.

Cdr. Hoke: Have you studied chemical protection against the pulmonary effects of O_2 toxicity?

Dr. Sanders: There is a pathologist who has been with our group for a period of time who has done some work along this line, but she has not reported it.

Dr. Wood: On the question of pulmonary damage, we have shown protection with the GABA. However, I think there are possibly two types of pulmonary damage. There is what we may call the slow reacting form, which is predominant at pressures below 2 atm. There is also very severe lung damage seen in animals that have suffered severe convulsion. I believe, as I think does Dr. Bean who was perhaps the originator of this type of thinking, that the convulsions trigger the nervous system and this is in itself a trigger for lung damage. Whether this is the same type as the slow reacting but just speeded up, or whether it is entirely different, I don't know.

Dr. Van Liew: I gather that some of the speakers, in particular Dr. Chance, believe that convulsions are the result of decreased energy production and thereby changes in the electrolyte balance between the inside and outside of the cell. On the other hand, GABA has been suggested by the neurophysiologists as being a specific transmitter of inhibitory synapses in the CNS, and its effects should not be related to energy levels. Isn't GABA thought to be a specific transmitter across inhibitory synapses, in which case it is not the energy metabolism which is important, but whether or not GABA reaches high or low concentrations?

Dr. Wood: This is true. However, a variety of substances can have the same inhibitory effect as GABA. For instance, glycine and β-alanine are just as potent as GABA when applied to the neurons. However, they are not present in the brain to the same level as GABA. GABA is in much higher concentrations than these other substances.

Personally, I feel that GABA in the CNS is perhaps not so much the classical inhibitor (released in little packages from the nerve endings, acting in the synaptic cleft and then being quickly destroyed), but rather that GABA bathes the neuron all the time. There is a fairly constant extracellular level of GABA, and it is a modulator rather than an inhibitor—if I can differentiate it that way.

About the role of oxidative metabolism and O_2 poisoning, this does come into consideration with GABA, even if GABA does act primarily in a neurophysiological manner; GABA has to be destroyed and, as I showed, this is done by the oxidative route, by the GABA shunt pathway.

Mr. Allen: I would merely like to say that the synthesis of GABA is also under the control of NADPH levels, I believe.

Dr. Chance: We have talked about energy levels and concentration, but we have not talked much about distributions, which Dr. Wood mentioned was a very important factor. Perhaps we can tie this up in the sense that we mentioned lipid peroxidation. Lipid peroxidation is bad for membranes, and it may be that some of the intracellular compartments are damaged very early in O_2 poisoning. While it seems a little bit oblique to bring the mitochondrion in, it does, after all, have a membrane and has compartments as well. The reaction which I believe to be inhibited by hyperbaric O_2 is the isolation of a pool of NADH which is under control of energy and may be maintained in reduced state as long as the membrane integrity is appropriate and the energy level is high. In order that this pool of NAD, which is under control of energy, be separated from that pool which is in active contact with the respiratory chain, one does need a form of compartmentation.

We have shown that a few moments at 16 atm will completely block the effectiveness of this compartmentation which is able to reduce NAD to NADH when ATP and succinate are added to a heart system. But the short exposure blocks the reaction, the ratio of the rates being over tenfold. One interpretation is that the compartment which prevents the reoxidation of this material is now damaged.

If we now turn to the GABA question, I can cite Garfinkle's computer representation of the whole sequence of GABA metabolism. His computer study demands a small compartment and a large compartment for the whole brain, on the basis of isotope studies. Perhaps it is the transfer between these compartments that is membrane-dependent, that is, altered in these events. Thus, not only would one expect to see the GABA now become available to enzyme systems to which it previously was unavailable, but also disequilibration of ion gradients as well. So I think these factors are all tied together.

Dr. D'Aoust: A point we were speaking of earlier related to the trigger mechanism for convulsions. It is generally considered that the trigger is displaced ion equilibrium in and around the synapse. However, it would be nice to conceive of a physical disturbance, and I want to suggest such a possibility.

Recently Koenig has shown that fluorocitrate-produced convulsions in the lumbar region of the cat are accompanied by mitochondrial swelling and rupture and lysosomal enzyme release in and around the axon where it leaves a neuron. I think it is a very plausible suggestion that mitochondrial swelling in and around either the axon, its exit from the neuron, or at the synapse would be an observation at least consistent with what is known of energy metabolism and its effect on mitochondrial configuration. This would also be consistent with the demonstration by Dr. Wood that hyperosmotic solutions inhibit the onset of O_2 convulsions.

If it were not the mitochondrial size that was affected, hyperosmotic solutions would be expected to generally shrink the neurons all over and would not cause a leaky membrane. However, they would inhibit the swelling of mitochondria due to such a process.

Chairman Davies: Two gentlemen have had their triggers for convulsion exceeded. The first is Dr. Chance.

Dr. Chance: I would like to raise some points about the photosynthetic example Dr. D'Aoust studied. The diffusion of O_2 in the cell is very effective, and it is very difficult even in milliseconds to establish a disequilibration of the O_2 gradient. Furthermore, photosynthesis is not the most appropriate mechanism on which to study hyperbaric states or at least physiological hyperbaric states.

The second point concerns the cytochrome content of the swimbladder. Actually, I do not think you had any cytochrome in either of these spectra you showed; I think you had a little hemoglobin in the spectrum which did show absorption close to that of oxyhemoglobin.

Lastly, I would say generally from the teleological standpoint that, if nature has had a problem, it probably has not been O_2 in high concentrations; it has been O_2 in low concentrations. I would imagine that peroxides would have been the biological problem and indeed we have the peroxysome which has been built more or less to take care of this problem. Maybe the oxysome should have been built to take care of the other period.

Chairman Davies: It is the teleost fish that produces this very high O_2 tension in the swimbladder, so teleologically there still exists that problem about high O_2 pressure in nature.

Dr. D'Aoust: This fact that in photosynthesis the light-induced proton pump is insensitive to higher O_2 pressures than 0.2 atmosphere is supported by experiments in which a pre-air-equilibrated system was subjected to 1,000 atm of hydrostatic pressure but, nevertheless, produced oxygen.

A recent experiment has shown that the equilibrium P_{O_2} of pre-equilibrated water and air rises with hydrostatic pressure by about 14%/100 atm. Thus one can calculate that at that pressure of 1,000 atm the P_{O_2} was approximately 0.8 atm. Therefore, O_2 evolution per se, while I admit, as Dr. Chance said, is not the best system for studying O_2 toxicity, certainly supports the idea that it is the dark reactions of photosynthesis that are oxygen-sensitive.

Chairman Davies: I think this problem of "what is the concentration" gets difficult if you think of what is the concentration in a sieve that just includes one O_2 molecule. You can arrive at apparently tremendous pressures. It is like "what is the pH of a mitochondrion" that Chance and others have discounted in the past because when you calculate it there is hardly enough room for even one H_2 ion. But the pH of a mitochondrion still has a significant statistical meaning.

Dr. Haugaard: I would like to make a plea that, in studies of agents that may protect against O_2 poisoning, we do not use the time of convulsion as the only way of measuring the effect of the agent. There are certain substances, particularly anesthetics, that may prevent convulsions and yet not at all slow down the really more damaging toxic effects on cells.

Dr. Sanders: We agree. However, we have also seen that when using succinate we have protected against convulsions and, when we check corresponding biochemistry as indicated by free lysosomal enzymes, by acid-soluble N_2, by depression of succinic dehydrogenase, and acid phosphatase, we find they are normal. So we are not relying only on convulsions as an endpoint in our protection mechanism.

Part II. OXYGEN. EFFECTS ON CELLS AND SYSTEMS

EFFECTS OF OXYGEN UPON OPHTHALMIC STRUCTURES

Charles W. Nichols and C. J. Lambertsen

Although man has now been purposefully exposed to several gases such as He, N_2, and O_2 at concentrations far exceeding those encountered in our environment, only O_2 has been found to alter vision and produce structural changes in the eye. The ocular consequences of exposure to increased P_{O_2} may be placed into two overlapping classifications according to their *physiological* or *pathological* effects. The physiological effects are those not due to irreversible chemical toxicity. They can be expected to occur immediately when O_2 breathing begins and reverse promptly on returning to a normal inspired O_2 tension. The range of pathological effects is broad, from transient influences of true enzymic O_2 poisoning to consequences of toxic effects upon chemical systems, physiological mechanisms, or anatomical structures. These effects persist or appear even after terminating the exposure of the subject to high O_2 tension. Most of the reported effects of O_2 upon the eye involve the neurosensory tissues and these will be discussed first.

Physiological Influence of Oxygen Involving Neurosensory Ocular Tissues

Oxygen at high pressure can cause constriction of the retinal vessels and peripheral visual field. The constriction of the retinal vessels in response to O_2 breathing has been observed in many studies, representative of which are those of Saltzman *et al.* (32) and Hickam *et al.* (15) (Fig. 1). Saltzman *et al.* found a mean decrease in the diameter of the retinal arterioles and veins of 8.5 and 10.7%, respectively, at a P_{O_2} of 1.0 atm abs. When the P_{O_2} was increased to 3 atm, there was a 19% decrease in the diameter of the arterioles and a 28% decrease in diameter of the veins. In a similar experiment with O_2 at 1 atm, Hickam found a decrease of 11.6% for the arterioles and 14.9% for the veins. The difference between these results is most likely due to a difference in the initial size of the vessels studied, for Dollery *et al.* (9) have pointed out that the degree of vascular constriction varies inversely with the initial caliber of the vessel and is directly proportional to the dose of O_2 (inspired P_{O_2}). Differences in the subjects used for such studies might also account for the differences in vascular reactivity for Sieker and Hickam (33) have shown that a decreased retinal vascular response to O_2 occurs with increasing age and in pathological states such as diabetes mellitis and hypertension.

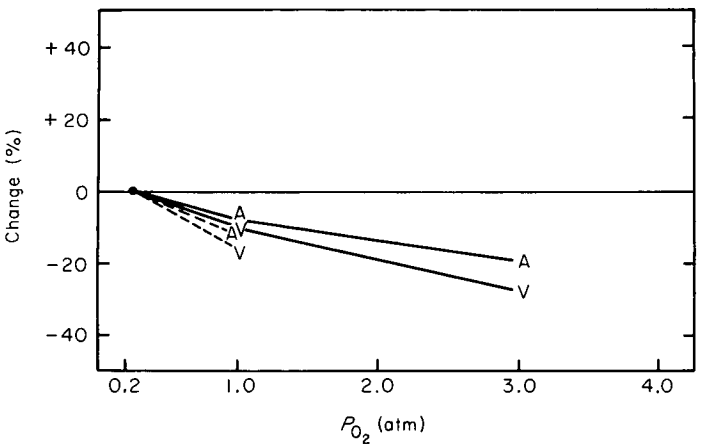

FIG. 1. Effect of O_2 inhalation upon the diameter of the retinal vessels in man. The results of two separate studies are compared on the graph. The dotted lines represent the alterations reported by Hickam *et al.* (15), and the solid lines are those reported by Saltzman *et al.* (32).

The relationship between the effects of hypocapnia and hyperoxia in producing cerebral and retinal vasoconstriction has been emphasized by Lambertsen (17) and Spalter, Nahas, and Len (34). When the Pa_{CO_2} is controlled, hyperoxic vasoconstriction of retinal and cerebral vascular beds is prevented; otherwise, the degree of vasoconstriction which occurs has appeared to be proportional to the hypocapnia normally associated with O_2 breathing (Table I) (18–20). Other authors (1) have failed to find an association of retinal vasoconstriction with hypocapnia. However, most of the subjects in these studies showed unexplained Pa_{CO_2} increases during O_2 breathing in contradistinction to the respiratory stimulation and hypocapnia reported consistently by others (18–20).

That the acute physiological vascular changes induced by O_2 do not adversely affect vision has been shown by Miller (23) and Gallagher *et al.* (12). They could find no changes in visual acuity, visual fields, electroretinographic measurements, dark adaptation, and stereopsis with O_2 exposures to 1 atm abs for periods of up to 24 hr. Of interest are two other studies of dark adaptation and O_2 breathing. Elsas *et al.* found no change in dark adaptation after

TABLE I

EFFECT OF O_2 BREATHING ON ARTERIAL P_{CO_2}

	Inspired P_{O_2} (atm)			
	1.0	2.0	3.0	3.5
Decrease in P_{CO_2} from air breathing (mmHg)	−1.4 (26)[a]	−5.1 (5)	−7.7 (7)	−5.4 (21)

[a] Numbers in parentheses indicate the number of subjects.

15 min of O_2 breathing at 3 atm abs (11). Kent (16), however, found prolongation of dark adaptation in two of four subjects exposed to O_2 at 2.8 atm abs for 20 min.

When O_2 is breathed at 3 atm abs, no change in the visual fields has been recorded for the first 3 hr. After that time, however, a bilateral symmetrical contraction of the peripheral fields occurs, as was first described by Behnke *et al.* in 1936 (6) and confirmed later by others (10, 31). The visual fields will contract to as little as 10° around fixation, but vision does not entirely disappear until unconsciousness occurs due to CNS toxicity (17). In all cases, recovery occurs in less than 1 hr after the subject returns to sea level and breathes air. That a decreased O_2 supply to the retina secondary to hyperoxic vasoconstriction is not a likely cause of the visual field changes has been discussed elsewhere (24). A more probable, but equally unsupported explanation is a decrease in nutrient supply to the peripheral retina with depletion of its limited glycogen stores. Such a nutritional deficiency could combine with O_2-induced inactivation of many enzyme systems to cause failure of normal neuronal function.

Asymmetrical constriction of the visual fields at pressures and times less than those reported by Behnke *et al.* (6) has been reported by two groups. In the case described by Zal'tsman *et al.* (36) both eyes were affected, but unequally and at a lower dose of O_2 than required to produce symmetrical contraction in a healthy individual. The constriction cleared completely within 1 hr after the subject stopped breathing O_2 at increased pressure. The cause of this excessive reaction of the individual diver to O_2 breathing was not determined.

In the second case, reported by Nichols *et al.* (25), the subject was a 21-year-old man with a previous history of retrobulbar neuritis in the right eye, manifested by mild temporal pallor of the right optic disc. After a 2-h O_2 exposure at 2 atm abs, he experienced deteriorating vision in this eye. The effect was carefully followed over the next 4 hr as the visual field, measured by perimetry with a 10 mm white test object at 330 cm, decreased to a small temporal island (Fig. 2). Over a period of several hours after O_2 administration was discontinued, the visual field in his right eye cleared, except for two paracentral scotomas which persisted for several weeks (Fig. 3). Over this period the scotomas gradually decreased in size.

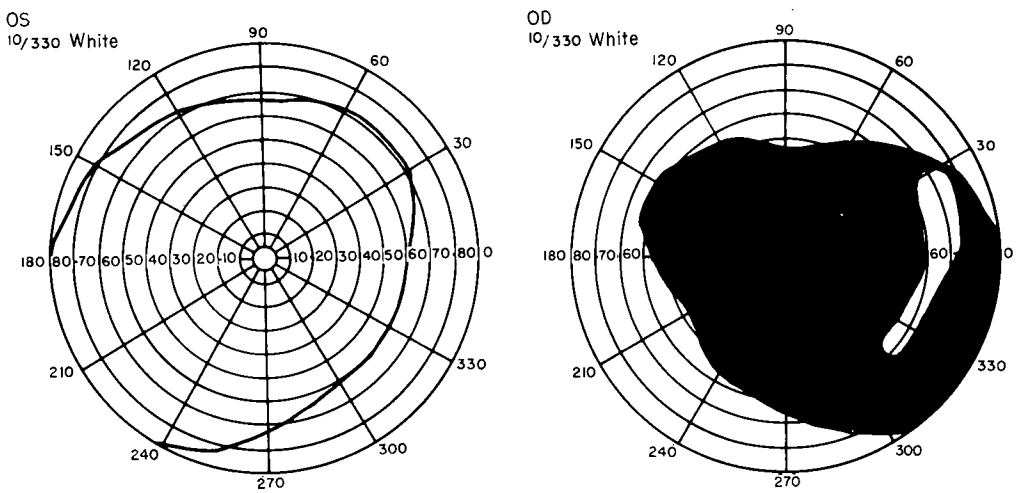

FIG. 2. Perimetric fields of both eyes of a subject after 4 hr of breathing 100% O_2 at 2 atm abs. Left field remained unchanged when O_2 breathing was terminated 2 hr later. Test object was 10 mm white at 330 cm.

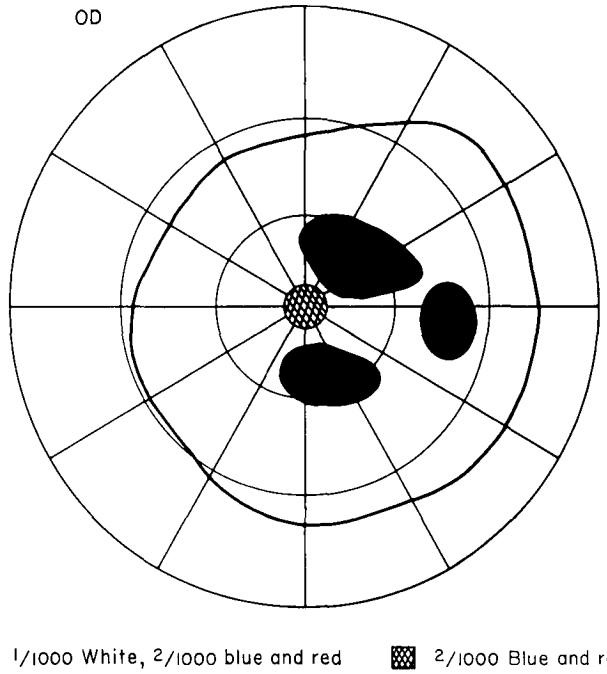

FIG. 3. Tangent screen field at 1 m, 14 hr after O_2 exposure, showing two paracentral scotomas with 1 mm white test object and 2 mm red and blue test objects. Central scotoma was present to colored test objects.

This unilateral effect of O_2 seemed to represent an exaggeration of a defect related to his previous retrobulbar neuritis. No visual changes were detected at any time in the left eye. These acute visual changes have been interpreted as representing an altered sensitivity to the metabolic effects of hyperoxia on the part of the neuronal enzyme systems or the vascular system or both. This subject demonstrates that while the previously discussed visual effects of O_2 in normal men are slow in onset and rapidly reversible, the effects may be quite different in individuals with ocular manifestations of disease processes.

Pathological Influence of Oxygen Involving Neurosensory Ocular Tissue

The pathological changes involving neurosensory tissues of the eye include enzymic derangement, visual cell death, retinal detachment, cytoid body formation, and retrolental fibroplasia. All these phenomena have been adequately described histologically or clinically. Although the direct relationship between retrolental fibroplasia and hyperoxia has been recognized and the condition once believed to have been abolished, its incidence is again believed to be increasing (30). Retrolental fibroplasia, however, is a disease of immaturity and will not be further considered here.

Noell (27–29) has described the visual cell death which occurs in the rabbit exposed to HPO, and Bresnick (7) has examined the phenomenon by means of electronmicroscopy. After exposure to 100% O_2 at 3 atm abs for 4 hr or 1 atm abs for 40–48 hr, the visual cells of the rabbit

are destroyed. Visual cell death also may be followed electroretinographically, as described by Noell (29) and Bridges (8). Oxygen exposure causes continual depression of the electroretinogram (ERG) until it is extinguished. Early in the exposure, the changes in the ERG are reversible; but after prolonged exposure, reversibility is lost. Iodoacetic acid will also abolish the ERG and will destroy the visual cells *in vivo* (27) or in tissue culture systems (13). The action of this compound, an inhibitor of glycolysis, suggests that O_2 may similarly destroy visual cells by blocking the glycolytic sequence required for their survival (14).

Retinal detachments, described by Beehler *et al.* (3, 4), occurred in dogs exposed to P_{O_2} of 680 or 760 mmHg for up to 72 hr. All the animals died, and 50% had large retinal detachments. Of another group of animals that were exposed under the same conditions but were allowed to breathe air for 1 hr each day, all survived for 85 hr and had large retinal detachments.

Recently Beehler and Roberts (5) reviewed this earlier work, in which phenothiazines had been used for sedation. In these experiments animals were exposed to 1 atm abs for 20 hr/day (the remaining 4 hr of exposure being to air). Some of the animals were not sedated, and some were given phenothiazine. In the unsedated animals, the incidence of retinal detachment was zero, and only 20% of them had focal elevations of the retina. In contrast, 73% of the sedated animals had focal elevations of the retina, and approximately 52% of them progressed to retinal detachment. Since phenothiazines are considered to alter the metabolism of the retinal epithelial pigment, the authors suggested that phenothiazines, in conjunction with O_2-induced enzymic alterations, intensify the production of focal retinal elevations, some of which may proceed to cause large retinal detachments. Yanoff *et al.* (unpublished observations), however, have observed a higher incidence of retinal detachment than reported by Beehler and Roberts without using drugs to influence O_2 toxicity. The two series are contrasted in Table II. All the dogs in Yanoff's studies were exposed to 1 atm of O_2 continuously for 50 hr and all survived. The primary lesion seen in the eyes on autopsy was a choroidal exudation. Sufficient subretinal

TABLE II

OXYGEN-INDUCED RETINAL DETACHMENTS

	O_2 only[a] (100% O_2 at 1 atm 20 hr/day)	O_2 and phenothiazines[a] (100% O_2 at 1 atm 20 hr/day)	O_2 only[b] (100% O_2 at 1 atm continuously)
Total animals	10	37	7
Number with focal elevations	2	27	7[c]
Number progressing to detachments	0	19	2[d]
Days to appearance of lesions	6	3½	2
Surviving exposure	80%	75%	100%

[a] Beehler and Roberts (5).
[b] Yanoff, Nichols, and Miller (in preparation).
[c] Bullous detachments.
[d] Complete detachments.

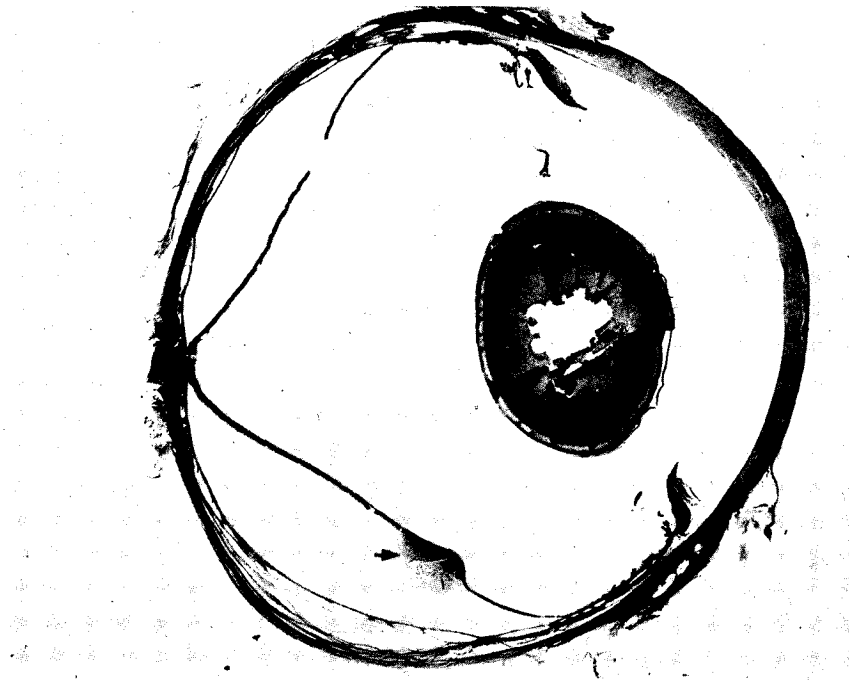

FIG. 4. Complete exudative retinal detachment seen in a dog's eye after 50 hr of breathing 100% O_2 at 1 atm. Arrow points to a remnant of the subretinal exudate. Rent in the retina is an artifact. ×4.

fluid accumulated in 12 out of 14 eyes to cause a bullous detachment which was complete in one eye from each of two different animals (Fig. 4). These findings indicate that O_2 induces a change in the permeability of the choroidal blood vessels in dogs, possibly through a toxic effect on the endothelium. From the study by Beehler and Roberts, it is apparent that retinotoxic drugs may increase the incidence of detachments. However, under somewhat different conditions the same or higher incidence may be achieved with O_2 alone.

Margolis *et al.* (21, 22) have reported the occurrence of cytoid bodies in the retinas of dogs exposed to 100% O_2 at 3.0 atm abs for 4–6 hr. These lesions appeared to be histologically similar to the cytoid body that is found in man, where it is believed to be secondary to arteriolar spasm and occlusion (2) and identical to the "cotton wool spots" seen ophthalmoscopically. No ophthalmoscopic examinations of the dogs are described by these workers, however. Development of the lesions in the dog may be prevented by adding 2% CO_2 to the O_2 inspired at 3 atm abs, which should prevent reduction of retinal blood flow. It seems likely, therefore, that these have a vasospastic basis and, as mentioned previously in discussing visual field contraction, a lack of nutrient flow may be an essential factor in their development, rather than decrease or increase in O_2 availability.

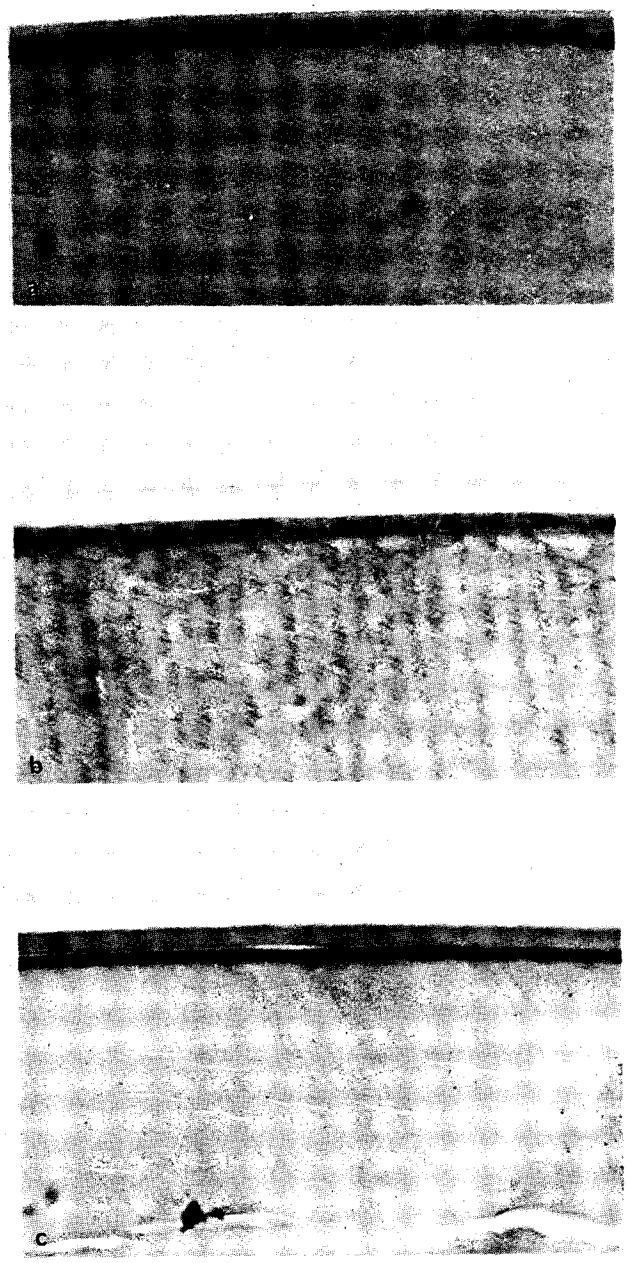

FIG. 5.(a) Normal guinea pig lens epithelium. ×600.

(b) Guinea pig lens epithelium following 5.5 hr of 100% O_2 breathing at 3 atm abs. Note dropping out of many nuclei and pyknosis of remaining ones. ×600.

(c) Guinea pig lens epithelium 2 months after exposure to 100% O_2 at 3 atm abs for 5.5 hr. Pyknosis and nuclear dropout are unchanged from (b). ×600.

TABLE III

RATE OF DEVELOPMENT OF OCULAR CHANGES WITH 100% O_2 AT 3 ATM ABS

Hours	Flattening of corneal endothelium[a]	Pyknosis and nuclear loss in lens epithelium[a]	Retinal edema
2	0	0	0
4	0	++	0
5.5	+ to ++	++	0
7	++	++	0
8.5	+++	+++	+

[a] (+) More cell changes than in control, but in less than 50%; (++) alterations in about 50% of cells; (+++) alterations in more than 50% of cells.

Influence of Oxygen on the Cornea and Lens

Thus far, we have concentrated on changes produced by O_2 in the neurosensory tissues of the eye. Effects of acute O_2 exposure have also been noted in the cornea and lens. Nichols *et al.* (26) have followed the progression of these changes in guinea pigs from the time O_2 exposure was initiated at 3 atm abs until death occurred 7.5–16 hr later (Table III). The corneal changes involved the endothelium and consisted of pyknosis of the nucleus with condensation of the cytoplasm resulting in flattening of the cells. The earliest changes were noted after 5.5 hr of exposure and became progressively greater until death occurred. In control animals, these changes could not be reproduced by anoxia over the same period or by altering fixation techniques (35). These endothelial changes were irreversible, the cells having essentially a similar appearance after 2 months in the animals permitted to survive that long.

The lenticular changes in the guinea pigs consisted of pyknosis and nuclear loss in the epithelium. These changes were noted as early as 4 hr after exposure began, and there was relatively little progression until death occurred. These changes also could not be produced by anoxia and were irreversible since they could be found 2 months after exposure. Figure 5 contrasts the normal lens of a guinea pig with that of an animal exposed to O_2 for 5.5 hr and then killed immediately, and with one exposed 5.5 hr and then killed 2 months later. The retinal edema as noted in Table III apparently was a terminal event; acute histological changes were not present in the retina, except in those animals dying of O_2 toxicity. Changes similar to those noted in the cornea and lens of the guinea pig could potentially influence human vision on chronic or repeated exposure, if corneal edema or cataract formation occurred.

ACKNOWLEDGMENTS

This work has been supported in part by grants (HE-08184 and HE-08899) from the National Institutes of Health; by contract (NONR 551 [58]) with the Office of Naval Research; by a training grant (5T01-NB-5577-02) from the United States Public Health Service; and by "Fight for Sight" Grant-in-Aid #351, Fight for Sight, Inc., New York, N.Y.

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ACUTE OXYGEN TOXICITY IN WORKING MAN

J. Murray Young

This investigation was designed to determine the incidence of symptoms of acute O_2 toxicity in men working to exhaustion in a dry atmosphere while wearing closed-circuit oxygen breathing equipment. It was carried out as a practical trial and not as a basic scientific experiment. The main object was to note any symptoms that might occur, and to determine the percentage of men who would be incapacitated by O_2 toxicity in relation to depth of exposure.

The investigation arose from a request from the local fire brigade, which has the obligation of entering pressurized workings and tunnels to deal with outbreaks of fire. It was suggested that the brigade provide the experimental subjects, and 45 volunteers started the series of dives 18 months ago. Thirty-five subjects completed the series, the remainder dropping out through lack of interest or medical reasons, or because they were posted away from the area. We have now completed 192 dives involving 422 exposures.

The tests were carried out in a pressure chamber at the Royal Naval Physiological Laboratory. They consisted of each subject's performing a standard exercise routine first at 20 FSW (8.9 psig) and then at successive 3-ft increments to a maximum depth of 47 FSW (20.9 psig). The chamber can accommodate up to six men comfortably, and in the shallow dives, four subjects exercised simultaneously while being closely observed by two attendants who breathed the air atmosphere of the chamber. On dives of 29 FSW and deeper, two subjects exercised simultaneously; they were accompanied in the chamber by two attendants who, with a recorder, kept a continuous log of the proceedings and recorded any signs or symptoms of O_2 toxicity in the subjects. The parameters measured on some subjects were cardiac pulse rate, end-tidal P_{CO_2}, inspired gas temperature, deep-body temperature, and inspired O_2 concentration.

The subjects wore their normal fire-fighting clothing, which consisted of socks, underclothes, trousers, shirt, waterproof trousers or leggings, a thick nonventile jacket, and a protective helmet. The breathing set was the PROTO Mk IV (Siebe Gorman and Co., Ltd.), which was first recommended by Professor J. S. Haldane as a mine rescue apparatus in 1914 (5). Since then, 12,000 such sets have been produced with only minor modifications, and they are at present in use by many rescue organizations in about 20 different countries.

The reducing valve of the apparatus is set to deliver a constant flow of 2.2 L/min from the O_2 cylinder to the breathing bag, which is worn on the chest. Nonreturn valves ensure circu-

lation of gas from the inspiratory compartment of the breathing bag via corrugated tubing to a mouthpiece, and expired air is returned by similar tubing to the expiratory compartment of the bag, the subject wearing a noseclip. To return to the inspiratory compartment, expired air must pass through a layer of CO_2 absorbent that lies loose in the bottom of the bag. A sodium phosphate cooler is inserted in the inspiratory pathway to attempt to cope with the heat of CO_2 absorption. The total weight of the equipment worn by the firemen in this investigation was 57½ pounds.

The standard exercise routine was of 40-min duration and so organized that the subjects would be approaching physical exhaustion at its completion. It consisted of:

1. Two minutes of bending and lifting a 20-lb reel of hose at 12 lifts/min, followed by 1 min of standing at rest.

2. Two minutes of marching "on the spot" at 120 paces/min while carrying the reel of hose, followed by 1 min of standing at rest.

This cycle was repeated six times, and the last 4 min of the routine consisted of continuing exercise 1 for as long as possible, changing to exercise 2 when necessary.

The O_2 consumptions ($\dot{V}_{O_2}$) varied among the subjects because a large proportion of the workload consisted of the subjects moving their own body weight, but $\dot{V}_{O_2}$ remained constant in any one subject.

The mean $\dot{V}_{O_2}$ was

Exercise 1: 2.33 L/min (S.D. ± 0.49)
Exercise 2: 1.19 L/min (S.D. ± 0.22)

Results

Inspired Oxygen Concentrations

The O_2 concentration in the inspiratory compartment of the breathing bag was measured with a paramagnetic analyzer (Servomex Controls, Ltd., type DCL 83); the sample from the bag was first led continuously through the chamber wall and then expanded to normal atmospheric pressure.

The subjects started most dives with a breathing mixture containing about 80% O_2 in their sets and ended the exercise with about 90%, the increase having been caused by the fact that the O_2 flow of 2.2 L/min into the set was greater than their overall $\dot{V}_{O_2}$. The mean value for all the subjects monitored over the 40-min exercise period on dives of 44 FSW and shallower was 86.6% O_2 (±6.1%). In the experiments at 47 FSW, the subjects were instructed to flush O_2 through their sets until the initial monitored concentration was at least 85%, and the mean O_2 concentration at 47 FSW was 89.2% (±3.5%).

Minor Symptoms of Oxygen Toxicity

No signs or symptoms attributable to O_2 toxicity were observed at either 20 or 23 FSW.

The first signs appeared in two subjects at the 26-FSW chamber depth (Fig. 1). Two other subjects also first exhibited similar symptoms at 26 FSW, but as these subjects were later excluded from the investigations for reasons unconnected with O_2 toxicity, their results are

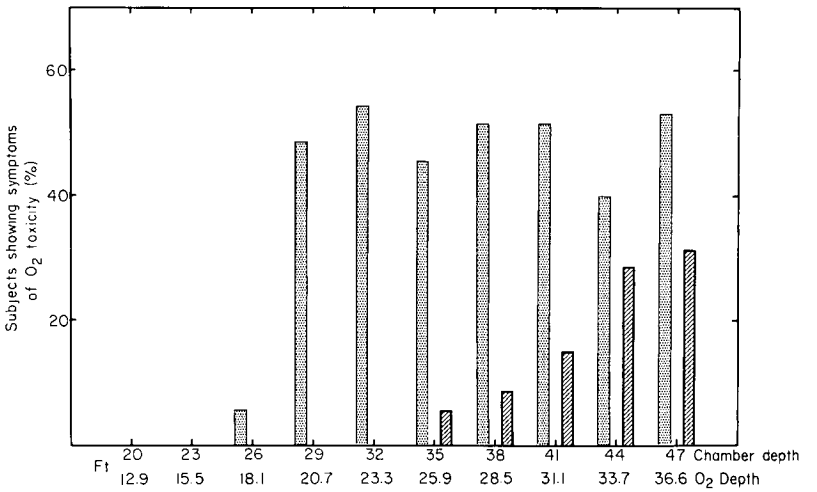

FIG. 1. Percentage of subjects showing symptoms of O_2 toxicity at chamber depths of 20–47 FSW. Symptoms: (▦) minor; (▨) major.

not included in Fig. 1. As the subjects were breathing a mixture containing only 86.6% O_2, their inspired P_{O_2} was equal to that which they would have experienced at 18.1 FSW if they had been breathing 100% O_2, and I have designated their "oxygen depth" as being 18.1 FSW (8.1 psig O_2).

The observed signs were fasciculations and twitches of the small muscles of the face, usually circumoral, commonly described by divers as "the lips."*

At the 29-FSW chamber depth (20.7-FSW O_2 depth) nearly 50% of the 35 subjects who completed the series of dives showed signs of "the lips" and at increased depths this figure remained of the same order (Table I). The muscle movements were rarely felt by the sub-

TABLE I

INCIDENCE OF SYMPTOMS OF OXYGEN TOXICITY AT CHAMBER DEPTHS OF 20 TO 47 FSW

	Chamber depth (FSW)									
	20	23	26	29	32	35	38	41	44	47
No. of subjects	34	35	35	35	35	35	35	33	35	32
No. with minor symptoms	0	0	2	17	19	16	18	17	14	17
% with minor symptoms	0	0	5.7	48.6	54.3	45.7	51.5	51.5	40.0	53.1
No. with major symptoms	0	0	0	0	0	2	3	5	10	10
% with major symptoms	0	0	0	0	0	5.7	8.6	15.1	28.6	31.3
Total no. with symptoms	0	0	2	17	19	18	19	21	20	20
Total % with symptoms	0	0	5.7	48.6	54.3	51.5	54.3	63.6	57.1	62.5

* Syndrome of circumoral muscle fasciculation and twitching is designated by the author as "the lips."

jects, but were observed primarily by the attendants in the chamber. Movement at the corners of the mouth was the most frequent sign, and the second most common was fasciculation of the upper lip. Twitches of the cheeks and lower eyelids were also seen, and one subject had an intermittent twitching of the platysma. No subject reported twitches in parts of the body that could not be observed.

The phenomenon of "the lips" was rarely apparent except during the rest periods in the exercise routine. As soon as exercise began again, the generalized increase in muscle tone apparently was sufficient to suppress the small movements. "The lips" was typically observed during the first rest period at 2 min, and sometimes was apparent during all rest periods from then on; but more commonly, it was intermittent in its appearance. No progression in extent or severity was observed either with time in any one dive, or between successive dives with increased depth.

No subject ever had the slightest difficulty in controlling his mouthpiece because of mouth twitching, and there was no decrease in exercise capability during the presence of "the lips" when it was the sole sign of toxicity. All subjects completed the 40-min exercise routine.

Time of Disappearance of "The Lips"

The interval between a return to air breathing and the disappearance of "the lips" was measured on 28 occasions in subjects who exhibited fine fasciculations of the lips at the end of the exercise routine.

While remaining at the dive depth, the subject was returned to chamber-air breathing by an attendant unscrewing the inspiratory tubing from the breathing bag while the subject retained the mouthpiece. The inspiratory tubing was partially supported to maintain a constant weight on the subject's mouth.

Signs began to disappear within 5–10 sec of the start of air breathing and faded rapidly from their original level of intensity. Occasionally there were small bursts of increased intensity along the decreasing trend line. Often all signs disappeared within 10–15 sec, but momentary signs of twitching at increasing intervals would then reappear. "The lips" was considered to have finally disappeared when an occurrence was not followed during the subsequent minute by any further signs. The mean time interval from the resumption of air breathing to the final disappearance of signs was 65.7 sec (range: 10–205 sec).

There was no obvious correlation between the time to disappearance and either the intensity of the previous signs or the depth of exposure.

Major Symptoms of Oxygen Toxicity

I have defined major symptoms as those that would tend to endanger a man in an actual exposure to increased pressure, remembering that he might be in a cramped environment, possibly surrounded by an irrespirable atmosphere, and must be fully aware of his actions and surroundings. Under this heading I therefore include severe nausea, dizziness, lightheadedness, incoordination, confusion, euphoria, dilatation of the pupils, and convulsions. The incidence of these symptoms is shown in Table II. No major symptoms were seen before 35 FSW (25.9-FSW O_2 depth), but their incidence rose markedly thereafter, in contrast to the incidence of "the lips" (Fig. 1 and Table I). Few of these symptoms actually caused the subjects to stop work, although their work rate sometimes decreased. The cases of nausea

TABLE II

TYPES AND INCIDENCE OF MAJOR SYMPTOMS OF O_2 TOXICITY AT CHAMBER DEPTHS OF 35 TO 47 FSW

	Chamber Depth				
	35 FSW[a]	38 FSW	41 FSW	44 FSW	47 FSW
	Oxygen Depth				
Symptoms	25.9 FSW[b]	28.5 FSW	31.1 FSW	33.7 FSW	36.6 FSW
Incoordination	—	—	1	2	—
Light-headedness	1	1	—	1	—
Dizziness	—	1	2	4	6
Nausea	1	2	3	5	5
Dilatation of pupils	—	—	—	3	7
Euphoria	—	—	1	—	—
Confusion	—	—	—	1	—
Convulsion	—	—	—	1	1
Total no. of symptoms	2	4	7	17	19
No. of subjects affected	2	3	5	10	10
% of subjects affected	5.7	8.6	15.1	28.6	31.3

recorded as major were those that occurred early in the exercise routine, that occurred in subjects who had not previously complained of nausea at shallower depths as a result of exhaustion, or that necessitated the subject's removing his mouthpiece.

Dilatation of the pupils did not per se affect the operational ability of the subjects. On seven occasions when this sign was observed, the subject also had either dizziness or nausea; on two occasions, the subject had "the lips" as well as dilatation of the pupils; and on one occasion, the dilatation appeared to be the only unusual finding. This manifestation is included among the major symptoms of O_2 toxicity because of its late appearance in the series and its marked increase in incidence between 44 and 47 FSW.

The time taken for the disappearance of the major symptoms was not specifically determined. Apart from the convulsive cases described below, all the subjects reported absence of any symptoms when they removed their breathing sets after decompression, which was usually about 4 min after the end of the exercise routine and 2 min after leaving depth. If dilatation of the pupils had been present, however, it was still apparent after decompression, and the pupils required a further 5–10 min to revert to normal.

CONVULSIONS

One subject had a grand mal convulsion after 38¾ min of the exercise routine at 44 FSW (33.7-FSW O_2 depth, 15.0 psig O_2). He had exhibited "the lips" manifestation continuously since the first rest period at 2 min, and this had become more pronounced at 31 min. However, the subject continued to work at his normal rate throughout, and felt no symptoms whatsoever until about 5 sec before his collapse. He then felt his lips twitching and signalled

this fact to the attendants; he completed one more lifting movement, and then slowly slid to the floor.

Another subject convulsed after completing a full exercise routine at 47 FSW during which he had exhibited "the lips", dizziness, and dilatation of the pupils, but had continued the routine with no reduction in his work rate. Two minutes after he had finished the exercise routine and was still at depth, his only residual sign was the wide dilatation of the pupils. Decompression to 10 FSW at 30 ft/min was uneventful and his condition remained constant. After 1½ min, he was instructed to remove his breathing set and he did so. Twenty seconds after the start of air breathing, he was observed to have gross oscillating movements of his right forearm; the subject himself saw these a few seconds later and called the attendants. He still appeared calm and fully rational; but the movements slowly increased and involved his shoulder, and he was assisted as he slid to the floor.

Both convulsions followed the normal pattern of a tonic phase succeeded by a clonic phase. There was then a gradual but complete recovery, within 90 min in the first case and 30 min in the second.

Exacerbation of Symptoms on Decompression

Decompression was carried out at 60 ft/min on 26 occasions involving 51 subjects who were still breathing O_2 from their sets; the depth of the dive was between 29 and 38 FSW, and the decompression was either to the surface or to 10 FSW. On 12 occasions a distinct exacerbation of already existing signs of O_2 toxicity was clearly seen by the attendants.

Decompression was carried out at 30 ft/min on 53 occasions involving 103 subjects who were still breathing O_2, the decompression being from a dive of 44 or 47 to 10 FSW. A distinct exacerbation of signs was seen on 11 of these occasions.

The exacerbation usually occurred within 5 sec of the start of decompression, and always within 10 sec. The intensity of the signs usually diminished rapidly during the decompression, and all signs had disappeared by the time of arrival at the stop.

No subject complained of any pulmonary symptoms during or after the decompression.

One of the subjects who repeatedly showed exacerbation of signs upon decompression was the one who had convulsed at 44 FSW, but the other subject who convulsed showed no exacerbation whatever. One subject showed exacerbation of "the lips" on six occasions, but never exhibited any major symptoms of O_2 toxicity.

Weight Loss and Body Temperature

The deep-body temperature was measured with a "radio-pill" (Electronic Instruments, Ltd.) in 23 subjects, using an aerial in the chamber and a receiver outside (3). Over the 40-min exercise period, the mean increase was 1.33°C (Fig. 2). After the end of the exercise the temperature continued to rise for about 20 min, reaching a mean maximum of 38.55°C.

The inspired gas temperature was measured by use of a thermistor (Yellow Springs, type 409) connected to a Sanborn 760-53 calibrated thermistor bridge, the thermistor being inserted into the T-piece of the subjects' mouthpieces. The mean inspired gas temperature reached a maximum of 38°C at the end of the exercise routine.

The subjects were weighed naked before each dive and again after it, sweat on the skin's

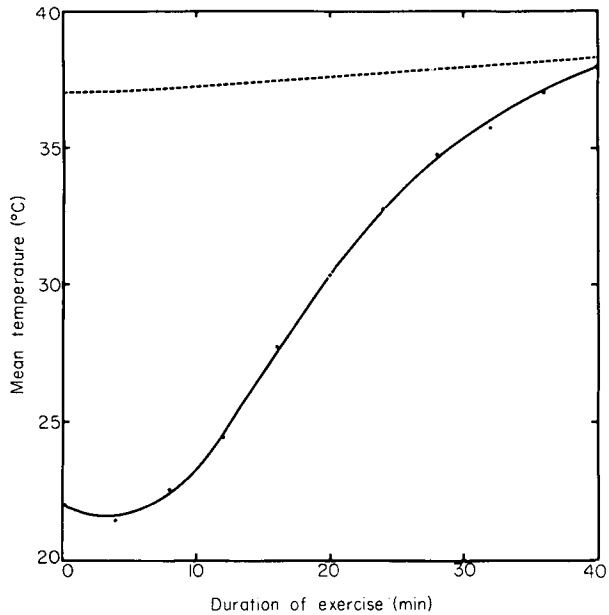

FIG. 2. Mean value of deep-body temperature in 23 subjects (dotted line) and of inspired gas temperature in 20 subjects (continuous line), plotted against time of exercise routine.

surface having been first removed with a towel. The mean weight loss over the series of dives was 25.8 oz, and there was no significant change in the mean with varying depth. The mean weight loss of the subjects whose deep-body temperature was monitored with the "radio-pill" was not significantly different from the overall mean, and so it is assumed that the mean increase in deep-body temperature determined is representative of all subjects in the series. Because of the increase in body temperature, all the subjects were flushed and sweating, and no reliance could therefore be placed upon facial color or degree of sweating as being signs of O_2 toxicity.

CARDIAC PULSE RATE

Cardiac pulse rate was obtained by hardwire ECG recording using three electrodes (MRC silver discs), two positioned on the sternum opposite the second and sixth intercostal spaces and a reference electrode positioned over the seventh intercostal space in the left anterior axillary line (7). The resulting ECG was displayed on the oscilloscope of a Sanborn Recorder and also was coupled to an instantaneous ratemeter (Devices, Ltd., type 2750) to provide a constant check on rate and waveform.

The mean heart rate for six subjects at 26 and 29 FSW is plotted in Fig. 3, and clearly shows the reactions of the circulatory system to the alternating periods of rest and different exercise levels. The terminal decrease in mean heart rate is caused by two of the subjects reverting to the less demanding exercise 2, because they were unable to maintain the last 4-min period of exercise 1.

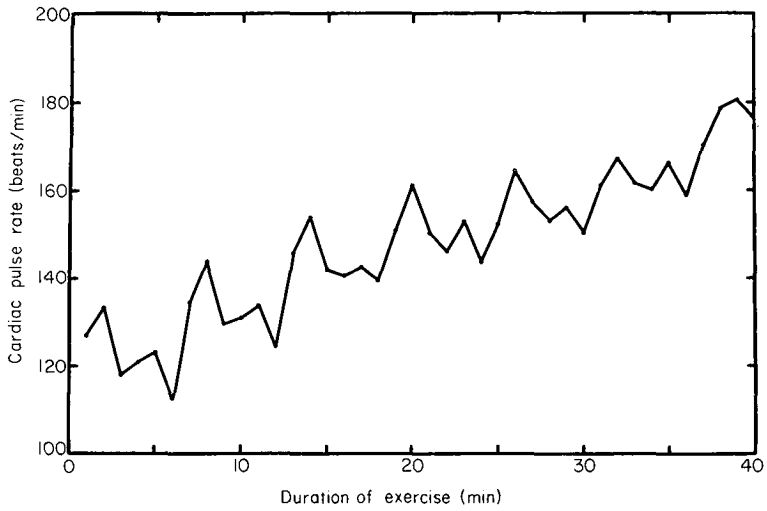

FIG. 3. Mean value of cardiac pulse rate in six subjects at 26 and 29 FSW chamber depth, plotted against time of exercise routine.

Taking the mean heart rate for each minute shown in Fig. 3 as a standard, Fig. 4 shows the difference between this standard and the heart rates found at 35 and 47 FSW. A degree of relative bradycardia was present at 35 FSW and increased at 47 FSW, being more apparent throughout during the rest periods. The apparent terminal reversal of the bradycardia is

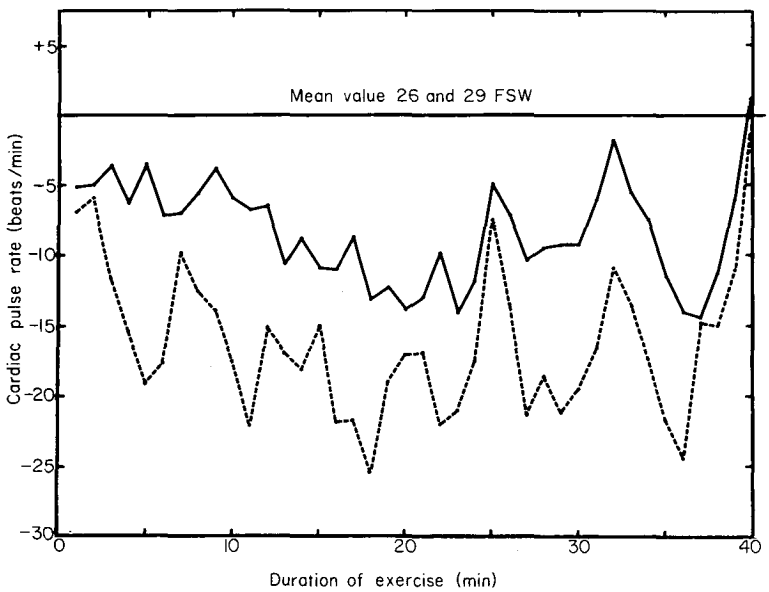

FIG. 4. Difference in cardiac pulse rate in six subjects between mean value at 26 and 29 FSW (shown in Fig. 3) and mean values at 35 (continuous line) and 47 FSW (dotted line), plotted against time of exercise routine.

caused by the subjects now having become more fit and better able to continue exercise 1 for the whole of the last 4 min.

End-Tidal P_{CO_2}

The end-tidal P_{CO_2} (PA_{CO_2}) was measured from "between the teeth" by continuous sampling through the chamber wall to an infrared analyzer (Beckman Instruments, Model LB 1).

The CO_2 absorption characteristics of the set were found to be good, and rarely did the inspired P_{CO_2} rise above 2 torr. No good evidence was seen of overall CO_2 retention in the body. During exercise 1, PA_{CO_2} typically rose to about 50 torr and then decreased to about 44 torr during the subsequent rest period. During the following exercise 2, PA_{CO_2} tended to remain steady at about 44 torr and to fall to about 38 torr during the next rest period.

Discussion

These investigations attempt to shed light on one of the "gray areas" in the effects of O_2 on the human organism, and provide some data applicable to men doing hard work under increased pressure but not actually under water.

It is known that an exposure of 2 hr at 60 FSW at rest in a "dry" chamber produces no symptoms of acute O_2 toxicity (6). It is also known that hard work decreases O_2 tolerance (1), and that O_2 tolerance while the subject is at rest or doing light work is greater in the "dry" chamber than under water in the "wet" chamber.

In these investigations the overall effect of working appears to be that it decreased the O_2 tolerance in the "dry" chamber to a level comparable with that seen in actual underwater dives, the first major symptoms appearing at an O_2 depth of 25.9 FSW, whereas the Royal Navy's depth limitation for hard work in the sea is 25 FSW on 100% O_2. The early appearance of the lip-twitching syndrome seems to have been revealed by the fortuitous arrangement of rest periods in the exercise routine.

The exacerbation of symptoms of O_2 toxicity upon decompression has been noted many times in animal experiments. Cases have also been reported in clinical hyperbaric O_2 exposures (2). I have collected records of 40 convulsive episodes in patients treated with hyperbaric O_2, and 16 (40%) of these convulsions occurred during a planned decompression at the end of treatment (J. M. Young, unpublished). It has been observed by both the Royal Navy and the United States Navy that most convulsive episodes in divers occur either during ascent or on the surface. Often divers have ascended because of symptoms occurring at depth, and it is not possible to assess the effects of decompression in these cases; but many convulsions have occurred in divers who had surfaced for other reasons. Convulsive episodes during decompression have also been observed in the U.S. Navy's O_2 tolerance test (4).

The cause of the exacerbation is uncertain. In these investigations the exacerbation occurred usually within 5 sec, which is a shorter time than probable lung–brain circulation time in these subjects. Exacerbation is therefore unlikely to be caused by a change in brain P_{O_2}. The other events that accompany decompression in a chamber are noise, cooling, air movement, and clouding, but exacerbations occurred before the clouding and any perceptible temperature change. We have never noticed any effect of sudden loud noises on the symptoms of the subjects; although we have on a few occasions opened the gas inlets and exhaust simultan-

eously to provide noise and air movement while the chamber has been maintained at a constant depth, we never managed to produce exacerbations.

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PART II. OXYGEN. EFFECTS ON CELLS AND SYSTEMS*

DISCUSSION

K. E. A. Seemann, Chairman

Dr. Houlihan: The mechanisms of O_2 toxicity discussed in the first session were associated with intracellular processes. In this session we are concerned with mechanisms of O_2 toxicity as they affect the whole organism. I wish to suggest a mechanism observed to occur in rats, and for which we have evidence to indicate a role in man.

In animals subjected to 4, 5, and 6 atm of HPO for periods of time which will lead to convulsions, there occurs a general sympathoadrenal medullary stress response—that is, a marked increase in epinephrine content of the urine and plasma, and depletion of norepinephrine of the brain. There also occurs a very high increase in the production of adrenal steroids.

Associated with these two findings, the metabolism of the epinephrine seems to change, undergoing a mode of metabolism other than the production of the usual DNA. There results an oxidation of the epinephrine and the result is the production of an adrenochrome.

The animals that have been subjected to very high O_2 tensions show signs of the adrenochrome normally undergoing further oxidation, with production of melanin. We have been able to extract melanin both from the plasma and from the lungs of the exposed animals. In addition, we also find that a peculiar type of hemolysis occurs in which, rather than a simple breakdown of red cells and accumulation of hemoglobin, the hemoglobin itself undergoes a cleavage and the result is the production of a nonhemoglobin pigment.

Altshuler has shown that when whole blood is incubated with adrenochrome, one can in this incubation procedure get a conversion of an indole, which leads to a soluble melanin, and the production of this nonhemoglobin type of pigment. What we are proposing here is that the high O_2 tensions, along with the tremendous increase in catecholamine and high steroid level, lead to the production of an adrenalutin-like indole and that this substance probably induces the kinds of reaction that we see in this preconvulsion stage in HPO.

Chairman Seemann: Are there any questions on this particular subject?

Cdr. Hoke: If adrenochrome or adrenalutin is produced during O_2 poisoning, it would seem that drugs like nicotinic acid or vitamin C should inhibit the production. Hoffer and Osmond have described an adrenochrome hypothesis of schizophrenia. They postulated that both nicotinic acid and vitamin C inhibited adrenalutin formation. Have you tried either one of these two drugs in O_2 toxicity?

Dr. Houlihan: We have tried ascorbic acid, and to get a high enough level to block O_2 effects, we actually got a degree of hemolysis from the ascorbic acid. We have not tried nicotinic acid.

Dr. Anderson: I would like to ask Dr. Nichols if he believes that the low arterial CO_2 is the primary cause of retinal vasoconstriction and his reasons. We have a little difference of opinion, and we might make this a subject for discussion.

Dr. Nichols: We have reviewed the studies that Dr. Lambertsen has done and these studies, which primarily dealt with the cerebral blood flow, were the basis for the summary that I showed. Those studies seem to agree with the work of Spalter, Nahas, and Len indicating that the amount of vasoconstriction parallels the decrease in arterial P_{CO_2} which is seen on O_2 breathing. In addition, maintaining the CO_2 tension constant apparently abolishes the retinal vasoconstriction that is associated with hyperoxia.

Dr. Anderson: Can you produce vasoconstriction by hypocapnia alone? We have not been able to.

* *Panelists:* C. W. Nichols, J. M. Young, B. Anderson, G. D. Blenkarn, R. T. Houlihan.

Dr. Nichols: This would suggest that there might be a second factor associated with the production of vasoconstriction. However, most of our work suggests hypocapnia is one of the major factors involved in producing this.

Dr. Anderson: Dollery finds that the CO_2 level is related to the degree of vasoconstriction, which would tend to discount this argument too.

We have photographs of choroidal and retinal vessels exposed to oxygen in a normal 20-year-old human being who had a central choroidal dystrophy. This is a dominant hereditary condition, not the result of an inflammation or an external destructive process.

The effect of O_2 on the retinal vessels has been photographed and the retinal vessels are found to be distinctly constricted, whereas the choroidal vessels appear less affected. The large arteries may not respond as much as do the smaller vessels to these high oxygen tensions.

Dr. Nichols: I have been asked how we can separate the effects of a decrease in delivery of metabolites by the blood from the intracellular biochemical effects of HPO. We cannot. Since the studies have been done on man, we can only postulate that possibly both of these factors are involved. We have no evidence to suggest one or the other as being the most important factor.

Dr. Anderson: Certainly low blood glucose in the ketotic hypoglycemia syndrome has been associated with cataract formation, and initiation of a cataract might be a lens epithelial defect.

Dr. Saltzman: I would like to confirm the suggestion of Dr. Nichols that the subjects chosen by Hickam and Frayser for studies of vascular responses to O_2 at 1 atm might have had a higher than average sensitivity to O_2. The apparent retinal vascular response is not striking at atmospheric pressure and they purposefully invested in subjects who responded dramatically. In our later studies at more than 1 atm, the response to HPO was dramatic in all individuals, and subjects were selected only on the basis of general good health and willingness to be exposed.

I will make the further suggestion that there is sufficient evidence as regards the anterior (or visible) retinal vascular response to O_2 to indicate that effects are produced by changes both in CO_2 pressure and O_2 pressure, with perhaps the effect of O_2 pressure response being more prominent. On the basis of the one individual described by Anderson, the posterior retinal or choroidal circulation may correlate more closely in its O_2 response with observations by Lambertsen and others of O_2 effects upon the cerebral circulation.

Dr. Houlihan: Dr. Nichols, relating to the retinal detachment question you discussed, you made reference to a transudate or a secretion which was forcing the retina loose. I want to know if you have any indication as to the possible cause and, further, whether it in any way could be similar to what we see in O_2-poisoned lungs. Rats kept at 1 atm of O_2 pressure for 3 days will have as much as 15 ml of transudate in the thoracic cavity.

Dr. Nichols: The retinal detachments that are seen associated with hyperoxia in the dog are really secondary detachments. They are secondary to what has gone on in the choroid, and if one examines the choroid of these O_2-poisoned animals, one finds that the vessels look atypical. There is a large amount of edema fluid or exudate between the vessels, and as this leaks through the limiting membrane, it pools subretinally and eventually the retina becomes detached. If these animals survive, this fluid resorbs and the retina reattaches.

These detachments are reminiscent of the retinal detachments that are seen in association with states such as toxemia of pregnancy in which, with very high blood pressures, usually on an acute basis, leakage from these choroidal vessels occurs and an identical type of detachment results. Several patients who have had toxemia of pregnancy with exudative detachments have been studied now with fluorescein angiography at New York Hospital and the vessels have been found to be markedly abnormal. Fluorescein does not remain within the vascular lumen as it normally does, but large amounts pool in the spaces between the choroidal vessels and large amounts pool under the retina. This indicates that there is abnormal permeability in this state, and probably such an abnormal permeability also exists in the choroidal vessels exposed to HPO to the point of toxicity.

Most of the vessels of the retina are merely very thin vessels lined with endothelium and with very few muscle fibers around them until one gets deep in the choroid. The change in permeability is probably a direct effect of O_2 on the endothelial cells, since this would be the major barrier to fluid getting out into the choroid.

Dr. Blenkarn: I think there is some evidence that the choroidal capillary does leak fluorescein normally. In fact, in the first phase of fluorescein angiography, the flush that you get is now felt to be the result of a slight fluorescein leak, and I think this is merely a matter of degree in terms of this exudated type of detachment.

Dr. Craig: Dr. Nichols, I wonder if there is any possibility that this retinal detachment might be related to the osmotic effects that you could have from O_2 in this situation, as suggested by Kylstra? In other words, perhaps you have a difference in partial pressure between the vitreous, for instance, and the choroid and thus could establish (at least transiently) quite high osmotic pressures. This suggests that if you introduced dogs into an O_2 atmosphere and increased the P_{O_2} much more slowly than usual, you might not get the retinal detachment.

Dr. Lambertsen: I gather that the concept of an osmotic effect of a gas involves the dissolving of large numbers of gas molecules in the tissues. This occurs at high pressures when the molecules are inert, such that they are not being consumed by the metabolism of the tissues they are dissolved in. However, when tissues are exposed to HPO by way of the capillaries, there exists a dynamic state in which O_2 delivered by way of the capillaries to the tissues is consumed at a massive rate by the cells themselves so that there is a very rapid decrease in O_2 tension through the tissue from the capillary to the point of metabolic activity. As a result, O_2 tensions do not rise uniformly high in the tissues and are quite low in much of the tissue mass.

Dr. Sobel: I would like to point out several matters of interest to those working with O_2 effects in humans. We are all concerned about the acute effects of hyperoxia, but there are certain "low dose" effects which are possible. You are all aware of the free radical theory of O_2 toxicity. There is also a free radical theory of aging. Because of these two concepts, we have considered whether high O_2 tensions might not increase the rate of aging, and we have been studying the skin of mice that have been exposed to HPO for long periods of time, and making other observations.

Among the things we did find in the skin (and there were many observations which did not show age dependency) was that the fluorescence of collagen increased greatly. Normally, collagen increases at the rate of about 1% a year. This is true for collagen and elastin and it is not species-dependent. It is true for man or mouse.

We found that after exposure of mice for three periods of 72 hr a week to 27% O_2 in N_2, at 45 psig, fluorescence of collagen increased 20%. We have now reproduced this effect *in vitro*, showing that acid-soluble collagen very rapidly develops increased fluorescence. I suggest then that measurement of the extent to which an individual has been exposed to the accumulative effect of high O_2 tensions could be estimated by measuring the fluorescence of collagen or elastin in the skin.

I do not know whether increased fluorescence of collagen is necessarily a harmful change. Nevertheless, this may be a clue to a means for going back in time and determining how long a person has been exposed to high O_2 tension.

A second thing we observed which may have applicability to man was that the mice that had come out of long periods of exposure to HPO showed a trait which we ascribe to aging phenomena, that is, reduced ability to retain recently acquired information, which is a well-known trait of older persons. While we do not know if this is reversible yet, it certainly appeared and was evident one week after the last O_2 exposure.

Dr. Schaefer: Dr. Young's studies showed quite a similarity to the original O_2 toxicity studies of Donald under resting conditions in that experiments were terminated by symptoms related to effects on the CNS. This endpoint is quite similar in exercise and under resting conditions. He mentioned in a way that the difference in O_2 tolerance in a dry chamber and under water would have been somewhat reduced.

We have done experiments in the wet chamber with a different type of exercise, swimming to exhaustion. In our experiments with pure O_2 exposure at a depth of 20–40 ft, we found that most of the experiments were terminated due to respiratory symptoms: dyspnea, spasm in inspiratory position, or hyperventilation. We know there is a different symptomatic effect of O_2 in the resting and exercising condition, as well as in the dry chamber as compared with under water.

Dr. Young: The symptoms we found in this series were definitely different in some respects to the ones reported earlier. The presence of dyspnea was impossible to determine because the subjects were dyspneic from their exhausting exercise anyway. About five or six people regularly complained of inspiratory difficulty. One of these has a family history of asthma, although he has never had any symptoms himself. With all of the subjects, it was noticeable that they always complained of inspiratory difficulty at a certain temperature of inspired gas, which we monitored. In closed-circuit O_2 equipment, the cooler is not very efficient and the temperature rose from a normal of about 21°C by the time they finished compressing up to a mean of about 38°C. One actually rose to 42°C. Therefore, it was rather hot gas they were breathing by the time they were finished. The subjects that complained of inspiratory difficulty always did so when the temperature rose above about 32°C.

Dr. Bühlmann: I can confirm the observations of Dr. Young. We studied the effect of exercise on decompression in 30 different subjects breathing 100% O_2 in a dry chamber by mask. Ten began exercise at 2.5 atm and three of the ten had symptoms, such as dizziness, beginning during the first minute. Another ten exercising in the range between 2.0 and 1.5 atm of inspired O_2 had nothing, and none of the 30 showed any symptoms in the range between 1 and 1.5 atm.

The respiratory symptoms came later in the second and third hour of breathing 100% O_2—with or without exercising made no difference. There were always CNS symptoms during exercise.

Dr. Segall: Could Dr. Young state how he measured the O_2 consumption and whether he was able to compare O_2 consumption in the same type of work under different pressures while breathing air or breathing O_2?

Dr. Young: The O_2 consumption could not be measured in the chamber during the actual experiments. Therefore, O_2 consumptions were done during air breathing under laboratory conditions using the subject's normal equipment and doing the same tasks as later done in the chamber.

As regards Dr. Bühlmann's study, it was very noticeable on several occasions that he saw no signs of any toxicity until an exercising subject went into a convulsion.

Of the convulsions in my series, one was an "off-effect" convulsion and actually happened at 10 ft after the end of the run. In another that occurred at depth, the man was working absolutely normally with no apparent subjective ill effects until 5 sec before he convulsed. He then suddenly felt his lips moving, drew this to the attention of one of the attendants and continued working until about one second before he took his mouthpiece out and collapsed.

Another case represented a convulsion that occurred long after the initial symptoms of O_2 toxicity. It was mediated by the exposure to O_2 at 47 ft. During that exposure he had dilatation of his pupils, dizziness, and lip twitching. As soon as he stopped exercising, the lip twitching and dizziness left, but he still had the dilatation of the pupils. We decompressed him at 30 ft a minute from 47 ft down to 10 ft while still breathing O_2, with no change in his condition whatsoever, and then waited for 2 min at 10 ft.

We then got him to take his breathing apparatus off. While doing so, he had a mouthful of saliva and didn't breathe for the first 9 or 10 sec while we were getting a towel to get rid of the saliva, and he then started breathing. About 20 sec after having begun air breathing, he saw (but did not feel) his right arm twitching and called the attendants to look at it. He continued perfectly clear mentally until about 2 or 3 sec before he collapsed.

Dr. Behnke: After hearing again of the reactions to O_2, not many of us would want to breathe O_2 routinely. However, O_2 has been breathed routinely for many years in the decompression of divers and in the treatment of divers' bends up to pressures of 2.8 to 3.0 atm without any symptoms. I know of no symptoms referable to the eye in man that have occurred as a result of breathing O_2 for such practical purposes. I know of no pulmonary or other untoward symptoms to have occurred in divers after breathing O_2 in this manner.

To exploit the advantages of O_2, we would like to say to tunnel workers (and we would revolutionize tunneling if we would do this) that they could breathe oxygen for decompression routinely day after day. We cannot now say that. We know that after exposure of animals to HPO there are histopathological changes of the CNS which give us great concern.

Small animals with their high metabolic rate apparently are much more sensitive to O_2 than man. We use small animals for such studies, but there has not been a single life span experiment that has been run involving O_2 exposures, not at toxic levels, but at what we call therapeutic levels or at the levels at which divers actually breathe O_2. There have been no studies of O_2 effects on life span comparable to studies in the field of radiation. More important, we do not know the consequences of breathing O_2 day after day at what we call therapeutic levels—up to pressures of 2½ atm abs or a little higher—for periods of 2–3 hr daily. After a period of 10 or 15 years there may be degenerative changes in the CNS.

Dr. Anderson: We have used therapeutic O_2 at 20 psig for treating patients with osteomyelitis and, although I cannot attribute the effect to O_2, we have had transient myopia (nearsightedness). I believe the group in Buffalo has had one patient with a similar finding. The patients become more nearsighted, and several weeks later the refraction returns to the normal preexposure level.

Dr. Glaser: My question concerns the suggested cross-linking theory of aging and the change in plasticity of collagen of the eye lens. I believe there have been experiments in which lenses were removed after exposure to various atmospheres and stress–strain properties determined. I believe there was a definite change in the elasticity of the material.

Dr. Anderson: We have observed no irreversible changes in humans. All the changes we have seen have returned to normal in the course of several weeks.

Dr. Lambertsen: It is extremely important to emphasize what Dr. Behnke has stated about the real usefulness of HPO, regardless of acute toxicity, and the requirement for obtaining information on chronic effects of repetitive exposures over a lifetime. In addition to these circumstances, we must take into account the possible adverse effects of continuous, perhaps multilevel, exposures to O_2 at relatively low P_{O_2} during the course of a single period, which may go on over the course of many hours or even many days in a saturation diving or decompression situation.

It has not been determined what is the effect of a continuous exposure to O_2 in which O_2 pressure is varied from a high level to a low, and perhaps back to the high pressure again.

The general point I am making is that, when one is exposed to HPO over a period of time, a pattern of increasing toxic effect upon the lung occurs.

The CNS influences and other influences of O_2 cannot be graded in the quantitative way we have graded pulmonary effects. However, these several kinds of damage—to lungs, to brain, and to other tissues—naturally are not expected to be the same in degree or in time scale.

It is important to recognize that residual effects of O_2 toxicity may be carried from one phase of an O_2 pressure exposure to another, thereby causing cumulative effects. If one is exposed to O_2 at 3 atm for the time it takes to produce a 4% decrease in vital capacity and then should go to the lower pressure of 2 atm, he should be considered as taking with him an effect of O_2 poisoning which he has just incurred at the 3 atm level. If he then goes on down to 1 atm and continues to breathe O_2, he takes with him a residual effect of O_2 poisoning equivalent to the rather severe situation which would have occurred if he had breathed O_2 continuously at 1 atm for over a day.

We are devising curves to predict this accumulating load of continuous acute exposure. However, it is not yet possible to define the effects of multiple exposures day after day.

Chairman Seemann: I couldn't agree more that we need more studies on the chronic effects of O_2.

Dr. Blenkarn: I want to describe some experience that Dr. Saltzman and I have had in assessing the use of succinate in protecting against O_2 poisoning in the conscious dog. You are aware that most studies of protection against O_2 toxicity have been conducted on small animals such as mice or rats, that the mortality or the time to convulsion has been the criterion for assessing effectiveness, and lastly, that the drugs have been administered as a bolus and usually intraperitoneally. We have used unanesthetized dogs to determine the effect of succinate infusions on preconvulsive and convulsive O_2 toxicity at an O_2 exposure of 4.3 atm abs.

Preconvulsed toxicity was manifested as unprovoked general arousal or agitation or as an isolated single myoclonic jerk. Control dogs received an intravenous infusion of glucose, while succinate-infused animals received a solution of sodium succinate, at dose levels that provide protection to rats when administered by the intraperitoneal route.

We could demonstrate no change in the mean latent period to the onset of either preconvulsive or convulsive O_2 toxicity in these awake dogs. The apparent increase in mean latent period to convulsive toxicity was not significant, and indeed was associated with gross postexposure neurological morbidity in four of the eight animals whose time to general convulsion exceeded the mean of 50 min. Control animals demonstrated no gross postictal morbidity.

In those dogs where the infusion of succinate appeared to delay the onset of convulsions, postexposure morbidity was sufficiently severe to preclude the use of succinate as a realistic protectant in this study.

For such reasons, it would appear that much data applicable to small animals is not readily transferable to larger animals. The awake large animal permits collection of much of the desired data (neurological, neuropathological data) in a more realistic setting and should provide a more definitive assessment of protectants from O_2 toxicity.

Part III. PHYSICAL EFFECTS OF PRESSURE AND GASES

HYDROSTATIC EFFECTS ON CELLULAR FUNCTION

J. V. Landau

Hydrostatic pressure has been an extremely useful tool in the analysis of various biological phenomena. Hydrostatic pressure experimentation may be considered to fall into two broad areas, one concerning morphological and other visibly measurable effects, and another concerning biochemical analysis of the fundamental processes involved.

The existence of a labile, three-dimensional protein network, or cytoplasmic gel with a high degree of potential contractility has long been considered necessary for the maintenance of characteristic cell shape and for such fundamental cellular activities as motility and cytokinesis (10). Protoplasmic gels have been shown in general to be endothermic (11) in character, involving an increase in volume upon gelation.

Application of hydrostatic pressure reversibly solates the gel component of living cytoplasm by opposing the volume increase accompanying gelation. This solation occurs in a precise, predictable pattern; and the degree of solation is directly proportional to the magnitude of

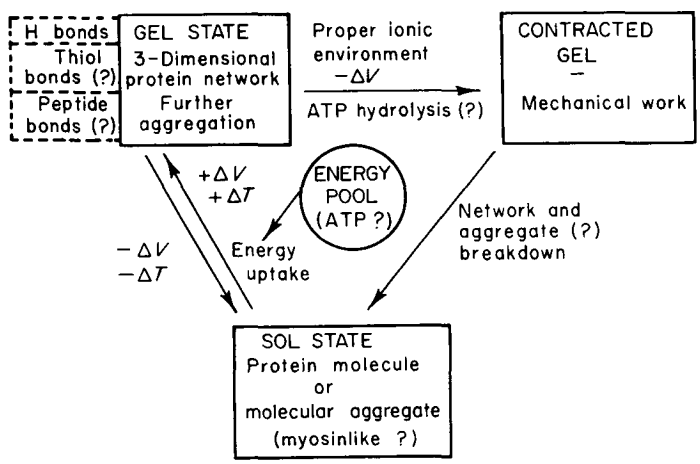

FIG. 1. A schematic representation of the sol–gel phenomenon. Note that the sol-to-gel stage is reversible. From Landau (3), by permission of the publishers.

the pressure applied at a given temperature over a given time period (10). A schema showing the role of the sol–gel equilibrium in the conversion of metabolic energy into mechanical work is shown in Fig. 1 (3). Changes in relative gel viscosity have been observed in pressures as low as 67 atm (9); but, for the most part, pressure effects are not directly observable below about 265 atm at room temperatures.

Typical examples of the pressure-imposed decrease in "gel strength" are the recession of the division furrow (12) and disaggregation of the mitotic apparatus (13) in dividing marine eggs, and the cessation of pseudopodial flow in the ameba and that organism's change to a spherical shape (8). Disaggregation of microtubular structures has also been observed through electron microscopy (16).

The effect of pressure on the biosynthetic capability of a living organism is presently being investigated and will be covered in some detail in this paper. Of immediate concern are the following questions:

1. Do human cells react to pressure in the same manner as do amebas, marine eggs, and other single cells? Is "relative gel viscosity" an important factor in the division, motility, and retention of the characteristic shape of these cells?

2. Are important biochemical processes affected by pressures between 1 and 265 atm? Is it possible, in some instances, that the minimum level at which pressure effects become manifest is *apparent* rather than *real* because of the particular parameters used to measure such effects? In other words, can the relatively small hydrostatic pressure increase brought about by the imposition of inert gas pressure in a deep dive situation affect biochemical function?

The answer to the first question is *yes*. Cells of human origin respond to pressure in the same

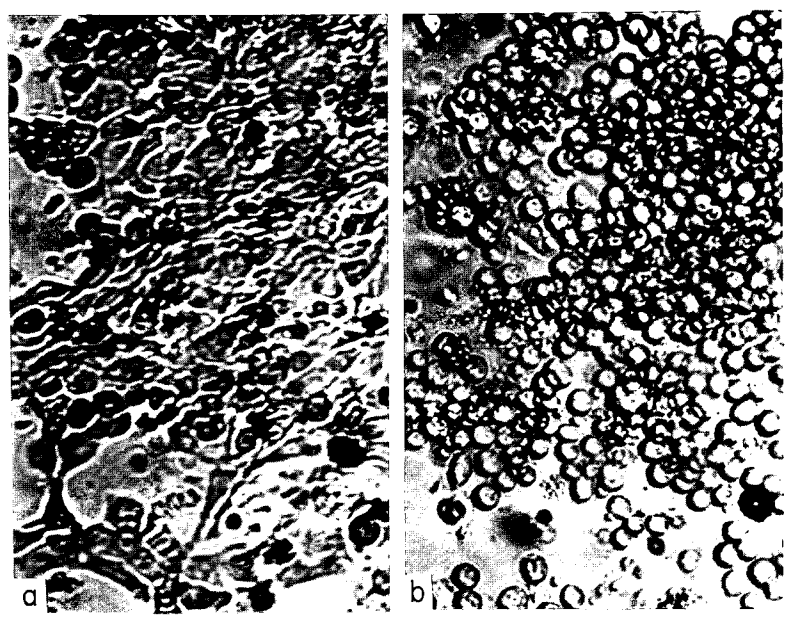

FIG. 2. (a) A monolayer culture of amnion cells at atmospheric pressure; (b) the same culture after 20-min exposure to 670 atm at 35°C. From Landau (4), by permission of the publishers.

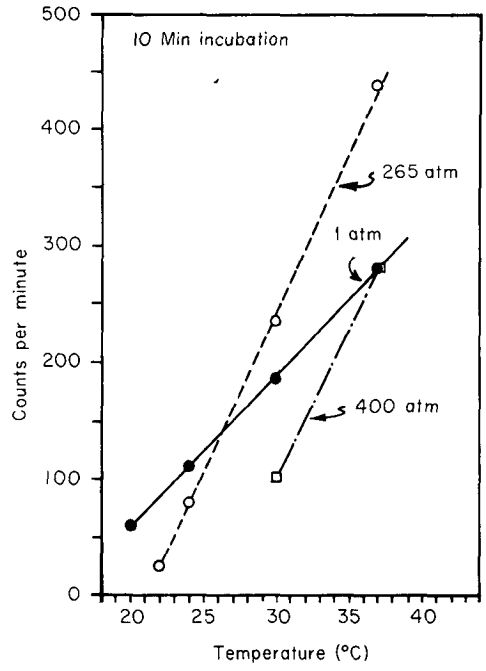

FIG. 3. ^{14}C-Leucin incorporation in *E. coli* during 10 min of pressure and temperature variation. From Landau (5), by permission of the publishers. Copyright 1966 by the American Association for the Advancement of Science.

precise, predictable manner that other cells do (4). Figure 2a shows a monolayer culture of human amnion cells prior to the application of pressure. Figure 2b shows the same field under 670-atm pressure. The cells round up as the gel strength decreases to a point below which it can no longer counteract tensional forces at the cells' surface. Within 15 min after release of pressure, the culture cannot be distinguished from that shown in Fig. 2a. Although little effect, if any, can be noted at pressures below 265 atm, there seems little doubt from an extrapolation of relative gel viscosity data to 1 atm that the "rigidity" of the system is decreased by 25% for each 67-atm increase in pressure (9).

We have studied (5) the effect of pressure on protein and nucleic acid synthesis in *Escherichia coli*. In general, we found that, depending upon temperature, the application of a specific pressure may result in either stimulation or inhibition of ^{14}C-amino acid incorporation into protein. For example: 265 atm stimulates at 37°C, has no measurable effect at 27°C, and inhibits at 22°C; whereas 400 atm has no effect at 37°C, and inhibits at lower temperatures (Fig. 3). These results are similar to those of experiments involving frequency of cardiac contraction (7) and luciferase activity in bacteria (1). The results have been interpreted in the following manner:

1. A primary rate-controlling reaction is postulated, which involves an activated enzyme and which is directly involved in protein synthesis; and
2. There is a reversible thermal inactivation of the enzyme that begins at about 25 to 27°C. Both the primary reaction and the enzyme inactivation are inhibited by pressure application, enzyme inactivation having the greater sensitivity.

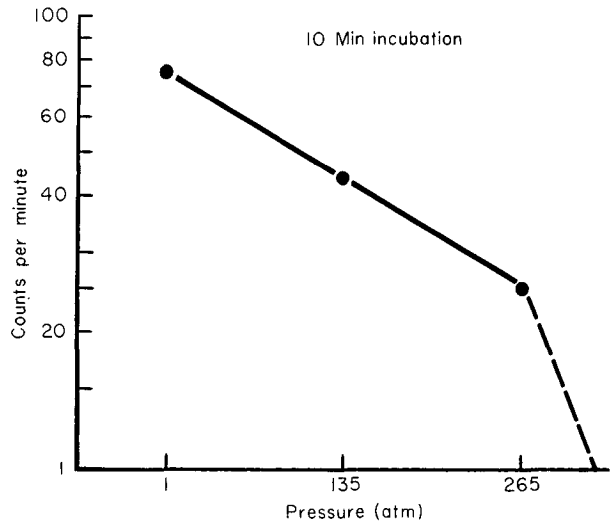

FIG. 4. The effect of pressure on ^{14}C-leucine incorporation in *E. coli* at 22°C. The slope of the line indicates the possibility of a rate-limiting reaction with a ΔV^* of 100 cm^3/mole.

According to this hypothesis, the application of 265 atm at 37°C would have little suppressive effect on the primary reaction and would, in fact, result in stimulation by making available a greater amount of activated enzyme. A pressure of 400 atm at 37°C might also result in a greater enzyme supply; but this pressure might also inhibit the primary reaction to a greater extent, thus yielding no net change in the rate of reaction. At temperatures below

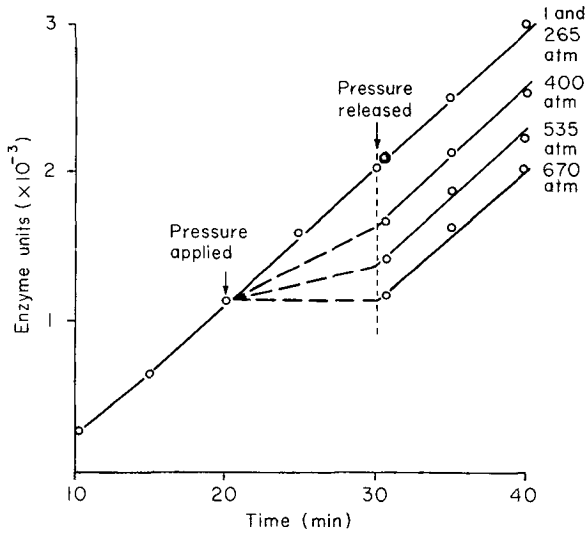

FIG. 5. The synthesis of β-galactosidase at 37° C and under various pressures. Note that normal rates resume immediately after pressure release. From Landau (6), by permission of the publishers.

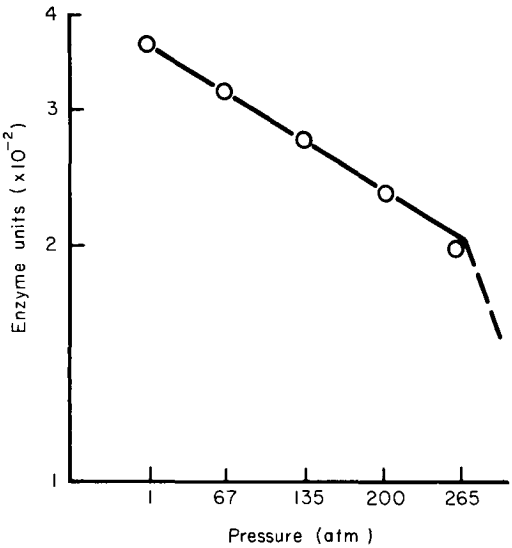

FIG. 6. The effect of pressure on the induction of β-galactosidase synthesis. The data indicate that inducer–repressor complex formation is accompanied by a ΔV^* of 55 cm³/mole. From Landau (6), by permission of the publishers.

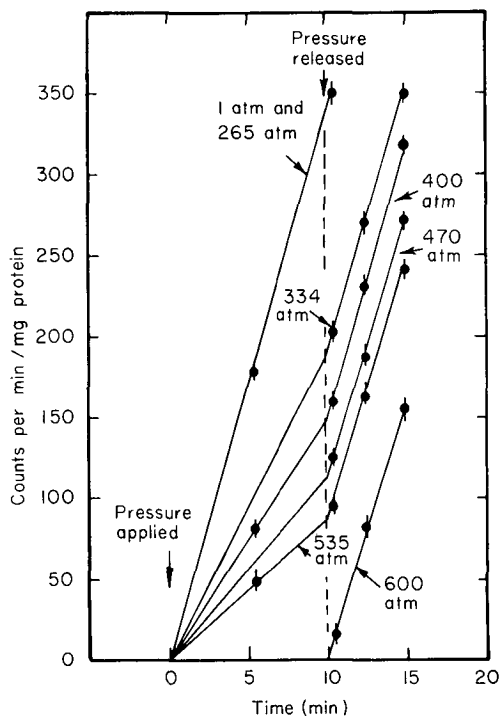

FIG. 7. The effect of pressure on the incorporation of ^{14}C-amino acids into protein of HeLa cells at 37°C.

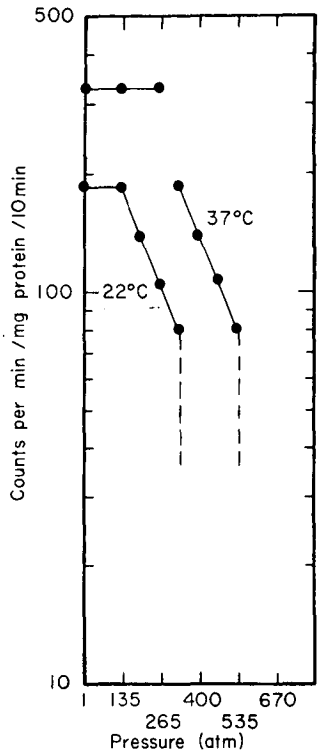

FIG. 8. The data in Fig. 7 at 37°C and additional data at 22°C redrawn as log rate of synthesis vs pressure. The slope indicates a ΔV^* of 100 cm³/mole.

25°C, however, the enzyme would be thermally inactivated, and pressure would directly inhibit the primary reaction. Figure 4 shows the data obtained at 22°C. The straight-line plot indicates the possibility of an effect on a rate-limiting reaction with a calculated volume change of activation (ΔV^*) of 100 cm³/mole. If this analysis is correct, it indicates that pressure effects exist at all levels, but are not directly measurable at the lower levels.

To determine which phase of protein synthesis may be affected by pressure, we turned to the induced synthesis of a specific and measurable protein, β-galactosidase (6). Through proper experimental manipulation, the induction, transcription, and translation steps involved in protein synthesis can be reasonably defined. Pressure effects on β-galactosidase synthesis are shown in Fig. 5. The effect is immediate when pressure is applied, and is instantaneously reversible when pressure is released. From the data gathered at pressures above 265 atm—where inhibition of synthesis is measurable—one may conclude that the effect is on a rate-limiting reaction with a ΔV^* of 100 cm³/mole. Under the specific conditions of these experiments, this volume increase of activation can be associated with the translational step of protein biosynthesis.

In this system, the production of β-galactosidase is normally prevented by a repressor molecule which does not allow the genetic information specifically concerning this enzyme to be utilized. When an inducer molecule is introduced, a complex is formed between inducer and repressor, thus effectively inactivating the repressor and allowing β-galactosidase synthesis.

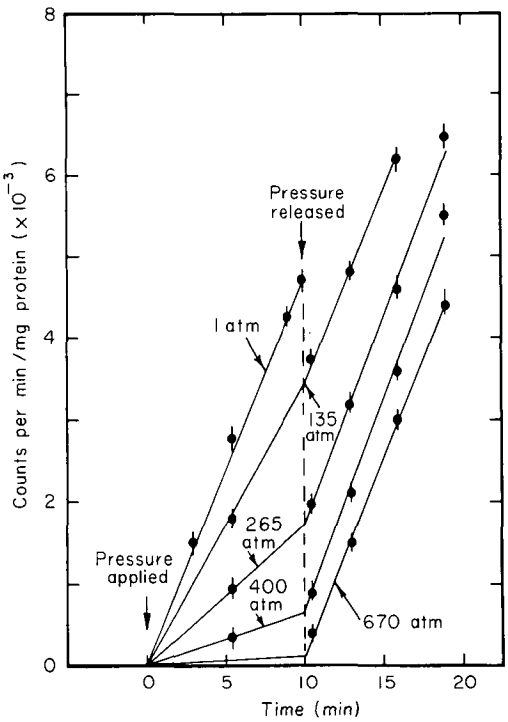

FIG. 9. The effect of pressure on the incorporation of ^{14}C-uridine into RNA of HeLa cells at 37°C.

The effect of pressure on induction, specifically the inducer–repressor complex formation (2), was next investigated. Such complex formation was found to have a ΔV^* of 55 cm^3/mole, indicating that a configurational change of the repressor molecule is induced by the small apolar inducer molecule when the two form a complex. Of specific interest was the fact that the pressure effect could be measured at all levels above 1 atm (Fig. 6). Thus there is direct evidence that important biochemical processes may be measurably affected by relatively small increases in hydrostatic pressure.

This question arose: Are these pressure effects unique to bacterial systems? Human HeLa cells were readily available, and our experiments were therefore extended to examine the effect of pressure on their synthetic capabilities (Landau, in preparation). The incorporation of ^{14}C-labeled amino acid into protein was affected in a manner very similar to that found in *E. coli* (Figs. 7 and 8). Once again, a reasonable interpretation of the results, based on the reaction rate theory, is that a ΔV^* of 100 cm^3/mole is associated with the translation step of protein synthesis.

The incorporation of ^{14}C-uridine into the RNA component of these cells reveals a distinctly different pattern from the one observed in *E. coli*. Inhibition of such incorporation at 37°C is indicated at all pressures over 1 atm, and the results yield a bimodal curve (Figs. 9 and 10). It is again of particular interest that measurable differences in incorporation can be found at relatively low hydrostatic pressure levels.

Rutberg (14) has shown that a 150-atm pressure applied to lysogenic *E. coli* K_{12} for a two-

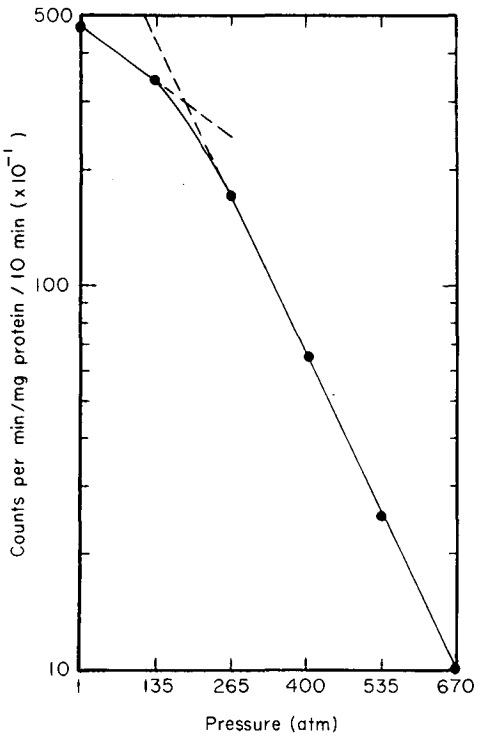

FIG. 10. The data in Fig. 9 redrawn to show the effect of pressure on the rate of incorporation.

minute period can cause phage induction and lysis of the cell. Schoffeniels (15) has reported marked changes in potential at 100 atm in experiments with isolated frog skin preparations.

Although the absolute magnitude of the effects at low pressures is relatively small, it must be realized that the present experiments involve rather short time periods (minutes). Whether the effects reported here would lead to more severe disruptions of cell function if pressure were maintained over extended time periods is not known.

It is now becoming recognized that these questions have considerable practical importance. Hydrostatic pressure was once considered a useful tool in biological analysis, but was of little concern to physiologists interested in deep-dive technology. It is now evident, however, that the effects of relatively low increments of hydrostatic pressure that have been generated by increased inert gas pressure merit major consideration by those concerned with the fundamental dynamics of living cells.

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EFFECTS OF INERT GAS PRESSURES ON PROTEIN STRUCTURE AND FUNCTION

R. M. Featherstone, S. Hegeman, and W. Settle

Very little is known about the effects of high pressures on protein structure. Two types of pressure can be considered:

1. Hydrostatic pressure, which is a stabilizing force as evidenced by increased hydrate stability and increased aggregation of monomers. Aoki *et al.* (1) have shown that these aggregations are both irreversible and reversible, depending on the number of monomeric units in association and the hydrostatic pressure applied. Extremely high hydrostatic pressures will denature protein much in the same way that heat denatures protein.

2. Gas pressure. Since gas is compressible, an increase in gas pressure can be considered an increase in the number of molecules per unit volume, i.e., an increase in concentration.

When an increased gas pressure is applied to a protein, the concentration of the gas in the protein is increased. In addition, there is a hydrostatic pressure increase. Hydrostatic effects on protein are observed only at high pressures, whereas the effect of increasing gas concentration is observed at much lower pressures.

It is usually assumed that functional changes in a protein indicate structural change. By structural changes are meant alterations in the protein ranging from conformational changes to those occurring on a subatomic level. In only a very few instances have changes in protein structure been directly determined. They are usually inferred by observing changes in function.

The concept of conformational change is familiar to everyone, but one must hesitate to ascribe this system to all proteins. Studies on myoglobin–CO binding in the presence of Xe indicate that the change in the protein structure is probably subatomic, rather than being an alteration in recognizable tertiary structure.

Several studies have been made on the effect of increasing gas pressures on protein function. Table I, taken from work done in our department by A.J. Trevor (6), shows the effect of the increasing pressure of N_2 and cyclopropane on rat cerebral cortex ATPase. Note that with increasing N_2 pressures, ATPase activity is stimulated, whereas cyclopropane under similar

TABLE I

EFFECT OF INCREASING PRESSURE ON ATPASE ACTIVITY OF RAT CEREBRAL CORTEX (6)[a]

Pressure (psi)	% of Control ATPase Activity	
	Nitrogen	Cyclopropane
Atmospheric	—	79
20	100	72
40	114	53
60	—	45
80	132	31

[a] The numbers represent the mean of five experiments. ATP was measured by orthophosphate release following exposure of the enzyme to the gases at varying pressures at 25°C.

pressures inhibits ATPase activity. This illustrates two points:

1. Increasing the pressure of gases can change the function of a protein.

2. The chemical–physical nature of the gas molecule is important, for it determines what kind of functional change will take place in a protein.

Table II shows that a wide variety of inert and other gases change protein function; the effectiveness of the gases is determined by their chemical–physical properties (5).

How does the gas–protein interaction cause a change in function? This question is currently being studied in our laboratory through the use of Xe–myoglobin–CO model system. Figure 1 is a picture of a model of the heme region of myoglobin showing the position of the Xe atom located by X-ray diffraction. Schoenborn and Nobbs (4) have shown that, in the presence of Xe, the myoglobin molecule is not altered at a level observable by X-ray diffraction tech-

TABLE II

EFFECT OF 30.4 ATM OF VARIOUS GASES ON PROTEIN FUNCTION (5)
(GAS ATMOSPHERES ARE SUPERIMPOSED ON 1 ATM OF AIR)[a]

Gas	Tyrosinase (% control activity)
None	100
Helium	84
Neon	70
Argon	68
Nitrogen	60
Nitrous oxide	37

[a] There are at least five observations for each gas. Tyrosinase (1.10.3:1) was obtained from Sigma Chemical Company. Activity, as measured by an increase in absorption at 280 mμ, was determined at 25°C.

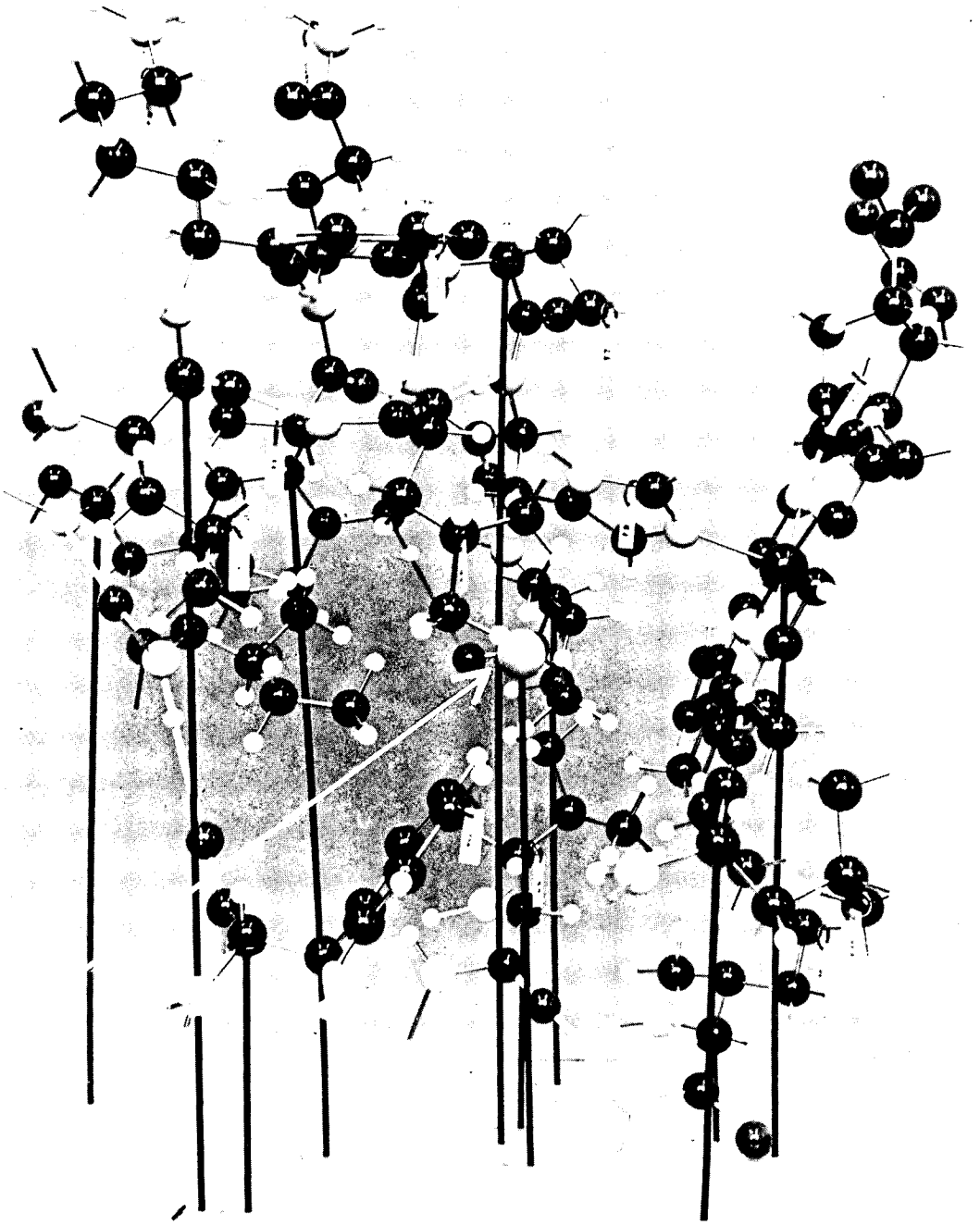

FIG. 1. Photograph of a model showing the location of xenon (↑) near the heme group of a myoglobin molecule.

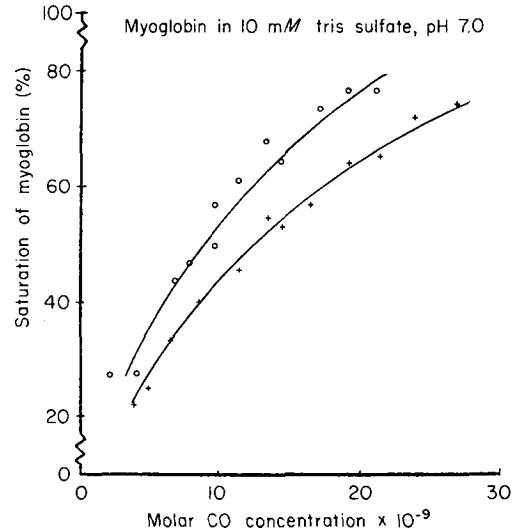

FIG. 2. Sperm whale myoglobin was equilibrated with either Xe or N_2 at 1 atm, and the percent saturation was determined spectrophotometrically as aliquots of CO_2 were added. (O), N_2; (+), Xe.

niques. However, Fig. 2 shows that the presence of Xe does alter the functioning of the myoglobin, as evidenced by the shift of the CO-binding equilibrium. This evidence leads us to a consideration of very subtle structural changes at a submolecular level.

The location of the Xe site as shown by X-ray studies could account for subtle changes in the electron configuration of the heme, which would lead directly to an alteration of the CO binding. Any electron shift must be very small since changes in the heme spectrum are not noted.

Work done by Keyes (3) indicates that there is more than one atom of Xe involved in the functional change. Keyes proposed that three or four Xe atoms are involved, all binding to specific sites. The position of the additional sites must be near the surface of the molecule because of the lack of suitable cavities within the protein. Keyes proposed that the Xe binding occurs at points that cause subtle rearrangement of the entire molecule. The possibility of a competitive inhibition with CO at the heme, or a binding of Xe with the residue side chains around the heme region, has yet to be entirely excluded. Wishnia and Pinder (7) have pointed

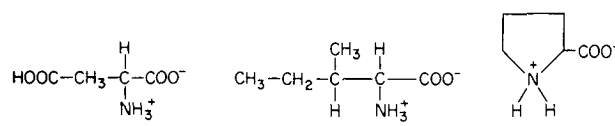

FIG. 3. Amino acids that singly overcome Xe and cyclopropane inhibition of anaerobic growth in *E. coli*. The amino acids were added singly to tubes of mineral broth containing $(NH_4)_2SO_4$, trace metals, phosphate buffer pH 6.8, glucose, and exponentially growing cells. The tubes were placed under cyclopropane, N_2, or Xe. After 24 hr, qualitative estimates were made of the amount of growth.

TABLE III

EFFECT OF XENON AND CYCLOPROPANE ON SUCCINIC DEHYDROGENASE, NADH OXIDASE, AND LACTIC DEHYDROGENASE OF MUTANT AND PARENT *E. coli* K_{12} 3000[a]

Strain	Gas	% of Control activity succinic dehydrogenase	% of Control activity NADH oxidase	% of Control activity lactic dehydrogenase
Parent $N = 6$	Xenon	83 ± 5	85 ± 5	100 ± 3
Mutant $N = 6$	Xenon	150 ± 5	131 ± 5	100 ± 3
Parent $N = 6$	Cyclopropane	79 ± 5	81 ± 5	100 ± 3
Mutant $N = 6$	Cyclopropane	143 ± 5	127 ± 5	100 ± 3

[a] Succinic dehydrogenase and NADH oxidase were prepared as membrane fragments of *E. coli*. Lactic dehydrogenase was obtained from the soluble fraction. Lactic dehydrogenase and NADH oxidase are assayed by following changes in absorbance at 340 mμ. The succinic dehydrogenase assay employs a substrate-dependent reduction of a blue dye (2, 6-dichlorophenolindophenol) to its colorless form.

out that even the possibility of a gas–hydrate type of interaction at the protein surface has not been wholly excluded in producing apparent binding sites.

At present we may draw two tentative conclusions: Only subtle changes in the molecular configuration are required for observable functional changes, and Xe appears to be binding to specific sites on the protein.

Are all proteins equally susceptible to functional changes by gases? Studies on the whole cell in our laboratory (2) indicate that there is a high degree of specificity of effect. Xenon and cyclopropane cause a reversible inhibition of aerobic and anaerobic growth of *Escherichia coli* when grown on a mineral–glucose or succinate medium (Fig. 3). The three structurally and biochemically unrelated amino acids shown in Fig. 3 singly overcome this inhibition, which indicates that on a physiological level, at least, the gas effect is specific. Perhaps the best evidence we have that there are only one or two cyclopropane- or Xe-sensitive sites in the cell is the isolation of a mutant that is capable of growth at otherwise lethal concentrations of cyclopropane. A cell can only survive one or two simultaneous mutations. According to the one gene–one protein concept, only one or two proteins will be altered unless, of course, a control gene is mutated. Then a few more proteins (up to six or seven) may be changed. A comparison of the parent and mutant enzymes is being made in our laboratories to determine the proteins that are the most sensitive to Xe and cyclopropane. Table III is an example of the kind of investigation under way.

Succinic dehydrogenase and NADH oxidase, both membrane-bound metalloflavoproteins, are stimulated in the mutant and inhibited in the parent. Whether this class of protein is the principal site in the cell that is sensitive to the gases remains to be shown.

In summary:

1. High gas pressures alter the function of a protein, the degree and the nature of the alteration depending on the physical–chemical properties of the gas rather than on pressure per se.

2. Gas binding to a protein is specific. It is not known whether the internal binding sites or the external ones cause the functional changes.

3. Proteins have vastly different susceptibilities to functional alterations by gases. In the whole cell, usually one protein (or, at most, ten) is susceptible to gases under pressure. Inhibition of any of these proteins probably accounts for the physiological effects caused by gases under high pressure.

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EFFECTS OF HYDROSTATIC PRESSURE ON MAMMALS

M. J. Lever, K. W. Miller, W. D. M. Paton, W. B. Streett, and E. B. Smith

The extent to which exposure to high hydrostatic pressures may limit man's underwater activities is as yet uncertain. The problem can be summarized in the form of three questions, one of which can be readily answered. The remaining two are the subject of much current research.

1. Does hydrostatic pressure affect animals deleteriously?
2. Do the narcotic effects of the gases breathed by mammals always make themselves felt before the effects of hydrostatic pressure do?
3. Can the effects of hydrostatic pressure on mammals be alleviated?

To answer the first question, we need only consider the results obtained when aquatic or amphibious animals are exposed to high hydrostatic pressures in the absence of potentially narcotic gases.

Effects of Hydrostatic Pressure

The effects of hydrostatic pressure on a wide range of biological systems have been the subject of extensive investigations. As early as 1884 Regnard (11) initiated a series of studies, the main conclusion of which was that hydrostatic pressures in the range of 200–300 atm can cause animals' muscles to lose their excitability, leading to a rigid paralytic state. Higher pressures (~400 atm) proved lethal. Even greater pressure was required to inactivate simpler organisms. Research undertaken since that time has confirmed and extended these conclusions. The effect of pressure on nerve cells is to stimulate at pressures below 200–300 atm (3). At greater pressures the height of the action potential is reduced and the duration is extended.

These effects of hydrostatic pressure have recently been reviewed comprehensively (4). The inference is quite clear: hydrostatic pressure inactivates aquatic animals at pressures at levels of 200–300 atm, and its effects are fatal at about 400 atm.

Mammals Exposed to High Hydrostatic Pressures

The "pressure barrier" for non-gas-breathing animals is approximately 300 atm. The barrier in the case of mammals is not so recognizable, as it is not always easy to distinguish be-

TABLE I

EXPERIMENTS EXPOSING MICE TO PRESSURES GREATER THAN 100 ATM[a]

Worker	Number of animals	Maximum pressure (atm)	Approx. time to 100 atm	Onset pressure (atm) for			Comments
				Tremors	Convulsions	Death	
MacInnis	16	107	3 hr	75–80	—	—	Mice
et al. (7)	24	122	3 hr				recovered after 4 hr at pressure
Brauer	11	102	1½–2 hr	60–70	90	—	
et al. (2)	12	116	1½–2 hr	60–70	109	—	
Miller	6	115	5 min	80	110	—	
et al. (10)	4	140	½ hr	55–80	110	126–143	
Brauer	12	116	1½–2 hr	60–70	109	—	H_2
et al. (2)	8	129	1½–2 hr	65–75	129	—	75% He, 24% N_2
Miller	1	136	½ hr	82	—	136	Ne
et al. (10)	1	125	½ hr	90	—	—	He/N_2O

[a] All experiments carried out at 30–32°C with O_2–He mixtures unless otherwise stated.

tween the effects of pressure per se and the narcotic effects of the gases breathed. Certainly when animals are exposed to high pressures of N_2, no effects are observed other than narcosis, which occurs at approximately 35–40 atm (9). It is probable that only three gases are suitable for studies of the effects of higher pressures: He, H_2, and Ne. There is now evidence that with He and Ne, the effects of pressure make themselves felt before narcosis sets in, whereas with H_2, pressure and narcotic effects appear to occur almost simultaneously.

Membery and Link (8) exposed mice to He–O_2 mixtures in the pressure range of 60–90 atm for up to 13 hr. They observed no adverse reactions, and the occasional deaths that did occur were attributed to hypoxia or decompression sickness. They noted that the mice shivered if the temperature of the chamber fell below 32°C. MacInnis *et al.* (7) exposed mice to pressures of 122 atm for 4 hr. (Table I). The rate of compression was less than 1 atm/min (somewhat slower than the rate used by Membery and Link). The mice appeared normal until pressures of 75–80 atm were reached, at which point slight tremors occurred. At higher pressures the tremors became coarse and persistent, and appeared to involve the whole body. The tremors ceased after 1 hr at pressure. At pressures above about 90 atm, the animals appeared to have some difficulty in breathing. Mice surviving the decompression procedure lived for several months.

Brauer *et al.* (1) showed that rhesus monkeys underwent convulsions of the grand mal type when pressures reached approximately 50 atm in both He–O_2 and H_2–O_2 breathing atmospheres. The pressures required to produce convulsions were marginally higher for H_2–O_2 mixtures and, in contrast to the animals breathing He–O_2, only half the monkeys convulsed.

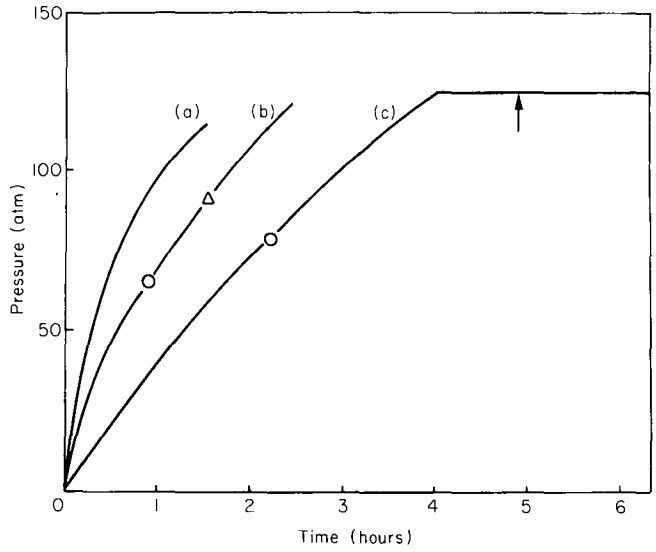

FIG. 1. Three compression schedules for mice. (*a*) Brauer *et al.* (2) (fast); (*b*) Brauer *et al.* (2) (normal); (*c*) MacInnis *et al.* (7). (○) Mean pressure at onset of tremors; (△) onset of convulsions; (↑) marks point at which tremors ceased. The pressure–time curves illustrated are schematic only.

Brauer and his co-workers extended these studies to mice (1, 2). Tremors began at about 60 atm, and convulsions were observed in all animals by 102 atm. Convulsions were not reported by MacInnis *et al.* The differences do not appear attributable to the somewhat faster compression rates used by Brauer in most of his experiments; it was therefore concluded that the discrepancies were due to the difference in the strains of mice used. The approximate compression schedules used by these workers are illustrated in Fig. 1.

Miller *et al.* (10), making a comparative study of Italian great newts and mice, attempted to confirm unequivocally the effects of the atmospheric environment under pressure. They first showed that the sensitivity of the mice and newts to anesthetics (N_2 and N_2O) at 30°C was comparable. The newts were shown to lose responsiveness between 165 and 245 atm, whether pressure was applied hydrostatically or by use of He or Ne. At pressures up to 125 atm, Ne (in one experiment only) and He failed to anesthetize mice; but at slightly higher pressures (135–145 atm) the effects were lethal. Within 5 min of reaching these pressures, the animals passed through a phase of prostration and respiratory difficulty to death. At pressures above 100 atm, distinct tremors were observed. It was concluded from these experiments that the effects of hydrostatic pressure appear both in newts and mice at pressures below those at which anesthesia occurs with He and Ne. The compression rates in this investigation were faster and the exposure times shorter than those used in the studies described in the preceding paragraphs.

In investigating the effects of pressure on mice breathing oxygenated liquids, Kylstra (6) observed tremors at 34 atm. At higher pressures (~70 atm) temporary cessation of breathing, caused by generalized muscular contraction, was observed. In other experiments, mice showed no apparent distress at 100 atm pressure, and in some animals "rhythmical respiratory movements of the chest" were evident at pressures as high as 160 atm.

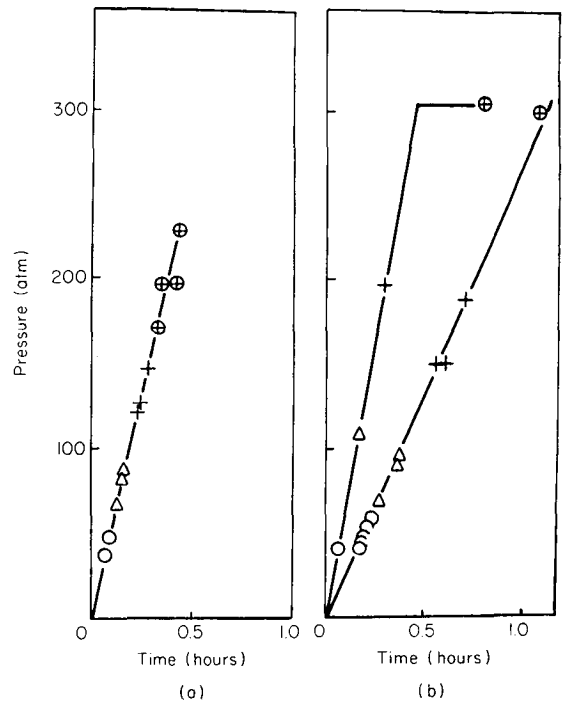

FIG. 2. Fast compression schedules for mice. (a) Chamber temperature, 30°C; (b) chamber temperature, 23°C. Oxygen tension, 1 atm. (○) Onset of tremors; (△) onset of convulsions; (+) death; (⊕) death of animals treated with pentobarbital.

Although the results of all these animal experiments are not entirely consistent, the main conclusions are clear. The effects of high pressure are fourfold:

1. Uncoordinated tremors ["fasciculation," as described by Brauer *et al.* (2)]. These have been observed by all workers except Membery and Link (8). The onset appears to be at pressures of 70 atm ± 10. There is some evidence that the tremors are dependent upon the rate of compression, although it is far from conclusive; and that the symptoms appear to remit after 30–60 min.

2. Convulsions. These have been most clearly observed by Brauer *et al.*, although the experiments of Miller *et al.* (10) did produce convulsions in a number of cases. The effect may depend upon the strain of mice used.

3. Respiratory distress. As MacInnis *et al.* (7) observed at pressures above 90 atm, the respiratory rate decreased and movement of the chest wall became obvious. Mouth breathing and gasping were observed. To what extent these effects were due to the increased density of the atmosphere or were caused by the effects of pressure on muscular activity cannot be stated.

4. Paralysis. Newts in the pressure range of 165–245 atm lost all spontaneous movements and frequently took up contorted postures. Spontaneous movement returned upon reduction of the pressure (10). Whether this paralysis was due to the effect of pressure on the nervous system or to pressure directly on the muscles is not known. In many experiments with mice,

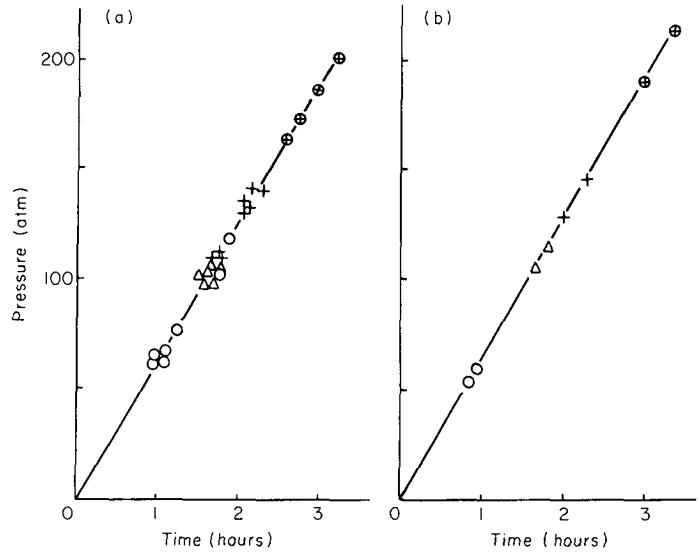

FIG. 3. Medium rate compression schedules for mice. (a) Chamber temperature, 30°C; (b) chamber temperature, 23°C. Oxygen tension, 1 atm. Symbols are the same as those in Fig. 2, except that ⊕ means death of animals in an anesthetic pressure of N_2.

the tremors and convulsions appeared to fade at higher pressures and most activity had ceased before death, suggesting paralysis.

The degree to which these symptoms depend upon the conditions of the particular experiment has been the subject of only a few isolated investigations. In general, it has been as-

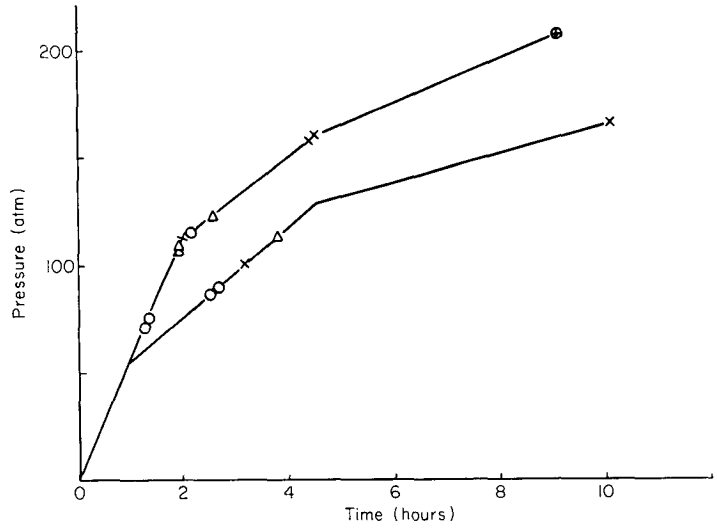

FIG. 4. Slow compression schedule for mice. Chamber temperature, 30°C; O_2 tension, 1 atm. Symbols are the same as those in Fig. 2.

TABLE II

EXPERIMENTS EXPOSING MICE TO PRESSURES GREATER THAN 100 ATM
(All experiments in O_2–He mixtures ($P_{O_2} \sim 1$ atm), unless otherwise stated)

No. of animals	Max. pressure (atm)	Approx. time to 100 atm (hr)	Onset pressure (atm) for			Comments	
			Tremors	Convulsions	Death		
3	150	0.2	35– 50	70– 80	120–150		30° C
6	160	1.7	60–110	90–100	110–130		30° C
3	160	2.0	68– 75	100–120	110–160		30° C
2	165	3.0	85	112	104 and 165		30° C
2	105	1.2	60	105	105		25° C
4	200	0.1–0.5	40– 54	70–110	190–205		22° C
2	150	0.5	40– 50	90–100	150		22° C
2	150	1.7	50– 60	105–115	130–145		22° C
4	250	0.1–0.5	—	—	170–240	Nembutal[a]	30° C
2	200	0.1–0.5	—	—	0/2 at 200	Nembutal[a]	30° C
2	200	1.1	—	—	184 and 200	Nembutal[a]	30° C
2	300	0.1–0.5	—	—	2/2 at 300	Nembutal[a]	22° C
5	136	0.2	—	—	0/5 at 136	Nembutal[a]	22° C
2	214	1.2	—	—	190 and 214[b]	Nembutal[a]	22° C
15	136	0.5	—	—	3/15 at 136	N_2O[a]	30° C
5	136	0.5	—	—	5/5 at 136	Ar[a]	30° C
2	170	1.2	—	—	160–170	N_2[a]	30° C
2	206	2.0	—	—	120 and 206	N_2[a]	30° C

[a] Anesthetic doses given.
[b] Animals cooled only when above 120 atm.

sumed that the adverse symptoms are reduced by maintaining fairly low O_2 tensions (<0.5 atm) and by utilizing slow compression, although neither of these suppositions was confirmed by Brauer's experiments. However, the range over which these O_2 tensions and rates of compression were varied may not have been sufficiently large to produce significant effects.

The results of our investigations (Figs. 2–4; Table II), in which the compression rates were varied by a factor of 10, suggest that the benefits of a slower compression are at best marginal in experiments carried out at room temperature. A preliminary study of the effect of temperature on animals exposed to high pressures did not produce statistically significant results. There is some evidence, however, that heat loss from the body is accelerated by high He pressures, and shivering has been reported by Membery and Link (8) when the chamber temperature fell below 32°C. In contrast to their observations, we have noted that death occurred at marginally higher pressures in mice when they were treated in vessels maintained at room temperature than when they were kept in vessels at 30°C temperature.

Drugs and High Hydrostatic Pressures

As the answers to our first two questions indicate that direct effects of hydrostatic pressure may produce harmful symptoms in mammals, it is necessary to consider how far these symptoms may be alleviated by such means as drugs.

The most important early observation concerning the effects of drugs in modifying the response of organisms to hydrostatic pressure was that of Johnson and Flagler (5). They noted that applying pressure could restore the luminosity of luminous bacteria that had been exposed to an anesthetic agent; they then tried a similar experiment using tadpoles narcotized with alcohol. The effect of 2–5% alcohol was to narcotize the tadpoles so that they stopped swimming and fell to the bottom of the vessel. If a pressure of 130–300 atm was then applied, the animals resumed swimming. (The effect of pressure on unnarcotized animals was increased activity at 130 atm, followed by paralysis at about 300 atm.) Similar results occurred when animals were anesthetized with urethane; but pressure did not remove the effects of amyl carbamate.

In an attempt to see how far general anesthetics and other drugs can modify the effects of pressure on other animals, we initiated a study using newts and mice. In the case of newts, the results were clear-cut. Narcosis produced not only with a general anesthetic (halothane) but also with pentobarbital (Nembutal) could be instantaneously reversed by the application of hydrostatic pressures of between 100 and 200 atm. Furthermore, newts treated with pentobarbital remained active to much higher pressures than the untreated animals. In one case the righting reflex remained to over 300 atm (9990 FSW) although the animals exhibited abnormal muscular contractions at that pressure.

Experiments were performed on mice anesthetized with pentobarbital or N_2O. There was uncertainty about the results when pentobarbital was used, because the time course of the experiment approximated the time required for the control animals to recover from the anesthetic. At room temperature the righting reflex was restored between 70 and 100 atm after about 40 min. The three control animals recovered their righting reflex after 80, 80, and 60 min. We also noted the tremors that have been observed in untreated animals by other workers, but in this instance they appeared to start at lower pressures and to continue to higher pressures. However, the lethal pressures for mice treated with pentobarbital were raised. In two experiments in which the chamber was kept at room temperature, deaths of anesthetized animals occurred at 300 atm. At 30°C, death occurred in anesthetized mice between 160 and 240 atm, compared with 120 to 150 atm in unanesthetized animals (Fig. 2; Table II).

All these preliminary experiments were carried out with rapid compression rates and in small chambers. We have initiated a further series of experiments using a new high pressure chamber. The results obtained to date confirm our earlier observations. With compression times to maximum pressure as long as 4 and even 10 hr, death in animals that received no drugs occurred between 155 and 165 atm (5100 and 5450 FSW), while the lethal limit was raised to as high as 215 atm (7100 FSW) by the use of anesthetics (Figs. 3 and 4).

Outlook for Man

Diving below 600 FSW on O_2–He breathing mixtures has resulted in tremors in man. However, there is evidence that with slow compression rates and with strict control of O_2 tension,

these tremors can be considerably alleviated, if not entirely eliminated. Recent reports of 4-hr dives to 1150 FSW (P. B. Bennett, private communication) suggest that the safe depth limit for man has not yet been reached. The animal experiments reported here suggest that at somewhat greater pressures severe problems will be encountered. A superficial examination of these experiments further suggests that this "pressure barrier" for man may exist at depths as shallow as 1500 FSW to 2000 FSW, since mice can develop severe tremors at these pressures. However, as long as the factors that determine the severity of animals' response to high pressures have not been critically evaluated, no reliable estimate of this limit can be made.

It is possible, however, that the apparent antagonism between anesthetic agents and high hydrostatic pressures may be important. Thus the addition of some N_2 to O_2–He mixtures at high pressures may prove beneficial. More extensive experimentation with animals is necessary before the potential and safety of such practices can be fully evaluated. A more detailed investigation of the effects of varying O_2 tension, compression rates, and temperature, together with a study of the influence of drugs on the condition of animals at high pressure, is at present in progress.

ACKNOWLEDGMENT

This work was supported in part by the Office of Naval Research, Physiology Branch, U.S. Navy.

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PART III. PHYSICAL EFFECTS OF PRESSURE AND GASES*

DISCUSSION

R. M. Featherstone, Chairman

Chairman Featherstone: I will call first on Dr. Fenn.

Dr. Fenn: I have one point to add about the activities of Xe and its entrance into special places in myoglobin. I have been measuring the volume changes in hemoglobin which occur when O_2 goes into hemoglobin. I find that the volume does not increase at all, so the O_2 seems to find places within the hemoglobin molecule which are really vacuums in among the other molecules.

Dr. Zimmerman: I will comment on Dr. Landau's presentation and add some facts from our own laboratory that complement his story concerning the effects of high hydrostatic pressure.

First, hydrostatic pressures in the range of 3000–6000 psi, or from 200–400 atm, at short pulses, for 1 or 2 min, will cause definite structural alteration. This structural disorganization is difficult to see with the light microscope, but with the electron microscope effects on microtubule formation are seen. Microtubules become disorganized. There are structural effects in the nucleus. We can relate these structural effects in the electron microscope with physiological effects.

These physiological effects may not be seen immediately; but if we record the time that certain cells take to divide, we see that the division time is delayed. We can also relate these very short pulses of pressure, as Dr. Landau has shown, to the biochemical sequence of events. We know that DNA is reduced. We know that RNA synthesis is affected and, of course, the associated protein synthesis is affected. Recently, we have been studying the effects of pressure on messenger RNA, or the heterogeneous high molecular RNA (as we prefer to call it). We can show that, with very short pulses of pressure, we are indeed affecting the cell's ability to synthesize these messages.

One other comment is appropriate here, and that relates to the microtubules that we know are found in various nerve tissues. If pressure disorganizes microtubules, perhaps many of the effects that have been discussed and many of the effects of high pressure on the complete CNS may indeed be related to a disorganization of microtubules in human tissue. This is not necessarily true, but perhaps this might be a limitation to extensive deep dives in the future.

Chairman Featherstone: Dr. Doebbler, you might comment further about your high pressure work with inert gases.

Dr. Doebbler: Accumulating numbers of observations now indicate that there are relatively simple biological systems ranging down to well-characterized macromolecules in which biologically significant separate effects of pressure and gases have been defined. However, it must be recognized that at high pressure gases dissolve in the liquid or the solvent phase of the biological systems and have effects on the liquid and thus on biological functions.

Certainly one cannot ignore water; and in all biological systems the most important component is water. Physical chemists and structural chemists have no agreement yet as to what a liquid actually is, much less what water is. The final resolution of the importance of what pressure and the high concentrations of gases in water do to the structure of water will depend on more complete understanding of normal water structure. But certainly the structure of water is dependent on temperature and pressure and is going to be dependent on almost any solute that one dissolves in it.

* *Panelists:* J. V. Landau, E. B. Smith, W. O. Fenn, G. F. Doebbler, B. Chance, A. M. Zimmerman.

In our investigations of the effects of gases on enzyme reactions, the possible importance of the effect of gases on the structure of the solvent was more evident when, instead of looking only at partial pressures of gas, one looks at the concentration of gas in a tissue. On this basis we have examined six or seven different isolated enzyme reactions, all of which have been quantitatively inhibited by heavy inert gases at pressures well below 100 atm. The effect has been dependent upon the nature of the gas rather than on the particular pressure used. We therefore have come to the conclusion that for 20 or 30% inhibition of isolated enzymes, the ratio between the number of molecules of the inert gas (e.g., Xe) and the enzyme molecules whose catalytic reaction we are studying is something like 50,000 to 1.

This raises a very interesting question because, in any other type of inhibition in which 50,000 molecules of an inhibitor are present for each molecule of an enzyme being inhibited, one would certainly have to consider what other effects this large amount of inhibitor would have by mass action in addition to a direct effect on the molecule.

In addition, these gases may in a biologically significant way modify the properties of the solvent in which the enzyme is functioning. So we have to consider that, where we are dealing with gases at high pressures, we may have significant concentrations of gas molecules in isolated enzyme systems within biological cells. The gases could affect the physical chemistry of the water as well, having specific effects involving interaction of the molecules.

At the last underwater symposium three years ago, we hypothesized that perhaps interactions of inert gases, at least the heavy ones such as Kr and Xe, might actually undergo chemical interactions other than van der Waal interactions in macromolecules. From the relation between biological responses in microorganisms and the ionization potentials of these heavy gases, we hypothesized that perhaps charge transfer complexes could exist. At that time we mentioned that possibly some of the coenzymes, such as riboflavin containing coenzymes and oxidized NAD containing coenzymes, might be likely things to consider.

We have searched for charge transfer complexes using model compounds, not coenzymes, and have been unable to demonstrate that xenon forms these. However, Featherstone's group has provided encouragement to reconsider the occurrence of charge transfer complexes, and certainly the quantum chemistry of riboflavin coenzymes and oxidized NAD coenzymes indicates that these might be acceptors for charge transfer interactions with heavy gases.

These important molecular studies may be peripherally related to diving, but certainly they are very important to the field of biology.

Chairman Featherstone: I will add that our laboratory has reported to the effect that when studying the dielectric constants of aqueous hemoglobin systems, we were able to calculate that every time one Xe enters a space in hemoglobin, about 30 to 40 water molecules become irreversibly bound to the aggregate; that is, they lose their ability to flip back and forth every time the current changes.

I will now ask Dr. Smith for comments. I found his statements about the protective action of barbiturates very interesting. It seemed to me we were going in opposite directions in our thinking about the heavy gases and the barbiturates, which are organic molecules and perhaps exert their activities in quite a different way from the inert gases.

Dr. Smith: It is curious that we would normally regard barbiturates and the general anesthetics as separate classes of agents. The fact that they have the rather remarkable feature in common—that both are apparently antagonized by high hydrostatic pressure—might lead us to reconsider and wonder how different they are. This is a clue that they are more closely related in their mode of action than suspected.

Dr. Brauer: I can describe work by Dr. Schoffeniels that involved the use of an isolated frog skin preparation and the demonstration that at pressures from approximately 50 atm up there is a sharp increase in transmembrane potential and an increase in sodium flux across the outer layers of the frog skin.

Dr. Zimmerman: I was hoping somebody would bring that up. I have something to add which I am unable to interpret.

Recently, in conjunction with Dr. Myukami from Japan, we have been placing cells into a chamber and subjecting them to an electric field under hydrostatic pressure. We find that the cells migrate toward the cathode and this is called cathodal galvanotaxis. As we increase pressure, this effect is reduced. At 8000 psi the cells change from being cathodally galvanotactic to anodally galvanotactic.

We have been puzzled with this problem for some time. This occurs within 30 sec of the onset of pressure. We are using voltages in the relatively low range of 3–4 V. We are unable to explain this phenomenon.

Dr. Fenn: I would suggest that perhaps very high pressures increase ionization because that produces a decrease in volume. Some new ions may be formed which change the charge on the cell.

Dr. Landau: Dr. Zimmerman pointed out the effect of pressure on microtubules. We are relatively convinced that this effect demonstrates the labile gel structure or a major component of labile gel structure.

With regard to nerve, we have subjected chick dorsal nerve ganglion in culture to increased pressure and observed some of the axons growing out under these conditions. This was a morphological study, but on applying pressure we saw development of a beautiful collection of beads along the axon, which is an indication of a solation or the formation of a liquid cylinder which then beads along the axon length. On release of the pressure the axon was restored to approximately its previous appearance.

Dr. Orsi: Dr. Landau, the human amnion cells that recovered after the hydrostatic pressure—could they be passed in tissue culture? Did they have the same plating efficiency as controls did?

Dr. Landau: Sterile technique here was very difficult, but coming out of the pressure chamber we dropped to about three passages and have not seen anything. However, it would take a deeper study than we did to actually answer you.

Dr. Orsi: The reason I ask is that we are investigating the effect of hyperbaric pressure on virus infection and are using HEp-2 cells and Coxsackie B-1 virus. If we take control cell monolayers and subject them either to 100% O_2 at 3 atm for a half-hour or 97% He and 3% O_2 for a half-hour, the monolayers seem to be normal and look normal.

When we try to pass them to a new culture, the unexposed controls grow and form a monolayer, but not the cells that had been treated with hyperbaric pressure. There seems to be an effect lasting enough to be detrimental to cell passage.

Dr. Brauer: We have done a series of experiments in which the first onset of convulsions has been used as the endpoint instead of death. We find this rather sharp and reproducible.

If mixtures of He with graded amounts of N_2O or N_2 or H_2 are used, it is possible to construct identical curves for the elevation of the convulsion threshold due to the inert gas admixture. Once this has been done, equipotency curves can be constructed. A midpoint can be taken which indicates that the relative narcotic potencies of H_2, N_2, and N_2O derived from studies of convulsion threshold are substantially identical with those derived from loss of righting reflexes in mice.

I remain uncertain about the effect of rate of compression on convulsive threshold. If we go from a compression rate of 40 atm/hr for mice to a compression rate of about 3 or 5 atm/hr, there is about a 25% increase in the convulsion threshold. There is a fairly perceptible shift which also occurs in monkeys.

The third point I wanted to make is that what we used to call tremors needs to be reexamined very carefully. Data I will present later show that the early helium tremors are not associated with electroencephalographic changes. However, there comes a point where they are no longer volitional and are associated with very definite spiking activity in the electroencephalogram.

Finally, once a monkey has been carried to the point where rather widespread spiking has occurred, this does not subside. Even over a number of hours of exposure, the electroencephalogram of an apparently quiescent animal will show spiking going on continuously some place. In mice these effects appear to show strain and age dependence. The sensitivity increases enormously in young animals so that, in mature CD-1 mice, convulsions begin around 100 atm. Five-day-old mice of the same strain will convulse at about 55 to 60 atm.

Dr. Buckles: Dr. Featherstone, I am interested in myoglobin–oxygen affinities. You showed the curve describing effects of N_2 versus Xe. I assume the myoglobin was in solution. Have you ever compared N_2 with He? Have you ever done this with hemoglobin within red cells or with cells that contain myoglobin so that you might look at the effect of the bound nature of the molecule?

Dr. Featherstone: No, we have not yet. We have done these in solution and it is a CO_2–myoglobin relationship we study, not O_2–myoglobin. We have not found any indication of a shift in O_2 binding in the presence of these gases.

Dr. Buckles: Have you ever compared N_2 to some other gas of lower molecular weight?

Dr. Featherstone: No, we have not. These, of course, are studies that would be very interesting to do and I think are somewhat mandatory as we go further into trying to relate effects of heavy gases to the effects of light gases. I have been fascinated with the effects of removing N_2 and putting in either He or Xe—the two extremes of this gas series—and all the things that can happen. We find these "tremors" on the one side and anesthesia on the other.

Dr. Butts: Dr. Smith, when you administered anesthetic agents to animals and then elevated the pressures, did you give a dose of the anesthetic and then raise the pressure in the chamber by adding extra gas?

Dr. Smith: With the gaseous anesthetics these were added in initially, in the initial gas mixture, and then the He was put in afterward.

Dr. Butts: But no additional anesthetic was added as you increased the pressure?

Dr. Smith: No.

Dr. Butts: For the gaseous and volatile agents the degree of anesthesia is proportional to the partial pressure of the agent in the CNS. Could it be a possible mechanism of the phenomenon you saw that, as the pressure elevated with additional He, the relative partial pressure of the anesthetic agent (disregarding the barbiturates) would have decreased below the level of general anesthesia, but perhaps maintained a level which would have provided a sedative and thus a protective action against the gross toxic symptoms?

Dr. Smith: Certainly if you regard the effective concentration of an anesthetic in terms of classical theory as that which produces a certain critical concentration in, say, a lipid or cell membrane, then this solubility will be pressure-dependent. Your proposal would be a possible but not sufficient explanation of the pressure reversible effects, particularly where the volume changes can be characterized or can be guessed at with some accuracy, as in the case of the experiments with tadpoles of Johnson and Flagler.

Dr. Wells: I have a comment regarding the reversal of polarity described by Dr. Zimmerman for growing cells. Dr. Fenn has pointed out that this may be due to the ionization of some macromolecules within the cell. Another possibility is the change in pH due to the change in the ionization of the medium, particularly if phosphate buffer is used. In this example pressures of the magnitude described would cause a change of about half a pH unit and, depending on what the isoelectric values of the proteins are, this could have an influence on the charge.

Part IV. FUNDAMENTALS OF INERT GAS EXCHANGE AND BUBBLE FORMATION

CONCEPTS OF INERT GAS EXCHANGE IN TISSUES DURING DECOMPRESSION

B. A. Hills

Every mathematical format for predicting the occurrence of decompression sickness is based upon a model, either directly or indirectly, and it is essential that this model be consistent with sound physical principles and physiological experience. In devising any method of calculation from fundamentals, there are at least three basic questions to be answered before an equation can be written. They are:

1. What are the number of actual or theoretical tissues to be considered, since this determines the number of equations to be used?

2. Which parameter is the most pertinent in estimating the imminence of decompression sickness, and what is its critical value for the occurrence of symptoms?

3. In what manner does this parameter change with time and the other conditions of the dive? Since the critical parameter must be related to gas tensions, this question is largely one of determining the relevant transport model (or models) at the various stages of a dive.

The last question involves the controversy over which process limits the rate of inert gas transfer between blood and tissue. This limiting factor is generally attributed to blood perfusion (13). If, however, one assumes that tissues are not media of homogeneous permeability (and if one can resolve the mathematical confusion in the physiological literature between transient and steady state processes), the data would seem equally compatible with the hypothesis that a major portion of the resistance is contributed by diffusion (8). Although this issue is fundamental to physiology in general, the net difference in decompression formats predicted by these models is nonetheless surprisingly small for normal dives.

The uptake of inert gas by a tissue can be described by a single exponential if blood perfusion is the rate-limiting factor. Uptake closely approximates the sum of an infinite series of exponential terms required to describe bulk diffusion, although one term predominates in normal exposures. In many respects the Haldane method of calculation (1) and the many subsequent modifications of it represent a routine by which the slower half-time tissues can be empirically introduced to place greater emphasis on the slower values of the Fourier series, which represent bulk diffusion relative to deeper gas penetration of the tissues.

Although the perfusion–diffusion controversy is a most interesting academic problem, a

much greater deviation in decompression computations results from the value selected as the driving force in gas transfer. If one assumes that the rate of inert gas assimilation is proportional to the difference between the inspired partial pressure and the mean tissue tension of the gas, the process can be considered a simple exponential function. While this assumption is reasonable for gas uptake, it is reasonable for gas elimination only if all gas remains in true physical solution during decompression. Only in the latter case can gas contribute to a driving force for its transfer. Any separation of the gas phase from solution in the tissues changes both the boundary conditions and the driving force, so that a function must now be used that is totally different from the exponential (7).

Any evidence of the presence of a gas phase during a conventional decompression also has serious implications with respect to the second question listed above, since it is generally assumed that no gas separates from solution in tissues during dives that prove to be safe. Thus, before one writes any equation for a decompression program, it is essential to ascertain the conditions under which the gas phase forms, and whether these conditions existed at the onset of marginal symptoms of decompression sickness (as is generally assumed) (15, 20). The most important question in this connection is, therefore, whether the decompression ratios—which are often designated as R or M values and which are known to be valid in hyperbaric exposures (6)—really represent the critical degree of supersaturation or the critical volume of separated gas that a tissue can tolerate. That is to say: Is the gas phase ever present during a conventional decompression that proves to be safe?

Most investigators have studied this problem only indirectly by computing a number of schedules and testing them, but relatively few experiments have been specifically designed to settle the issue. One classic example of the latter is the work of Willmon and Behnke (19), who measured the washout of total body N_2 at different absolute pressures following the same change in inspired P_{N_2}. At the time, the results were not regarded as conclusive, since the elimination rate was found to be neither independent of the decompression (as would be predicted on the basis of the supersaturation concept) nor constant (as would be expected if the whole body had come to phase equilibration). However, the significant decrease in N_2 washout was consistent with the concept of random nucleation that is now known to apply to almost all cases of suppressed transformation, of which the supersaturation of a liquid by a gas is just one example.

Nucleation of the "suspended" phase has been shown to be a random process in which the probability of nucleation becomes finite after gas in solution exceeds equilibrium (16); the probability then increases progressively. Supersaturation theories of decompression sickness seem to be the only ones still based on the metastable limit concept that was originally introduced by Ostwald (a contemporary of Haldane).

Other experiments designed specifically to test the popular concept of limited tissue supersaturation include "titration" of a schedule based upon the conventional supersaturation concept, in which the last stop is made at 10, 20, or 30 FSW (9). The fact that 30 FSW proved to be more effective against decompression sickness than 20 FSW, and that 20 FSW was more so than 10 FSW, is totally incompatible with conventional reasoning. This observation indicates regions of phase equilibration. The regions may be very few relative to the number retaining their supersaturation, but, as the weakest link in the chain, these are the zones for which decompression must be programmed.

The foregoing method of differentiating between mechanisms has the advantage of using

the specific symptoms as evidence per se that the tissues in which they occur are the ones primarily affected. This cannot be said of techniques employing a direct physical search for the gas phase. The research technique currently in vogue involves an ultrasonic means of detection; the devices must be clamped to flaps of tissue, the most popular being the ear lobe (18) and the hind leg of a hamster (2). These techniques are probably quite successful in the detection of intravascular bubbles; but there is considerable doubt that the gas responsible for marginal symptoms forms within the vascular system (4), and it is even more unlikely that the gas would form in such vascular tissues as those mentioned above. Ultrasonics can also induce cavitation, although the intensities employed in the above studies were several orders of magnitude lower than the ones normally selected for this purpose.

Bubbles—considered per se now for the first time—have a curved geometric form, which is likely to resonate and to give an "echo enhancement factor" of as much as 10^3 (12). Thus it is much easier to detect bubbles than the films of gas predicted thermodynamically (7). These films might coalesce, with movement, into the irregular masses of gas that Harvey (5) observed deep in tissues prodded with a rod. Hence the failure of ultrasonic methods to detect bubbles in decompressions whose ratios are below those popularly advocated can in no way exclude the presence of extravascular gas separated from solution. This possibility is particularly strong in those tissues that are more likely than others to cause marginal symptoms—tissues that are probably less vascular than those that have to date been monitored ultrasonically.

Gas in the avascular tissues—the more significant ones in decompression sickness—appears in irregular geometric forms, and is much more likely to be detected as a change in conductance. Gas has a much higher electrical resistivity than tissue. The separation of any gas from solution should therefore cause an appreciable change in net transtissue resistance, particularly if the separation ocurs in planes perpendicular to the direction of the current flow. The purpose of the present experimentation, therefore, was to monitor avascular tissue in an effort to detect any change in conductance that would be indicative of bubble formation. The pressure for the onset of gas phase separation from solution can then be compared with values predicted by supersaturation and equilibration theories to determine which of these fundamentally different approaches is correct.

Methods and Procedures

The first problem is the selection of the tissue to monitor conductometrically. We do not know which tissue produces marginal symptoms of decompression sickness, but current data indicate that the tissues of the tendon are more likely than any others (10). A most convenient source of this tissue is found in the tails of rats, and we therefore chose rats for our experimentation. Rat tails have a loose skin beneath which thin platinum electrodes (½ in. × 3/16 in. × 0.010 in.) can be very easily slid into position on opposite sides after two small incisions have been made. Thus no clamp is necessary to hold the electrodes, so that any gas phase that is forming can grow and expand against the cohesive forces naturally provided by the skin. This is a particularly effective and simple preparation (Fig. 1) that can be completed within 30 sec.

Each rat (weighing 400 ± 10 gm) was exposed to a particular breathing mixture (air, $He–O_2$, or $N_2O–O_2$) for 6 hr, killed within 30 sec by chloroform, and placed in a box at 37.5°C. Its tail was cut off and mounted on a tray attached to the end plate of a small pressure chamber

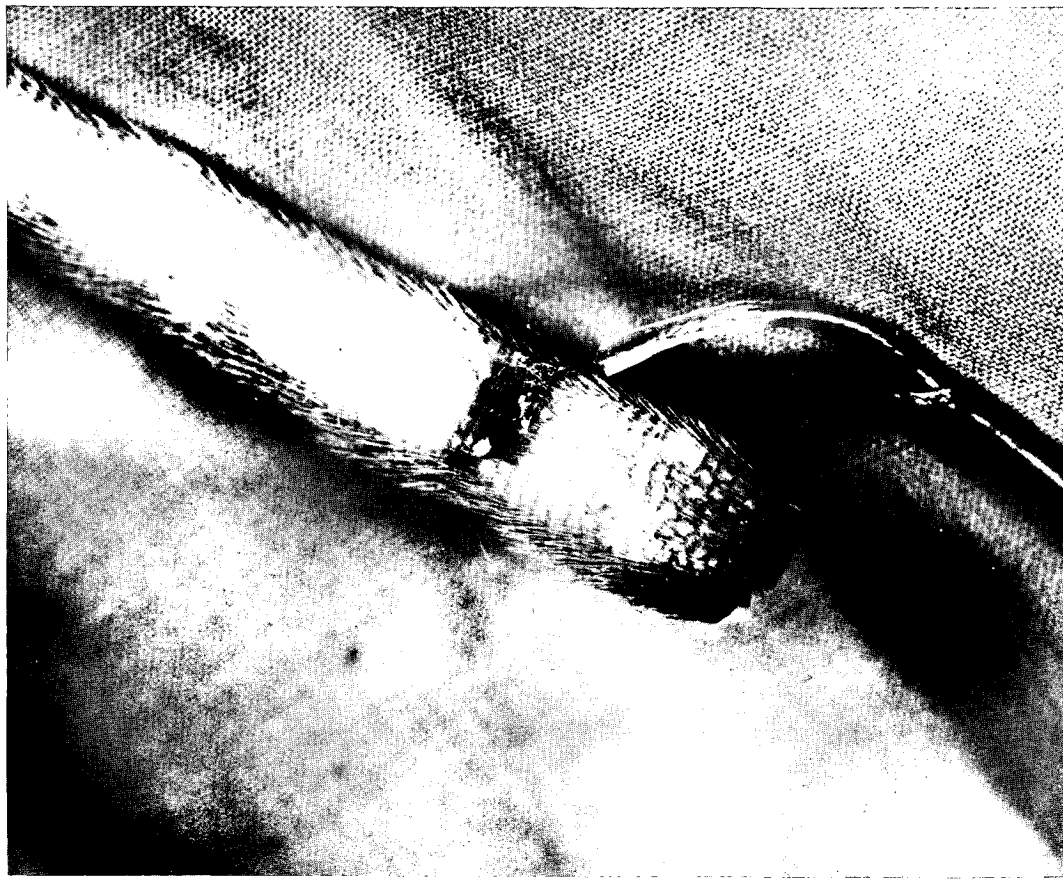

FIG. 1. A rat tail showing platinum electrodes inserted subcutaneously on opposite sides and in direct electrical contact with the tendonous fibers.

to which were attached electrode leads. After the electrodes were inserted, the whole preparation, still in the same warm box, was slid into the barrel of the plastic pressure chamber after which the chamber's end was sealed. The chamber was then transferred to a water bath held at 37.5 ± 1°C, and the coaxial electrical leads were connected to a conductance ac bridge with an accuracy of 0.01%. From the chloroforming to the time that conductivity readings could be taken required no more than 2 min. The electrical resistance was monitored for several minutes. The chamber was then decompressed from ambient pressure at the rate of 50.8 mm-Hg/min, readings being taken every minute.

Results

The foregoing procedure was repeated many times with the tails of rats that had been breathing air, and the plotting of electrical resistance vs decompression, or time, invariably produced

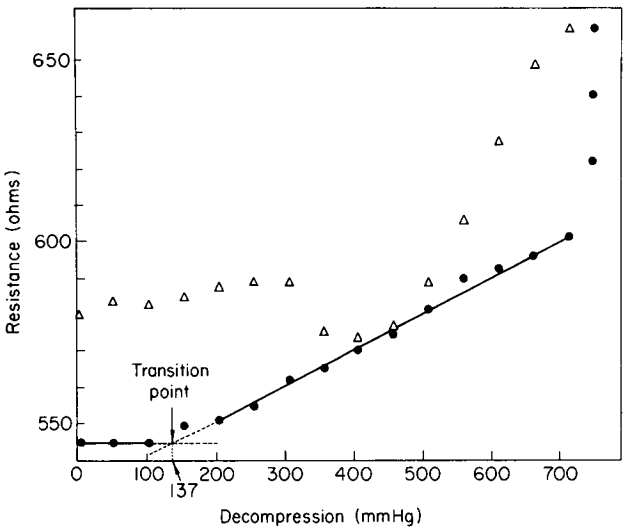

FIG. 2. The variation in electrical resistance across the tail of a rat upon decompression from normal atmospheric pressure at the rate of 50.8 mmHg/min, held at an absolute pressure of 15 mmHg for 2 min, and then recompressed at the same rate. (●) Decompression; (△) recompression.

a transition point—i.e., a sudden increase in resistance. No noticeable change in resistance was noted when the rat tail was momentarily pressurized to 500 mmHg positive before a run, or when the electrical leads were reversed (which indicates that gas was not introduced with insertion of the electrodes).

A typical run for the tail of an air-breathing rat is shown in Fig. 2. A transition point oc-

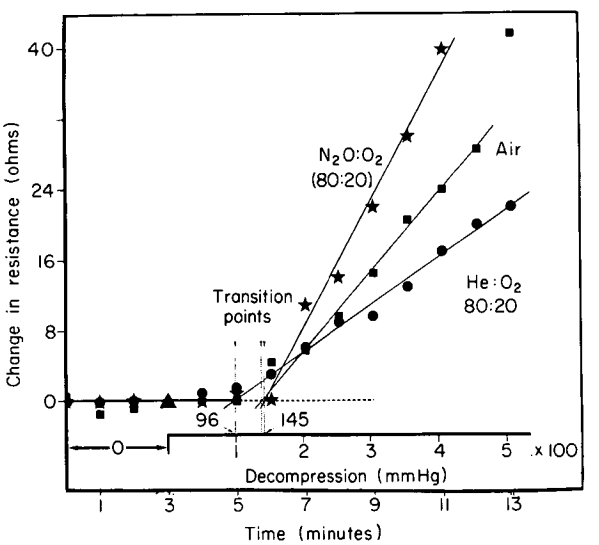

FIG. 3. The change in resistance across the tails of rats that had been breathing mixtures of 80% He–20% O_2, 80% N_2O–20% O_2, or air for 6 hr before death.

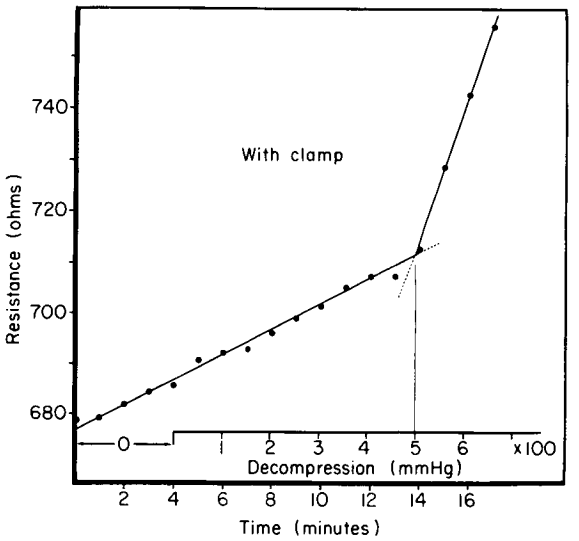

FIG. 4. The variation in resistance across the tail of an air-breathing rat when an insulated clamp was placed across the electrodes outside the skin. The variation was considerably different from that of a rat's tail on which no clamp had been placed (Fig. 2). In particular, the lower absolute pressure of the transition point was different.

curred when decompression reached 137 mmHg. This sudden increase in resistance indicated the onset of the gas phase at or below this stage of decompression, whereas the irregular decrease in resistance with recompression was consistent with simultaneous bubble movement and size reduction.

Repetition of the procedure with the tails of rats that had been breathing 80% He–20% O_2 and 80% N_2O–20% O_2 mixtures revealed similar transition points (Fig. 3).) The gradients increased in order of increasing solubility of the inert gas. This increase is consistent with the concept that the volume of a more soluble gas separating from solution in a tissue during a given decompression should be greater than that of a less soluble gas, and hence should develop a greater change in resistance.

Another run was performed upon the tail of an air-breathing rat in which an insulated clamp was placed across the electrodes outside the skin in much the same manner as is done in ultrasonic testing. This gave a totally different curve (Fig. 4), and the transition point occurred at a much lower pressure.

Discussion

These experiments have demonstrated that the magnitude of the decompression required to produce a phase change in the tissues of the rat tail is reproducible, varies with the gases in solution in the tissues, and suggests that the results are more relevant than those obtained by the ultrasonic techniques. The results displayed in Fig. 4 show that an artifact can be introduced by placing a clamp around the rat tail—a procedure that is necessary for most ultrasonic recordings.

In the other runs, however, in which the gas phase could grow and expand against the

natural cohesive forces of the skin, the transition points occurred at absolute pressures within the range of 610–670 mmHg for all three breathing mixtures. This indicates that nucleation of the gas phase can occur at absolute pressures significantly higher than those predicted by conventional decompression ratios, which are based on the maximum supersaturation that tissues are assumed to tolerate without risk of decompression sickness. In fact, the onset of cavitation would appear to occur in decompressions just a little greater than the natural or "inherent unsaturation" that has been measured in living tissue (11).

The variability among transition points of about 40–100 mmHg may be attributed to the fact that the resistance was measured in excised tissue that would have consumed all available O_2 at death. In any case, this O_2 consumption should have reduced the absolute pressure for nucleation of the gas phase, thereby bringing the transition points closer to the pressure thresholds predicted by conventional theories.

As they stand, the results indicate that the gas phase forms during decompressions to pressures which differ from those predicted on the basis of phase equilibration by no more than 50–100 mmHg, while they differ by 230–290 mmHg from those predicted on a supersaturation basis. This conclusion is consistent with the results of other methods used to test this vital issue: gas elimination (19), titration of decompressions with goats (9), and changes observed to occur in "soft"-tissue X-ray roentgenograms at absolute pressures as high as 483–512 mmHg (4).

This evidence indicates that the gas phase is present following decompressions involving far smaller pressure changes than any known to give rise to symptoms, and hence, during those decompressions based upon conventional supersaturation theories (17). These schedules could well be considered therapeutic treatment for a latent gas phase that does not become manifest as symptoms by virtue of residual compression at the staging pressure. However, the phase conditions are then totally different from those assumed in the model generally used to derive the simple exponential function; use of the latter, therefore, to describe gas elimination during decompression (15, 20) would no longer be valid. Use of latent gas phase data for calculation of decompression schedules requires a totally different mathematical approach (7), which has been shown to offer a better correlation of much diving data—including the results of trials programmed according to the conventional (supersaturation) theories.

From a more positive standpoint, gas should be eliminated more effectively from the tissues when the diver is kept much deeper than is advocated in conventional schedules, particularly at the start of his ascent to surface. This is borne out by the remarkably short overall decompression times used in very deep dives by Bühlmann (3) and the much shorter surfacing times in the schedules developed empirically by Okinawan pearl divers working off the northern coast of Australia (14).

An incidental implication of the foregoing experimental results is that cavitation should occur in air-breathing rats (Fig. 2) for decompression to an altitude of about 6000 ft, which is within the pressure range used in the cabins of commercial aircraft.

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DECOMPRESSION CHARACTERISTICS OF INERT GASES

M. J. Lever, W. D. M. Paton, and E. B. Smith

Nitrogen and helium have long been regarded from a practical and economic standpoint as the most suitable gases for use in diving. In recent efforts to improve diving performance and to elucidate the mechanisms of decompression sickness, there has been renewed interest in the properties of other gases. Research has primarily been concerned with three issues:

1. The gas-exchange technique. If, during the latter part of a dive or during decompression, the gas breathed by a diver is replaced by O_2 or by gases that are more slowly absorbed and eliminated by body tissues, the overall rate of desaturation may be accelerated, thus permitting more rapid decompression. The technique of following He dives with, first, air breathing and then O_2 breathing has been used successfully by the U.S. Navy (3). This technique has been tested critically by Keller and Bühlmann (14), who achieved significant reductions in decompression times by using Ar as well as N_2 and He.

2. The advantages of gas mixtures in decompression. If breathing mixtures are changed during diving the question arises whether two or more inert gases in the tissues can interact to modify the fundamental processes of bubble formation and growth after decompression. Webster (25) recognized that the sum of the partial pressures of inert and metabolic gases in the tissues was the major factor that determined the occurrence or absence of decompression sickness, but suggested that some advantages might accrue if mixtures of inert gases were used. This principle has been tested by various workers. Workman (26) found that the decompression times for men in a variety of dives could be at least halved when 50% He–50% N_2 was used as the inert gas diluent rather than He or N_2 alone. Similarly, Zal'tsman (27) claimed that there is a slightly higher no-stop decompression threshold for men when He–N_2 mixtures are breathed than when either gas is breathed alone. It has been shown, however, that when rats breathed He–N_2 mixtures, the incidence of decompression sickness was lower than when N_2 was breathed alone, but that the incidence was indistinguishable when they breathed He–N_2 mixtures or He alone (16). Bennett (4) found that when rats breathed He–Ne mixtures, the incidence of decompression sickness was slightly lower than when they breathed either component separately.

3. Decompression characteristics of single inert gases. Ever since Hildebrand (11) suggested that He might be preferable to N_2 in the prevention of decompression sickness, many attempts have been made to compare the properties of the two gases quantitatively. Argon and, to a

lesser extent, neon have also been studied. Such studies have recently been intensified in the search for a suitable gas medium for space-cabin atmospheres. Roth (22) has reviewed the factors that determine the efficacy of different gases in preventing decompression sickness. On theoretical grounds, he concluded that Ar is less satisfactory, and Ne more satisfactory than N_2, but that the relative efficacies of N_2 and He would depend on the specific mechanisms governing the appearance of symptoms.

In cases of decompression of small animals from raised pressures, it has been observed that He breathing results in a lower incidence of decompression signs than N_2 breathing does (4, 8, 16), while Ar breathing results in a higher incidence (4, 8). In experiments with Ne (4), the incidence fell between those for He and N_2. Similar results have been observed in men and goats that have been exposed to pressure for long periods. In dives longer than 4 hr, the threshold pressures for safe no-stop decompressions have been found to decrease in the order: He, N_2, and Ar (9, 10). For shorter exposures, the efficacy of He deteriorated relative to that of N_2; and in 16-min exposures, the threshold pressure with N_2 was greater than that with He. This crossover in potency was explained by the faster rate of He uptake by the body.

In contrast to the findings in experiments with high-pressure exposures, an experimental assessment of the relative efficiacies of He and N_2 in subatmospheric pressure changes revealed that the incidence of decompression sickness was lower when N_2 was breathed (1). Differences in results between altitude and high-pressure studies are not altogether surprising, however. Proportionately, CO_2, O_2, and water vapor will exert a much greater influence on bubble formation during decompression to altitude than during decompression from deep dives, since these gases represent a much greater fraction of the total gas tension (5). The main contribution of the inert gas component in such circumstances may be toward the growth and stabilization of seed bubbles, which for a given pressure change are dependent on the product of the diffusion coefficient and the concentration gradient between the tissues and the bubbles (7). In aqueous media, at least, He would be expected to diffuse into bubbles more rapidly than N_2, and this may be the determining factor in altitude decompression sickness. Because of the greater solubility and slower rate of elimination of N_2 compared with He, a far greater excess of N_2 will be retained in the tissues after saturation diving to a particular depth. This excess may be more important in the total incidence of decompression sickness than the faster growth of He bubbles.

The other chemically inert gas whose decompression characteristics have been recorded is N_2O (18). Because of the very great solubility of this gas, tissues can be expected to tolerate only small pressure changes. Decompressions from 3 to 1 atm have proved fatal to dogs breathing N_2O, whereas when dogs breathe air, a decompression ratio of 4:1 is considered safe (2).

Most studies of the comparative behavior of inert gases in decompression are fraught with inadequacies. Often the number of experiments is too few and the experimental design is too complex. These inadequacies are usually the result of a compromise between doing basic research into the behavior of gases and formulating an economically realistic and workable decompression schedule for practical diving purposes.

Inert Gases in the Elucidation of Decompression Sickness Mechanisms

The present study was undertaken to test a range of gases with widely differing physical properties to determine whether they displayed any regular pattern of characteristics during

TABLE I

RELEVANT PHYSICAL PROPERTIES OF VARIOUS INERT GASES AND O_2 IN DECOMPRESSION SICKNESS

Physical properties	N_2	He	Ar	N_2O	CF_4	SF_6	O_2
Solubility in water[a] α H_2O at 37	0.0125	0.0095	0.0295	0.45	0.0044	0.0047	0.024
Solubility in fat[a] α Olive oil at 37	0.069	0.017	0.159	1.82	0.072	0.295	0.12
Fat–water partition coefficient	5.52	1.79	5.39	4.05	16.3	62.8	5.0
Diffusion coefficient in H_2O at 25° C (cm^2 sec^{-1} $\times$ 10^5)	2.01	5.9	2.22	2.6	1.45	1.16	2.2

[a] Represents volume of gas at 37° C and 760 mmHg absorbed by 1 vol of solvent.

decompression procedures. If, for example, the solubility of gases in a particular tissue is the major factor in determining the appearance of decompression sickness, the order of potencies of various gases in causing the sickness should parallel their solubility values. The gases studied were N_2, He, Ar, N_2O, O_2, CF_4, and SF_6. The properties which are probably the most relevant in decompression mechanisms are diffusion and solubility in various solvents. The very wide range in these properties exhibited by these gases is shown in Table I.

Owing to their rather weak intermolecular forces relative to their size, SF_6 and CF_4 have extraordinarily low solubilities in aqueous media. Their solubilities in nonpolar solvents are less anomalous than those in polar solvents, and so their fat–water partition coefficients are much greater than those of the other gases tested. The diffusion coefficients of simple gases in liquids are an approximate function of molecular size (21). Thus in the same solvents, the diffusion coefficients of the smallest molecule He are approximately five times greater than the diffusion coefficients of the largest molecule SF_6. The diffusion coefficients of gases in tissues may be very much smaller than those in water (13), but the relative values for the different gases should remain the same.

The gases were all tested in experiments with mice. Numerous tests were performed so that a detailed statistical evaluation could be made of the properties of each gas during decompression. Unfortunately, mice do not exhibit some of the marginal symptoms of decompression sickness that are typically exhibited by men. Bubble formation and growth, however, are undoubtedly the primary cause of the sickness in both species, and the results of studies in mice should (with some reservations, according to anatomic and metabolic differences) be applicable to men. The usual signs observed in mice after decompression are: (*1*) gradual loss of muscle tone leading to prostration; (*2*) irregularities in breathing (usually there is slowing and deepening of respiration with occasional intermittent periods of tachypnea); (*3*) generalized convulsions which usually lead rapidly to death. Sometimes paralysis of the hind limbs intervenes, from which recovery is rare. Death was selected as being the most unsubjective endpoint in quantitative studies of the incidence of decompression sickness.

If a comparison of the physical properties of different gases and their quantitative decompression characteristics is to be a valid guide in elucidating the mechanisms of decompression sickness, it must be assumed that different gases bring about the sickness by the same mechanism. Qualitative differences have been observed in the diurnal signs produced by different gases, and these differences must be considered first to test the foregoing assumption.

Gross Decompression Signs Produced by Different Gases

After mice had been exposed to each gas at raised pressure, decompression caused convulsions and death after a variable induction period. These symptoms could be averted by recompression.

With most gases there were no signs of anoxia after decompression, but after decompressions from raised pressures of SF_6 the mice were rather cyanotic and sensitive to low P_{O_2} in the postdecompression breathing mixture. When mice breathed O_2 after decompressions from raised pressures of SF_6, the incidence of death was much lower than it was when air was breathed. The time of onset of adverse symptoms was also much longer with O_2 breathing than with air breathing. After N_2 decompressions, there was little difference in the incidence or time of onset in O_2 or air. Taken together, these results probably indicate that anoxia contributes to fatalities after SF_6 exposures, but to a much lesser extent after N_2 exposures.

Spinal paralysis was rarely observed after exposures to CF_4 or SF_6, but was seen in 5–10% of all cases after He, N_2, and Ar exposures. Since the mice were deeply anesthetized during exposure to N_2O at raised pressure, the early stages of decompression sickness were masked during their recovery from the anesthetic. However, convulsions and respiratory signs were a common occurrence. Owing to the convulsive effects of hyperbaric O_2, experiments were performed after treatment of the animals with a nonvolatile anesthetic. It was possible to expose tissues and blood vessels under these conditions, and the formation and decay of bubbles could be followed visually.

Bubble Formation and Distribution with Different Gases

Following the decompression of mice from raised pressures of the inert gases, dissection was carried out postmortem or under anesthesia, and all exposable surfaces were examined under a dissecting microscope. Bubbles with diameters of less than 50μ could be seen.

Extensive studies with N_2 showed that the extent and distribution of bubbles were dependent on the decompression ratio used and the severity of the sickness. In mice only slightly affected by sickness, bubbles were seen in subcutaneous fat and sometimes in the major lymph vessels that drain the abdominal skin. When respiratory distress and prostration had been observed, intravenous bubbles were invariably present, usually caudally, and most commonly in the veins draining into the inferior vena cava from the large pelvic fat bodies. In those mice which died, the vascular system was typically filled with bubbles, and the venae cavae and right side of the heart were often distended with gas. Extravascular bubbles in connective and adipose tissues were regularly present.

Other gases differed markedly in the extent and distribution of the bubbles observed after decompression. Helium decompression appeared to produce either very few bubbles or very extensive bubble formation in all blood vessels, fat, and viscera. Often bubble development had advanced sufficiently to cause hemorrhage.

Bubble formation in fatty areas was usually greater after Ar or CF_4 exposures than after N_2 decompressions. However, fewer bubbles were seen in the blood vessels after CF_4 decompressions.

The greatest differences in bubble formation were seen after SF_6 exposures, although in many respects, the external signs had been similar to those observed after exposures to other

gases and were reversible upon recompression. Often, no bubbles could be seen in animals which had exhibited signs of decompression sickness. When bubbles were seen, they were usually few in number and were often confined to the lymphatic system, although occasionally entered the blood vascular system through the thoracic duct. The results of recompression experiments, however, did suggest that small seed bubbles might be widely distributed after SF_6 decompressions. When N_2 or He was used to recompress the mice, the signs of sickness disappeared at rather low pressures (20–25 psig). These pressures are much lower than those which are normally required to cause sickness in mice upon decompression to 1 atm. However, subsequent decompression from these therapeutic pressure levels caused severe signs of sickness and very extensive bubble formation. Later experiments with mixtures of SF_6 and N_2 indicated that these signs were unlikely to follow a single decompression even though the tissue gas tensions were similar to those that were expected in the recompression experiments. It is concluded that the extensive bubbles observed after therapeutic recompression must have arisen by the diffusion of the gas used in recompression into seed bubbles that were formed after the first decompression. Although these seed bubbles may have contributed to the signs observed after the initial SF_6 decompressions, the major factor was more likely ventilatory impairment due to the white foam, containing lung surfactant, that is usually observed in the bronchi and sometimes in the trachea after decompression. The presence of this foam may explain the sensitivity of mice to hypoxia after SF_6 decompressions.

Quantitative Differences between Gases

The experiments described above show that the decompression sickness produced by the several gases exhibited marked qualitative differences. Since bubble formation is undoubtedly the major contributor to the external signs, however, a quantitative comparison of the gases is pertinent in investigations of the mechanisms involved.

Rate of Saturation

With regard to decompression sickness in mice, it is necessary first to consider the rates at which different gases are taken up by critical bubble-forming tissues. These rates have been determined by following changes in the incidence of decompression sickness as a function of exposure time at a particular pressure which was selected for each gas. The results indicated that the rate of gas uptake increases in the order: SF_6, CF_4, N_2, Ar, and He. This order is consistent with the perfusion-limited gas uptake model (15). Very large fat–water partition coefficients could be the cause of very slow uptake of SF_6 and CF_4 by the fatty regions of the body by the perfusion-limited mechanism alone, and some intertissue diffusion (19) would therefore be expected to contribute to the overall uptake rate. A model based on diffusion-limited uptake would predict the same order for the uptake rates of the different gases, and the present study does not provide a clear distinction between the conflicting diffusion and perfusion theories.

Time to Onset of Symptoms

The animals were kept in the pressure chambers after decompression, and the times of onset of the first major signs of decompression sickness were recorded. When plotted as histograms

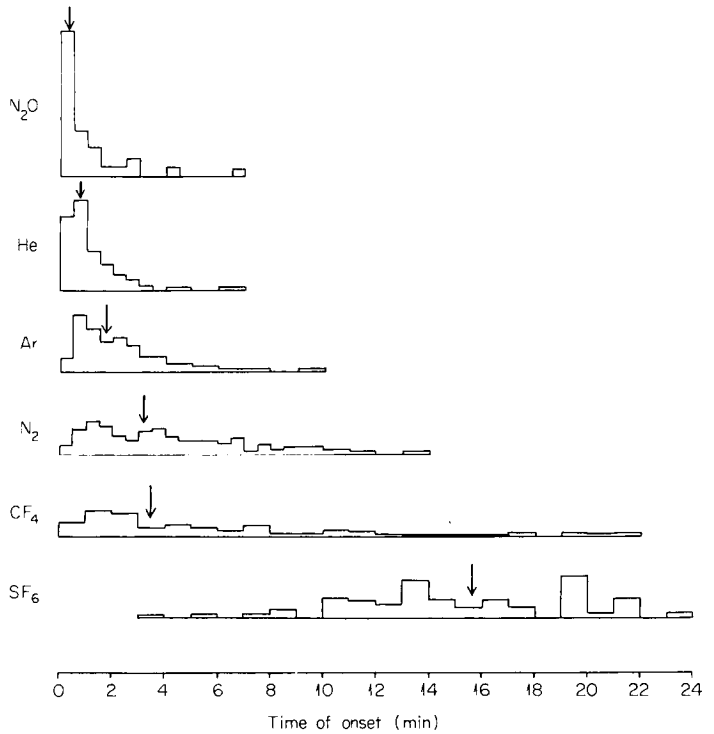

FIG. 1. Histograms showing the times of onset of major decompression signs after exposure of mice to raised pressures of N_2O, He, Ar, N_2, CF_4, and SF_6. (↓) shows median time.

for each gas, the times of onset were found to follow a roughly log-normal distribution (Fig. 1).

For all gases, signs of decompression sickness appeared earlier in those experiments in which the incidence was high. The induction periods seen in the histograms are dependent on the rates of formation and growth of bubbles in the body. The position of the median is determined by the relative rates of the opposing processes of bubble development and inert gas elimination by the body. Elimination rates are the main factors in determining the length of the "tail" of the distribution curve of times of onset, and the times at which 90% of the signs had appeared closely reflect the uptake times. The very long time which elapsed between SF_6 decompressions and the appearance of signs may have been the result not only of the slow rates both of bubble development and inert gas elimination, but also of the development of hypoxia. If the mice were transferred from an O_2 to an air atmosphere after SF_6 decompression, convulsions and death ensued very rapidly.

DECOMPRESSION RATIOS WITH DIFFERENT GASES

Gases differ in the critical decompression ratio that will elicit adverse signs, and the main object of these experiments was to determine whether the measured ratio obtained for each gas would correlate with any particular physical property. To eliminate one variable—the rate of gas uptake by the body—near-saturation exposures of 90 min were used with each gas,

and mice were exposed to a range of pressures before rapid decompression in 15 sec to 1 atm abs. By measuring the incidence of signs produced by each decompression, the decompression ratio (the ED_{50}) required to cause signs in 50% of the animals was determined.

If we now assume that a single tissue or group of tissues is the primary site in the body for the formation of bubbles that lead to signs of decompression sickness, the absence or appearance of these signs after decompression will depend partly on the relative rates of bubble formation and gas elimination, but primarily on the total quantity of gas held in supersaturated solution in that tissue. When rapid decompressions follow saturation exposures, the total quantity of gas in a tissue will be the product of the exposure pressure (P) and the solubility (α)—i.e., $P\alpha$. When the ambient hydrostatic pressure is 1 atm, the quantity of gas that can be retained in the tissue is α, and the level of supersaturation, expressed as a volume of gas, will be $(P-1)\alpha$. If it is assumed that decompression sickness will ensue when $(P-1)\alpha$ exceeds a certain value, then a plot of log α against log $[1/(P-1)]$ for different gases should be linear with unit slope. For each gas, P is the critical decompression ratio (ED_{50}) which was measured in the experiments described above. The nature of the critical tissue is unknown, and so its solvent properties, on which the value of α will depend, cannot be defined. However, Figs. 2 and 3 show graphs of log $[1/(P-1)]$ against log α for two extreme types of tissue—one entirely aqueous, the other entirely fatty. When SF_6 and CF_4 are included with the other gases, the correlation fails in aqueous tissue (23). Despite their very low solubility in water (the

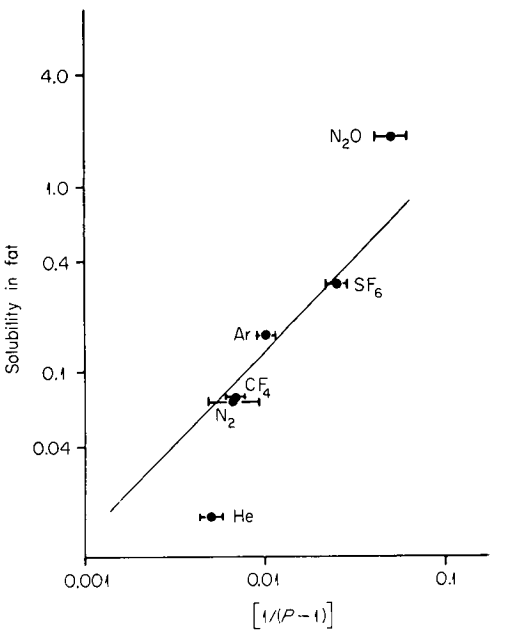

FIG. 2. Correlation of potency of gases causing decompression sickness with their solubility in fat. After long exposures to a gas at a pressure (P) from which decompression to 1 atm abs causes symptoms in 50% of a population of animals, the total quantity of gas in fat is $(P-1)\alpha$ in which α is the solubility of the gas in fat. If this represents a critical quantity of gas necessary for the onset of symptoms, then a graph of log α against log $[1/(P-1)]$ should be linear and of unit slope.

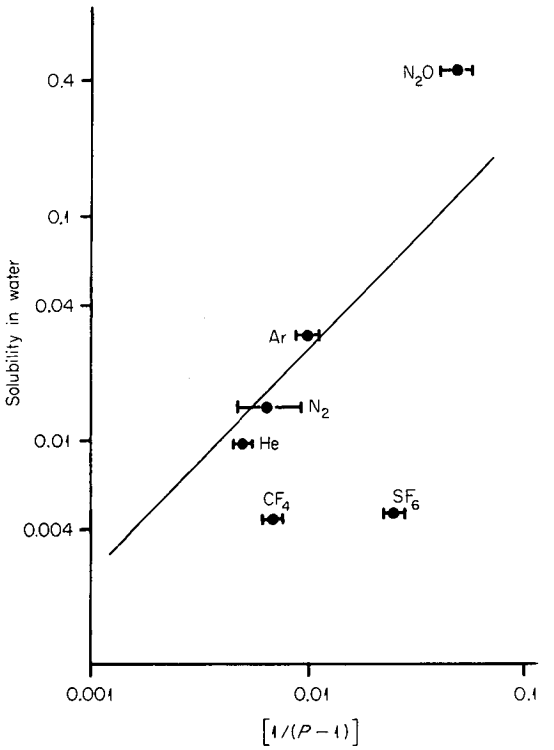

FIG. 3. Correlation of potency of gases causing decompression sickness with their solubility in water. After long exposures to a gas at a pressure (P) from which decompression to 1 atm abs causes symptoms in 50% of a population of animals, the total quantity of gas in water is $(P-1)\alpha$ in which α is the solubility of the gas in water. If this represents a critical quantity of gas necessary for the onset of symptoms, then a graph of log α against log $[1/(P-1)]$ should be linear and of unit slope.

major constituent of the body), SF_6 and CF_4 rather easily caused decompression sickness. The degree of solubility of gases in fat, however, can be used as the basis for predicting the correct order of their potencies. This does not necessarily imply that bubbles in fat are always the direct cause of signs of decompression sickness; the fatty regions may act as a reservoir of gas, which can contribute to bubble development in neighboring blood or lymph vessels, for example.

The importance of fat in bubble formation is also supported by other observations:

1. Bubbles are often seen in fat and in veins draining fatty regions in animals that have escaped major signs of decompression sickness. These bubbles often can be observed long after decompression.

2. After long exposures, it has been found necessary to assume very long tissue half-times when the Haldane approach is used to construct decompression tables. Such long half-times are typical of fatty tissues. Lundin (17) suggested that the effect of varying preoxygenation times on altitude decompression sickness was consistent with gas elimination from fatty tissues.

3. In a perfusion-limited model for gas uptake, the rates of saturation of aqueous tissues are similar for most gases. In contrast, marked differences are expected in the rates of uptake of different gases by fatty tissues. Such differences were apparent from the various exposure times required to cause maximum incidence of decompression sickness after exposures to different gases.

4. It has long been recognized that fatter animals are more prone than thin ones to decompression sickness (24). Philp (20) has concluded that body fat is by far the most important feature of body composition in determining an individual's susceptibility to decompression sickness.

Despite the apparent importance of fat solubility when it is linked with the concept of a critical supersaturated volume of gas, the behavior of inert gases in decompression cannot be predicted quantitatively. The behavior of He and N_2O in particular fails to comply with this model.

Because of its very small molecular size, He has a much lower fat–water partition coefficient than the other inert gases (12). A greater proportion of the total gas content in the body is therefore dissolved in aqueous tissues, and fat may therefore play a less important role in the mechanisms causing decompression sickness with He. However, since aqueous solubility is less than fat solubility, a higher critical decompression ratio would be predicted in order to maintain the volume of gas in supersaturated solution at a critical level. However, the behavior of He deviated from that which would be expected from the fat solubility model because the measured critical decompression ratio was too low. Although N_2 is more soluble than He in water, He has been shown to form bubbles upon decompression of aqueous solutions more readily than N_2 (6). If solutions in blood behave similarly, the ED_{50} for He would consequently be lower than that expected from the fat solubility model.

The rate of bubble growth is roughly proportional to the product of the concentration of gas and the diffusion coefficient (7). The large diffusion coefficient of He causes a high initial rate of bubble growth after decompression, and although He is normally eliminated rapidly from the body, the process may be inhibited by the rapidly expanding bubbles. This hypothesis is supported by the observations of the early onset of symptoms after decompression, and the enormous extent of He bubbles in affected mice. If bubble development does not start soon after He decompressions, the rapid rate of gas elimination prevents the onset of signs and few bubbles will be found.

Although the concept of a critical volume of supersaturated gas has been assumed, it is almost certainly inapplicable in the case of N_2O. Mice can tolerate much larger volumes of this gas than other less soluble gases injected intravenously. Therefore, greater volumes of liberated gas are probably needed to produce signs of decompression sickness, and decompression ratios higher than those predicted must be employed to cause adverse signs.

The Behavior of Gases in Slow Decompressions

To investigate the rate processes involved in the mechanisms controlling the appearance of decompression sickness, slow linear decompressions (1, 5, and 10 min) have been studied in addition to rapid decompressions. The rapid rate of He elimination was reflected in a marked change in the ED_{50} for slower decompressions. In contrast, although SF_6 and CF_4 were eliminated from the body more slowly than N_2 or Ar, there was not much difference between

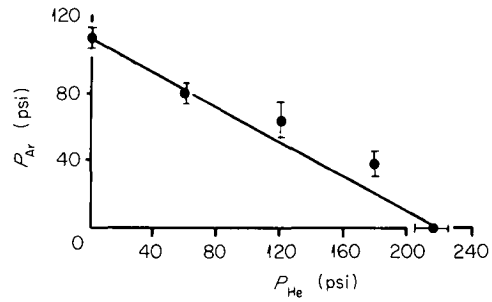

FIG. 4. Partial pressures of Ar and He in mixtures breathed at increased pressures from which decompression to 1 atm abs caused symptoms of decompression sickness in 50% of the experimental animals.

the gases in the observed changes in the ED_{50} of each gas as decompression times increased. Because of the low solubilities and moderately low diffusion coefficients of SF_6 and CF_4, bubbles would be expected to grow very slowly in solutions of these gases. During slow decompressions, therefore, large quantities of these gases may be eliminated before bubble development has time to reach critical levels. With N_2 and Ar, bubble development proceeds more briskly; and since elimination of these two gases does not proceed as rapidly during decompression as it does with He, bubble development may predominate and cause the onset of sickness. With N_2 and Ar, the first signs of decompression sickness appear sooner after slow decompressions than after fast decompressions and occasionally they appear during the last stages of decompression, indicating that in some animals at least, bubble growth is under way before the final pressure level is reached.

The Behavior of Gas Mixtures in Decompression Sickness

In view of the qualitative differences in signs of decompression sickness produced by the individual gases studied, and the deviation of N_2O and He from a gas solubility model, mixtures of N_2 and SF_6, N_2 and N_2O, and Ar and He were investigated to test the additivity of the contributions of each component to the appearance of decompression sickness. Charac-

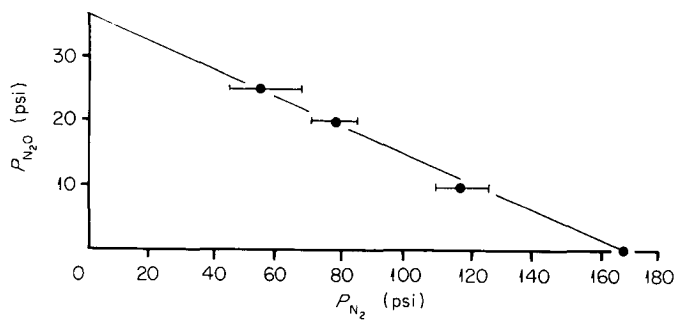

FIG. 5. Partial pressures of N_2O and N_2 in mixtures breathed at increased pressures from which decompression to 1 atm abs caused symptoms of decompression sickness in 50% of the experimental animals.

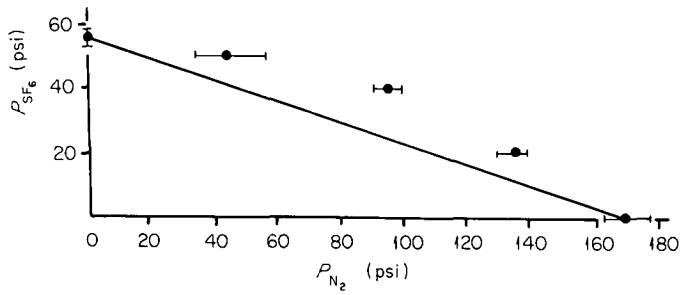

FIG. 6. Partial pressures of SF_6 and N_2 in mixtures breathed at increased pressures from which decompression to 1 atm abs caused symptoms of decompression sickness in 50% of the experimental animals.

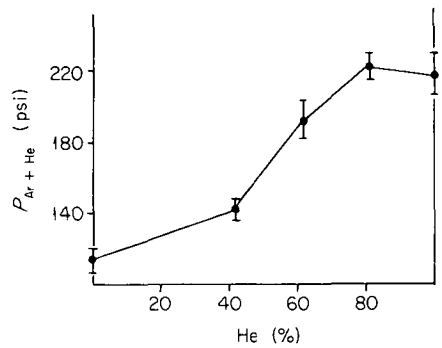

FIG. 7. Total pressure of Ar and He mixtures from which decompression to 1 atm abs caused symptoms of decompression sickness in 50% of the experimental animals.

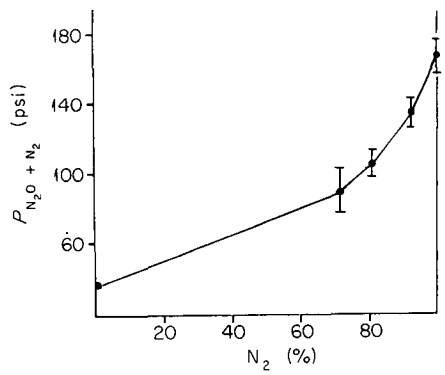

FIG. 8. Total pressure of N_2O and N_2 mixtures from which decompression to 1 atm abs caused symptoms of decompression sickness in 50% of the experimental animals.

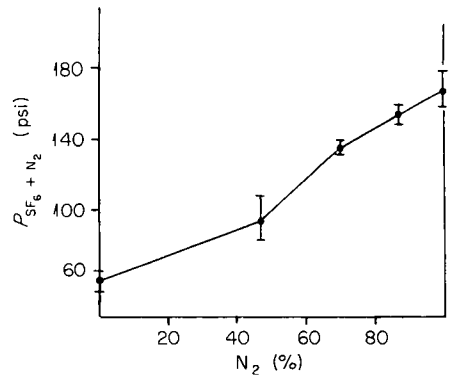

FIG. 9. Total pressure of SF_6 and N_2 mixtures from which decompression to 1 atm abs caused symptoms of decompression sickness in 50% of the experimental animals.

teristics such as the profusion and distribution of bubbles and the time of onset of signs in decompression experiments with gas mixtures were found to be intermediate between those characteristics observed when each component had been used alone and were dependent upon the ratio of the gases in the mixture. If different gases and gas mixtures bring about the signs of decompression sickness through the same mechanisms, then it would be expected that the quantitative characteristics which describe the behavior of different gases would be additive. If the contribution of a gas at partial pressure P_i is P_i/P, where P is the ED_{50} for the gas used alone, a graph of the partial pressure of one gas component against the partial pressure of the second gas component in mixtures at a critical pressure (ED_{50} mixture) should be linear and pass through the critical decompression ratios (ED_{50}) of the pure components. Figures 4–6 show that deviations from additivity occur with mixtures of Ar plus He and with SF_6 plus N_2, but not with N_2O plus N_2. Figures 7–9 show the overall critical decompression ratios as a function of the percentage composition of the mixtures. Of those used, only Ar plus He might offer any practical advantages.

Conclusions

By a critical assessment of the relative decompression characteristics of several inert gases, the following conclusions have been reached.

1. All the gases tested produced similar adverse decompression signs in mice, including respiratory abnormalities and convulsions, which could be mitigated by recompression. But the various gases exhibited qualitative and semiquantitative differences in (*a*) the characteristics of the sickness, especially with respect to bubble profusion and distribution; (*b*) sensitivity to hypoxia; and (*c*) the time of onset of symptoms. The differences are most marked when SF_6 is breathed.

2. The most important single factor governing the appearance of decompression sickness seems to be the excess of gas in solution in fatty tissue. Consequently, the critical decompression ratio for a particular gas depends upon its fat solubility (Fig. 10).

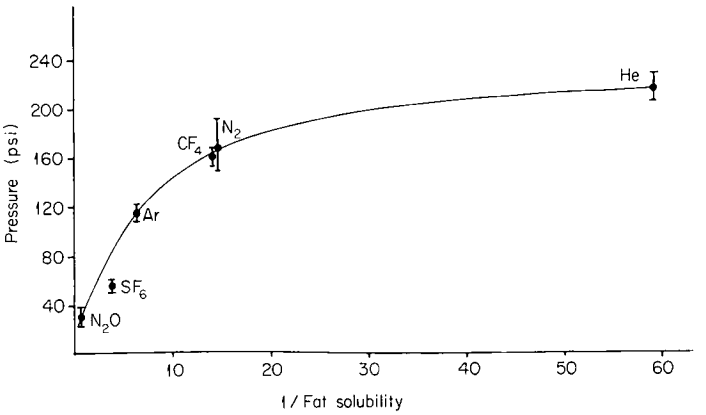

FIG. 10. The relationship for different gases between fat solubility and the pressure from which decompression to 1 atm abs caused symptoms of decompression sickness in 50% of the experimental animals.

3. Experiments with mice indicate that the failure of a simple fat solubility model to predict quantitatively the potency of gases in causing decompression sickness may depend in part on the tolerance of the animals to the bubbles of the different gases, and in part on the relative rates of gas elimination from the tissues and rates of bubble development which are characteristic of the different gases.

ACKNOWLEDGMENT

This work was supported in part by the Office of Naval Research, Physiology Branch, U.S. Navy.

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CRITERIA FOR BUBBLE GROWTH

Ruport Hester

Diving medicine has been concerned primarily with a single criterion for bubble formation and growth, the onset of symptoms associated with the decompression syndrome. This paper will examine a possible criterion for so-called "silent bubble" growth in decompression from helium saturation diving.

The theoretical foundation of standard diving practices is the principle of inert gas exchange associated with the name of J.B.S. Haldane (4), based upon the premises that, in the absence of symptoms, all gas in the blood and tissues can be considered as remaining in solution and that supersaturated states of gas solution can exist. In many respects, however, the supersaturation theory can be considered only a convenient doctrine. Some years ago Behnke (2) stated: "It may well be that bubbles form as soon as a state of supersaturation is initiated and that what appears to be a ratio of supersaturation tolerance is in reality an index of the degree of embolism that the body can tolerate." This question is still open.

Silent Bubble Theories

Bateman (1), who apparently coined the term "*silent bubble,*" emphasized "that the most fundamental gap in our knowledge of decompression sickness is still the exact nature of the relationship between effective desaturation rates and the production of symptoms. . .." He further suggested that the most promising experimental approach to the problem would be to study individuals who are fully equilibrated with N_2 at various known partial pressures. Nims (9) based his formulation on findings by Inman and Saunders (8) that symptoms perfectly analogous to decompression sickness could be induced by the injection of isotonic buffered Ringer's solution into deep tissues. The onset and severity of symptoms were related to the pressure of the fluid as well as to the kind of tissue involved. Nims assumed a simple mechanical model for decompression sickness, and theorized that pain is related to tissue deformation or displacement (d) caused by the volume of separated gas. Hills, in his recent and impressive analysis of the silent bubble issue (7), has advanced the provocative hypothesis that extravascular phase equilibrium rather than tissue supersaturation with gas is the relevant condition in decompression sickness.

Relevant Data

The response of the whole organism to reduced pressure is a complex psychophysiological event that is perhaps several steps removed from the relevant biophysical process. Thus, to use an expression of Workman (10), definition is relatively "gross." In the present state of knowledge, however, direct observation of the physical and physiological mechanisms appears to be precluded, mainly because the most common symptom, bends, is restricted to a relatively small amount of semirigid tissue in the neighborhood of the joints and involves only a minute fraction of the total inert gas exchange by the body. Evaluation of gas concentration in specific tissues is especially difficult because the radioisotopes both of He and N_2 have extremely short half-lives (10). What is of statistical importance, therefore, are the specific conditions under which an attack of bends occurs.

Theoretical Issue

The choice between the classical doctrine of supersaturation and the silent bubble hypothesis is not difficult. Although the tensile strength of an aqueous solution appears to be rather high, the argument against a stable zone of supersaturation in a physiological system is convincing (7). This view is consistent with observations of Harvey (6) concerning injured or stretched tissue in Nembutal-anesthetized cats and of cat tissue manipulated mechanically after decompression. Also, it is in keeping with observations by Blinks *et al.* (3) concerning tissue of bullfrogs following violent activity induced by electrical shock.

Some workers do not, in fact, preclude the possibility of nucleation at moderate levels of excess inert gas tension in their computations of decompression tables. Workman (10), for example, has suggested that the probability of nucleation is a function of time as well as of the degree of supersaturation. Thus a higher degree of supersaturation can be risked for a short period than for a long period of time. Apparently a formal model for nucleation based upon the hypothesis of random negative mechanical pressures as an energy source has not been used. However, in principle it should not be difficult to develop one. In any event, finding a practical difference between a silent bubble hypothesis and a stochastic concept of supersaturation is not easy. In many respects, both suggest the same conclusion: in decompressions sustained over a long period, gas tension excess should be held at a low level.

If bubbles are formed in critical tissues but do not induce symptoms, the only practical consequence would presumably be the retardation of inert gas transport, and the consequent asymmetry between uptake and elimination. If, as Hills (7) has suggested, critical nucleation is in an extravascular diffusion field, the mechanism would be loss of effective tissue tension by equilization with bubbles.

If one assumes that a single bubble is formed in a tissue mass of volume, V, and that q denotes the concentration of gas in the mass prior to phase separation, and that q' denotes the concentration following phase equilibration, the quantity Q of inert gas thus separated (expressed at body temperature and standard pressure) is given by

$$Q = V(q - q') \tag{1}$$

The partial pressure p' of inert gas inside the bubble may be expressed:

$$p' = H + Z \text{ (absolute pressure units)} \tag{2}$$

in which

$$Z = P_\sigma + \delta - p_{O_2} - p_{CO_2} - p_{H_2O} \tag{3}$$

where

H = hydrostatic pressure
P_σ = surface tension
δ = tissue deformation stress (or resistance)
$p_{O_2}, p_{CO_2}, p_{H_2O}$ = partial pressures inside the bubble

Water vapor, O_2, and CO_2 pressures are assumed to stay in approximate equilibrium with surrounding tissue tensions. The bubble volume, v, may be obtained by applying Boyle's law to the partial pressures of the inert gas:

$$p'v = H_0Q \tag{4}$$

where (H_0 = 33 FSWG) is standard pressure.

Now, assuming that p denotes tissue tension in the inert gas prior to phase separation and that α denotes the absorption coefficient for the inert gas in tissue fluids, if $q = \alpha p$ and $q' = \alpha p'$, we may thus rearrange Eqs. (1) and (4) as

$$p = (1 + w)p' \tag{5}$$

in which

$$w = v/\alpha H_0 V \tag{6}$$

In this notation w corresponds to the expression (F/SP_0) by Hills (7).

Now let us assume a saturation dive with some inert gas. Let us further assume that an initial ascent is made to some depth D_1 followed by a linear rate of ascent to the surface R in feet per minute. We have $H_1 = D_1 + H_0 = p_1 - \Delta p$, H_0 = 33 ft, wherein p_1 is tissue tension at saturation. A simple Haldane-type inert gas transport function will be assumed:

$$dp/dt = kF \tag{7}$$

in which

p = tissue tension in inert gas
t = time
k = decay constant
F = driving force (pressure)

Suppose that in the initial ascent from bottom to depth D a bubble of volume, v, is formed and that a subsequent linear ascent rate, R, is such that v remains constant; hence, by Eq. (6), w remains constant, $D_1 \geq D \geq 0$. Ascent time t, from D to surface is defined in the relationship

$$p_1' - p_0' = Rt \tag{8}$$

in which

p_1' = residual tissue tension at D_1 following equilibration
p_0' = residual tissue tension at surface assuming equilibration

Strictly speaking, a linear rate R that satisfies the conditions for the last expression exists only when a constant relationship between the ambient P_{O_2} and the sum of tissue $p_{O_2} + p_{CO_2}$ obtains. Later it will be assumed that a constant P_{O_2} is a sufficient condition.

Substituting by $p_0 = (1 + w)p_0'$ and $p_1 = (1 + w)p_1'$ in the last expression and multiplying by R^{-1} yields

$$t = (p_1' - p_0')R^{-1} = (p_1 - p_0)R^{-1}/(1 + w) \tag{9}$$

Now

$$\begin{aligned} R &= dp'/dt = d(p(1 + w))/dt \\ &= (1 + w)kF \text{ ft/min} \end{aligned} \tag{10}$$

wherein the driving force for inert gas elimination, F, is given by

$$F = p' - P \tag{11}$$

in which

p' = tissue tension following phase separation
P = ambient partial pressure of inert gas

Assuming a constant P_{O_2} throughout decompression, $P = H - P_{O_2}$, wherein H is the hydrostatic pressure. It follows that the rate of linear ascent in minutes per foot is

$$\begin{aligned} R^{-1} &= (1 + w)/kF \\ &= (1 + w)/k(p' - P) \\ &= (1 + w)/k(Z + P_{O_2}) \text{ min/ft} \end{aligned} \tag{12}$$

Substitution of the last expression in Eq. (9) yields

$$t = (p_1 - p_0)/k(Z + P) \tag{13}$$

in which t is total decompression time. Thus, if nucleation occurs in the initial ascent from bottom to $H_1 = p_1 - \Delta p$, it can be seen from Eqs. (3) and (13) that total decompression time, t, will be least when Z is kept as large as possible, thereby keeping the bubble as small as possible.

The above result was obtained by assuming nucleation in an extravascular space. Analogous relationships may be obtained by postulating vascular cavitation in a critical tissue zone, although the mechanism would be different. The driving force, F, for inert gas transport from extravascular tissue to the blood would not be reduced; but occlusion of blood vessels by gas bubbles would decrease the perfusion of tissues by blood, thus depressing the effective value of the decay constant, k. For vascular emboli in a given zone of critical tissue,

$$k' = (q_\beta'/q_\beta)k \tag{14}$$

in which

k = decay constant without phase separation
k' = decay constant with phase separation and a given vascular distribution of emboli

q_β = blood flow per unit volume of tissue per unit time without phase separation
q_β' = blood flow per unit volume of tissue per unit time with phase separation and a given distribution of emboli

Vascular nucleation without occlusion, of course, would not imply Eq. (14).

Further details do not appear necessary. It should be evident that either extravascular or intravascular cavitation in tissues that are critical in saturation diving could slow down the permissible rate of linear ascent by increasing the effective half-time of the tissue.

Zurich Group Data

As a rule, the maximum half-time for saturation diving is estimated according to the requirements of the decompression format. A series of He saturation dives reported by Bühlmann *et al.* (5) provides a rare estimate of the rate of He uptake by slow tissues. A series of 19 dives to 4 atm was conducted—a total of 40 experiments with 26 different subjects—in 80% He–20% O_2. The exposure times ranged from 3 to 72 hr. Decompression was continuous at a ratio 1:1.6, the subjects breathing 100% O_2. Bends occurred in both subjects decompressed in 120 min following an 8-hour exposure, and in one of the two subjects decompressed in 150 min after a 12-hr exposure. A decompression time of 180–200 min was sufficient protection against decompression sickness after dives with bottom times of 12–72 hr in 22 experiments with 15 different subjects.

The experiments were not, of course, addressed to the issue of interest here and were not titrated; nevertheless, the findings are of considerable value. Since one subject in the 150-min decompression following a 12-hr exposure suffered an attack of bends, the minimum time—180 min—for an uneventful decompression for 12- to 72-hr exposures does not greatly exceed the time actually required for ascent following a 12-hr exposure at 4 atm. An estimate of 95% saturation with He in 12 hr yields a half-saturation time of slightly over 160 min.

Bühlmann *et al.* showed graphically that a 160-min half-time for He, corrected for residual N_2 tension, was quite consistent with their decompression times over the entire series of exposures with 3- to 72-hr bottom times. Residual N_2 tension was calculated on the basis of an initial tension of 0.79 atm at the start of each dive and an N_2 half-time of 420 min for the slowest tissue.

Any attempt to generalize about the half-saturation time required for safe decompression after He dives would be unwarranted, since the effect of the very high P_{O_2} used in these experiments is not known. In any event, it appears safe to conclude that the rates of He uptake and elimination in this series of dives were essentially symmetrical. Conceivably, there could have been perivascular bubbles, but scarcely could there have been bubbles in the deep extravascular space or extensive vascular occlusion by emboli in the controlling tissue.

In the same paper, Bühlmann *et al.* reported a series of dives in which a 79% N_2–21% O_2 mixture was used. Exposure time ranged from 3 to 48 hr at 4 atm, followed by decompression in 100% O_2. For present purposes it will suffice to say that an N_2 saturation time was not established within these exposure times. It might also be added that the experience of the Tektite I project suggests that the slowest rate of N_2 elimination (maximum N_2 half-time) is still unknown.

Evaluation for Symmetry of Uptake and Elimination

Given the half-saturation time for uptake of a gas, the problem of evaluating for symmetry of uptake and elimination is closely related to the choice of an optimum decompression format. Available data are too limited to support a firm conclusion.

The U.S. Navy multistage saturation–excursion dive to 1025 FSW (G. F. Bond; memo report dated April 22, 1968) and the U.S. Navy–Duke University 1000-FSW saturation dive (working papers), both conducted during 1968, had very similar decompression profiles. For the Navy–Duke dive, total time for decompression to the surface from 1000 FSW was 279.5 hr, or 16,770 min, yielding an average rate of 16.77 min/ft. The ambient P_{O_2} was held approximately constant at 0.3 atm. If one assumes the He half-time (160 min) used by the Zurich group and further assumes supersaturation throughout ascent, the average excess tissue tension would be $\Delta p = p - P = 13.8 - 9.9 = 3.9$ ft. From the Navy's 1025-FSW saturation–excursion dive, we have estimated an average rate of ascent of 16.35 min/ft, the P_{O_2} being held at 0.3 atm. This yields an almost identical value for Δp. Whether or not the rates of ascent were optimum does not have to be considered here. Symmetric gas uptake and elimination could be assumed if the tension excess of 3.9 ft (or perhaps lower) should prove consistent with satisfactory linear decompression formats for a range of ambient P_{O_2} using a half-time for He of approximately 160–180 min.

Discussion and Summary

The evidence upon which the above observations are based is admittedly circumstantial, since the optimal rate of ascent from a very deep saturation dive has not been determined. Whether linear ascent is the best method remains to be proven; but it has some logic in that only a small excess of inert gas tissue tension can be sustained over a long period of time without undue risk of nucleation. For linear decompression over a long period of time, the same logic suggests that excess inert gas tissue tension should be held very low.

As previously indicated, there is no simple criterion for distinguishing between a silent bubble theory and a stochastic concept of nucleation. Fortunately, there is no urgent reason to make such a distinction. In looking ahead, several aspects of decompression deserve attention:

1. A detailed decompression model projected upon a concept in which facts are nebulous can be deceptive. For example, Nims (9) recognized that gas might become separated into a rigid cavity, in which case one would not expect a simple pressure–volume relationship to obtain. (In any case, there is no compelling reason to assume that the rate of inert gas elimination would be appreciably retarded if a rigid cavity were formed.)

2. The perfusion–diffusion issue has been entirely omitted from the foregoing comments, which seems entirely justified when attention is restricted to saturation dives. Current transport models based upon a field of extravascular diffusion [for example, the concentric cylinder model suggested by Hills (7)] assume transport functions that can be approximated by an ordinary exponential decay function. A practical problem of far greater urgency is the estimation of maximal half-saturation times, for only when this problem is solved will it be possible to examine in a reasonably precise manner the symmetry (or lack of it) between inert gas uptake and elimination.

3. As Bateman (1) clearly recognized, the problem of predicting bubble growth cannot be separated from that of estimating effective saturation curves. Decompression theory is based upon the model of a uniform homogeneous transport system; in practice, there are few alternatives. Almost certainly, however, a more realistic concept is a multiple, small-scale heterogeneous system relating to our formal model only in a statistical sense.

4. It may be, of course, that half-saturation time is a function of a number of conditions, for example, ambient P_{O_2}, level of activity of the diver, and, possibly, hydrostatic pressure. Thus a critical test of the hypothesis of silent bubble growth requires fairly rigorous controls.

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DISSOLVED GAS WASHOUT AND BUBBLE ABSORPTION IN ROUTINE DECOMPRESSION

Hugh D. Van Liew

The variability and unpredictable nature of decompression sickness are evidence that many factors are involved—there is more to the problem than a simple supersaturation ratio which a diver must not exceed. In experiments where animals or men are subjected to standard supersaturation ratios, some factors have been identified—surface tension of the blood (15), state of the autonomic nervous system (3), body size (8), exercise (1), and solubility of the supersaturating gas (2). The experiment presented here focuses on another factor, the probability of bubble formation and growth.

Piccard (9) presumed that bubble formation was a chance phenomenon—that whenever there was supersaturation, there was a probability that bubbles might form, and that the probability increased as the degree of supersaturation increased. Accordingly, there is at least a chance of bubbles forming during any decompression, and once formed, a chance that the bubbles may do damage. In a symptomless decompression, therefore, either bubbles do not chance to form, or else the bubbles that do form are present in small quantity or in insensitive locations.

To test the idea of the chance nature of bubble formation, we subjected rats to a maneuver that would drastically increase the probability that bubbles would form or grow. Rats were given a highly soluble gas (N_2O) to breathe after they had reached surface following a decompression. Nitrous oxide should act as a "bubble amplifier" to reveal "silent" bubbles by causing them to expand. Nitrous oxide will diffuse into a N_2 bubble 11 times faster than N_2 will diffuse out (10), and therefore it can be expected to cause rapid expansion of any bubbles that exist (14). It is also possible, however, that the N_2O may facilitate nucleation of new bubbles. The N_2O was never supersaturated; the animals breathed 80% N_2O–20% O_2 at atmospheric pressure. Because they breathed the mixture instead of air, washout of N_2 was actually expected to be more rapid in the N_2O–breathers.

Methods

Female rats, six at a time, were put into a U.S. Navy chamber, and compressed with air to 165 ft (6 atm abs) at a rate of approximately 25 ft/min. If the animals showed unusual

excitement or evidence of pain, rate of compression was slowed. They remained at 6 atm abs for 110 min, then were decompressed at a rate of 50 ft/min. On a few occasions, decompression was stopped for a few seconds when the rats showed great excitement, probably due to noise.

After decompression, the rats were brought out of the chamber and divided into two groups. One group of three was left in a cage breathing air and the other put into a glass jar through which a mixture of 80% N_2O–20% O_2 flowed at a rate of approximately 5 L/min. Both groups were observed for signs of decompression sickness. Of the rats receiving N_2O, some were put into the environment as soon as possible (approximately 1 min after surfacing), and others were purposely left in air for a 3-, 5-, or 10-min delay before being put into N_2O. In all cases they remained in the N_2O environment for 60 min.

In the N_2O environment the rats were narcotized, but not completely anesthetized. They usually sat upright and sometimes crawled around, but not in a well-coordinated manner. When brought out of the N_2O environment, they regained full consciousness and alertness in 5–10 min.

All rats were observed for 90 min after the decompression, after which they were put into their home cages and checked the following morning. Only severe, unmistakable signs of decompression sickness were recorded. Besides death, such signs noted were markedly labored abrupt breathing, limping, and convulsions. Sometimes death was the first sign to occur. In a few cases in which signs were very severe, the rats were purposely put to death. It is not certain that these would have died, however, for several times rats with severe signs recovered, apparently completely, after a day. Less certain signs of decompression sickness which were not recorded were coughing, standing on the hind legs in the corner of the cage, exceptional grooming, industrious tail grooming, moderately labored breathing, irregular breathing, piloerection, and jumping as if from pain (especially in hind quarters).

Forty-two of the rats were used twice, with at least 21 days intervening between uses. In all, 155 Wistar strain rats were used; weights were between 150 and 290 gm, and mean weight was 216 gm.

During compression the rats huddled together, appeared anxious and uncomfortable, shook their heads, sometimes ran about the cage in great excitement, and on one occasion a rat convulsed. At pressure, they usually explored, groomed, and engaged in social activity during the first hour and slept during the second. During decompression they usually buried their heads, tried to hide, and exhibited piloerection.

Results

Table I gives numbers of rats that showed severe signs (including death) and numbers that died. In both categories, incidence was higher in rats given N_2O after reaching the surface than in control rats that continued to breathe air. The increased incidence was usually two- or threefold, as in the overall totals, in which incidence of signs including death was approximately 30% for controls and 70% for N_2O-exposed rats. The effect remains after a delay of 10 min between surfacing and the beginning of N_2O exposure. Detailed analysis of the differences between the 1, 3, 5, and 10-min delay groups is probably not warranted, since there was such large variability between the air-breathing controls for each group.

TABLE I

EFFECT OF BREATHING N_2O AFTER SURFACING ON DECOMPRESSION SICKNESS INCIDENCE

Delay time (min)	N_2O breathing			Air breathing		
	Exposed	Total hits[a]	Deaths[a]	Exposed	Total hits	Deaths
1	15	9	3	15	3	0
3	18	12	7	18	9	6
5	25	20	6	23	8	2
10	18	10	4	18	3	0
Totals	76	51	20	74	23	8
Totals, %	—	67	26	—	31	11

[a] Both number of hits and number of deaths in N_2O are different from controls with high statistical significance, except in the 3-min delay group, in which the incidence of decompression sickness in the control group was exceptionally high.

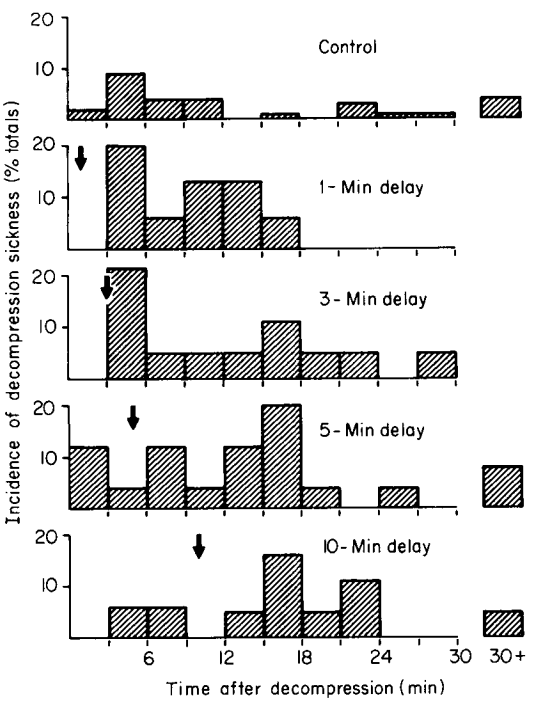

FIG. 1. Time lapse after decompression before onset of signs of decompression sickness in rats exposed to different regimens.

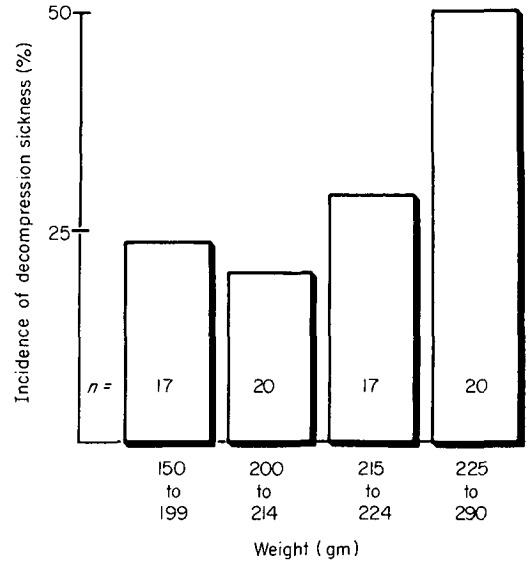

FIG. 2. Influence of body weight on the incidence of decompression sickness among the air-breathing control rats.

The severity of the decompression sickness incurred with N_2O appeared to be the same as for the controls: about 40% of those stricken died.

Figure 1 shows time of onset of signs. Abscissa is time after surfacing and ordinate is percent of total number of rats exposed to a given regimen. With the control rats, most signs appeared within 12 min after reaching the surface, whereas with the N_2O-treated rats, many times signs appeared after 12 min. In the 5- and 10-min delay groups, signs appeared in some cases before the exposure to N_2O, as would be expected from the appearance times in the control group.

Part of the variability seen in Table I may be due to unintentional differences in the weights of the rats in different groups. Figure 2 shows incidence of decompression sickness in the 74 air-breathing controls divided into four groups according to weight. Incidence is about 20% from 150 to 214 gm, then increases until it is 50% for 225–290 gm rats.

Discussion

The results suggest that bubble nucleation and growth can be very important under the conditions of this experiment, i.e., when the animals are on the verge of decompression sickness. It seems that after surfacing, many of the rats maintain a "potential" which can cause decompression sickness when the animals are given N_2O. The potential may be "silent" bubbles, the gas films that Hills has alluded to (6), or perhaps some other form. Other investigators have observed (5) or suspected (8) that their decompressed animals had bubbles even though there were no severe or lasting signs of decompression sickness.

Washout of dissolved gas in a tissue is determined by the blood perfusion rate, the blood/tissue solubility ratio, and the difference between the P_{IG} reached by the tissue during the preceding stage and the P_{IG} in the blood at the current stage.

The determinants of bubble size, however, are more complicated. Bubbles increase in size due to pressure decreases on going from one decompression stage to another. During a stage, bubbles may grow at first when partial pressure of inert gas in the tissue is greater than partial pressure of inert gas in the bubble, and then later shrink as gas diffuses out from the bubble. The eventual absorption of bubbles occurs because as the tissues become washed out, partial pressure of inert gas in a bubble becomes greater (by virtue of the O_2 and CO_2 tensions of the metabolizing tissues) than the inert gas in the blood, which is equilibrated with lung gas (4, 6). This "inherent unsaturation" varies from tissue to tissue, being greatest in tissues having a high arteriovenous O_2 difference (12).

It is not claimed that the existence of a "bubble potential" is the only possible explanation for the results of our simple experiment. However, if such a potential exists, the logical application is to include some of the aspects of the cure of decompression sickness (11, 13) into routine decompression procedures. Tables could be modified to minimize the hazard of decompression by emphasizing prevention of growth and promotion of absorption of bubbles. Modification might include more use of O_2, especially after surfacing; slower ascent rates between stops; and longer stops at depth, as Hills has suggested (7). However, modifications of workable decompression tables should not be undertaken lightly.

Acknowledgments

These studies were supported in part by Contract NONr 969(03), and Contract N00014-68-A-0216 (NR 102-722), made between the Office of Naval Research, Department of the Navy, and the State University of New York at Buffalo.

The author also wishes to acknowledge, with gratitude, the assistance given him by Christa Buentig, Edward A. Gard, Charles H. Smith, Jr., and Edward H. Lanphier.

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DETECTION OF BUBBLES IN TISSUES AND BLOOD

R. Stuart Mackay and George Rubissow

One way to study bubbles is to look at them. The studies of Pudenz, Sheldon, and Restarski (11) involving a plastic calvarium placed on monkeys, and the more recent microscope observations of bubbles in the vasculature of the hamster cheek pouch (1) might be mentioned as examples. In such cases, surgery or other disturbance to the subject is usually involved, and this can render the results misleading. Though such methods have given valuable results, an alternative nondestructive method for observing bubbles in the intact animal would be desirable.

An interface between a gas and either a liquid or a solid is a very good reflector of sound because of the great difference in acoustic impedance (product of density and sound velocity) across the boundary. Thus bubbles are well detected using ultrasonic energy. The velocity of sound in soft tissue is such that the wavelength of 15 Mc sound is 0.1 mm, and many sound imaging systems can resolve roughly one wavelength. The sensitivity of detection suggests using sound waves through the intact skin to study bubble formation during decompression, either by imaging individual bubbles, or by noting the attenuation or scattering of a sound beam traversing the tissue. Bubbles might have to become of some minimum size to exercise an effect, and the existence of "silent bubbles" might be proven or negated by such methods. Degree of supersaturation might be measured for different sites and the onset of too much overpressure at any decompression stop indicated in the preparation of a diving table. These considerations were noted and prompted the report at the 1963 Symposium of the successful observation of bubbles produced by the decompression of a rat, while using a low intensity pulsed ultrasonic imaging system (6).

The cycling pressures associated with an intense sound wave can trigger the formation of a bubble or "pump up" an existing one. The sound intensity mentioned in the above was 0.001 W/cm^2, and this still seems like a rather appropriate value in many cases. The ultrasonic transducer and the subject were both immersed in degassed water to assure good acoustic coupling, the animal having been shaved and a depilatory cream applied to minimize external bubble collection. The appearance of this equipment has been reproduced elsewhere (10). The method was suggested as supplying an objective endpoint in decompression or supersaturation studies, as well as having direct applicability in diving practice.

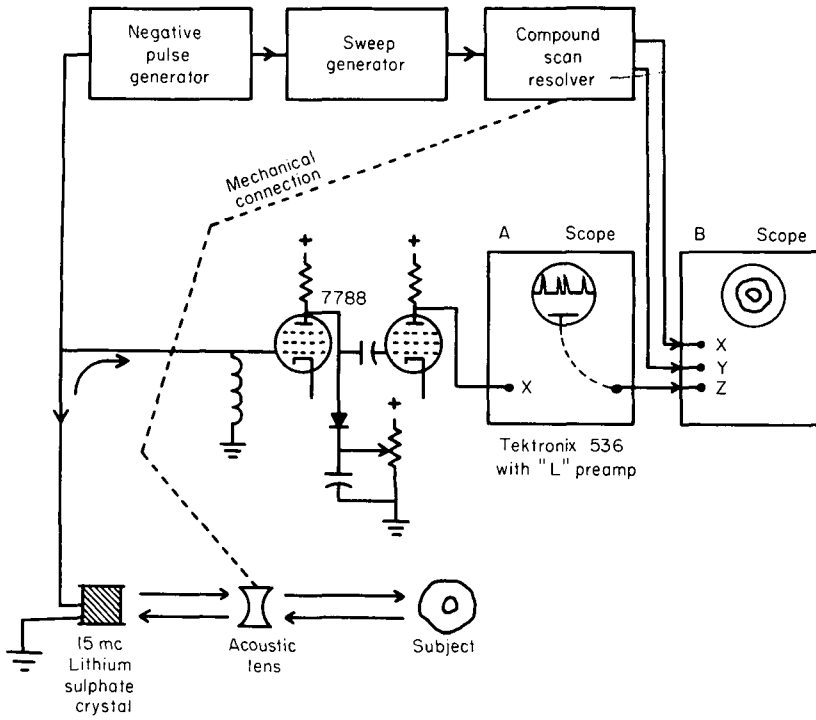


FIG. 1. Ultrasonic echo system for obtaining cross-section images of body structures through the intact skin. Scanning allows the entire pattern to be mapped on the second variable intensity oscilloscope, while the first displays the pattern of echoes as a function of time or depth for any one position. By permission of the publishers (8).

Pulsed ultrasonic energy has previously been used to image internal body structures using sonar-like systems [for example, see Howry (5)]. In soft tissues more detail can be seen than in an X-ray image, and repeated observations need not produce the harmful effects that can accompany radiography. In such systems, a short electrical impulse applied to a piezoelectric crystal generates an outgoing sound wave, part of which is reflected back at each successive interface traversed. The successively returning echoes strike the crystal to generate voltages that are applied to a television system to display the various regions in the subject, a cross-section image being built up with suitable scanning (Fig. 1). The scanning action can consist of two separate motions of different speed, which allows the viewing of each point in the subject from all directions in the scanning plane so that echoes reflected off in any direction are eventually seen and recorded. Such a compound scanning acoustic probe was used initially, but we have recently found a simple scan useful and much less demanding in mechanical precision. A concave lens may be placed before the piezoelectric element to focus the sound (Fig. 2). Coupling to the subject must be through a path of liquid or grease for good sound transmission. Low sound frequencies generally provide greater penetration and less resolution than high frequencies.

Others have worked with these methods. Walder, Evans, and Hempleman (14) used a commercially available pulsed ultrasonic system with the transducer held in contact with a

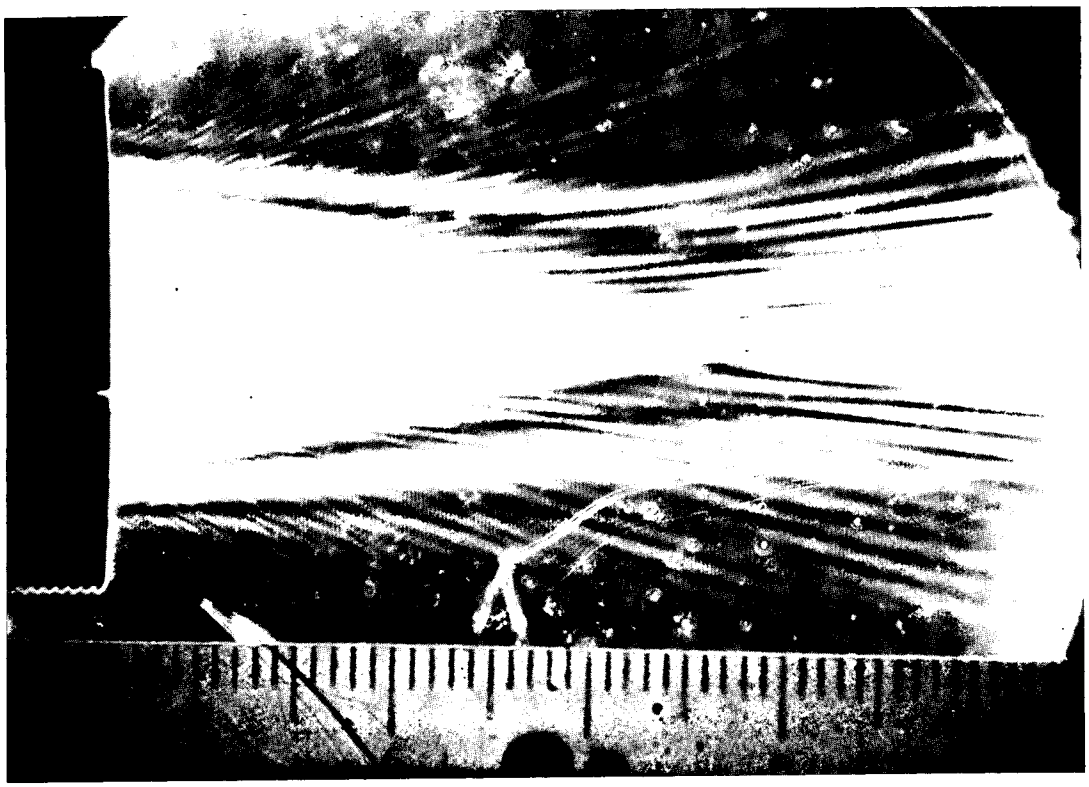


FIG. 2. Focusing action of a concave lens on ultrasound as visualized in a Schlieren light optical system. The streaks in the lower part of the figure are due to imperfections in the camera lenses used to construct the optical system. Similar sound image visualization can be used with biological subjects at reduced intensities if the brightness of a laser is used as the light source. By permission of the publishers (8).

fold of back skin of the test animal. They noted that after decompression, the proximal reflection was unchanged, but the distal reflection decreased in size after a small delay. Recompression restored the original pattern. Again, the decrease in distant echo amplitude presumably was due to increased tissue opacity accompanying the formation of minute bubbles in the intervening region.

The measurement of blood flow using the Doppler shift principle was demonstrated by Franklin, Schlegel, and Rushmer (2). Briefly described, a moving interface returns a slightly different sound frequency from that incident. At a piezoelectric crystal energized at, for example, 10 Mc, there will be a combination of the direct signal frequency and one slightly different reflected from anything moving, and these two frequencies will beat to give a difference frequency proportional to the velocity. Such units are suitable for either implanting or transcutaneous use, and for typical velocities of the formed elements in blood returning the sound, the difference frequencies generated conveniently fall in the human audible range. Such devices have been developed and are commercially available at a relatively low price.

Gillis, Peterson, and Karagianes (4) used a Doppler unit to indicate moving bubbles in the vascular system. A unit implanted on swine vena cava gave audible "chirps" as gas bubbles

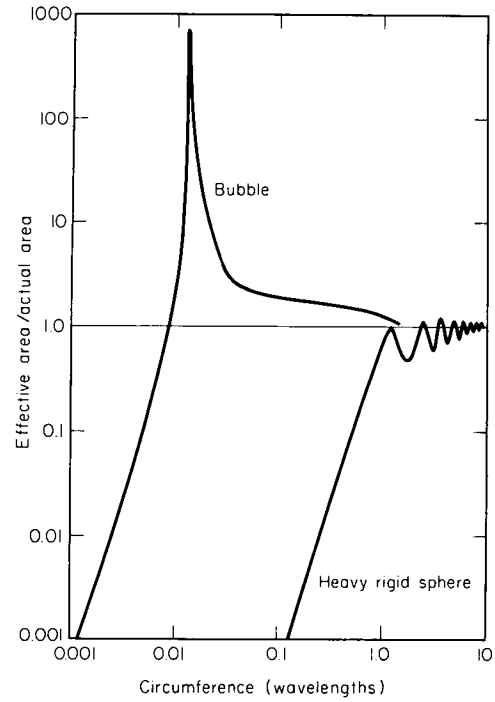


FIG. 3. Scattering by bubble and by rigid sphere. A bubble is a much more effective scatterer of sound than a similar size rigid sphere if the circumference is less than twice the wavelength. At the left, the curves display the familiar fourth-power falloff, and at the right, the curves coincide. At a frequency of 15 Mc, the resonant bubble size is slightly under $\frac{1}{2}$ μ.

passed through the vessel following decompression. There was occasional total signal loss. Circulating emboli were noted before the first decompression stop recommended for man. Transcutaneous observations were also made on dog, swine, and a goat (3).

Spencer and Campbell (12) implanted Doppler flow transducers on the inferior vena cava and descending aorta of sheep, and observed "whistles" and then a "roar" during decompression. An implanted electromagnetic flowmeter on the aorta disclosed "artifact voltages" simultaneous with the Doppler-detected bubbles. Bubbles were found in the venous blood of sheep at pressures greater than the usual first decompression stop for man.

We might note that these Doppler meter clicks are not only easily noticeable, but are an interference to blood flow observations in at least the monkey in altitude chamber studies. In studying bubbles in the blood, such a system has the advantage that no information about stationary structures appears, and thus there is not a confusing mass of echoes to interpret. But for the same reason, there are no reference points to tell where the bubble is, a given bubble cannot be followed, and if a bubble expands to fill a part of the vascular system and becomes stationary, it then becomes invisible. Similarly, stationary bubbles in tissue cannot be seen.

Most Doppler systems work at an unspecified sound intensity, often just under a level which would produce periosteal pain or burns. This is higher than that mentioned in connection with the pulsed system, and could encourage bubble formation; intensity could be reduced if bubbles rather than the formed elements in blood were the only scatterers of interest.

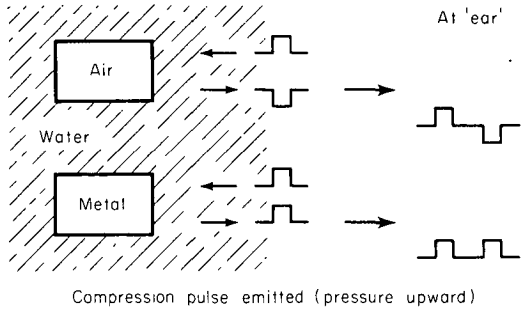


FIG. 4. Example of the inversion of a sound pulse at a more dense to less dense reflection, and no phase change when the reflection is of the opposite kind.

Tucker and Welsby (13) have noted that a sound wave applied to a bubble will result in the production of some second harmonic, that is, some sound of twice the original frequency will be returned along with that at the basic frequency. In compressing a gas bubble, one would expect significant nonlinearity, that is, this process could be relatively effective. However, low sound intensities must be employed, and this minimizes the effect of that nonlinearity; the mathematical expression for the second harmonic term should involve the square of the incident sound intensity, and thus that expression is reduced by involving the square of a small fractional number. In practice, any nonlinearity in the overall system could also generate a second harmonic which might drift in magnitude to confuse readings. It will be interesting to follow this proposal.

If bubbles approximately uniform in size are involved in these observations, then adjusting the frequency to resonance could give a much increased signal (Fig. 3). This well-known curve also shows the drop in noticeability of a bubble which is smaller than a wavelength of the sound (the Rayleigh scattering range).

Other possibilities exist in connection with imaging systems. Sounds reflected at a less dense to more dense interface are returned without a phase change, whereas a reflection in the opposite sense, as from a large pocket of gas, results in an abrupt change in phase of 180° (Fig. 4). Thus a strong return of inverted phase might generally be interpreted as representing a region of gas, and this could be displayed in a different color on a colored cathode ray tube to make a more noticeable display. For example, the signal could be integrated to display areas rather than just boundaries, and all echoes of the original phase could be shown in blue, whereas those of reversed phase might appear in red. (Some weaker reflections from tissue can also have reversed phase.) A similar discrimination can be made by transmitting a continuous wave mixed with some of its own second harmonic; this will return with a different wave shape after reflection from a gas interface.

Pulsed Doppler systems have been constructed that show not only the position but the approximate velocity of reflectors. Such a device could have applicability in the present case for specialized studies, but probably the simplest possible indicator of the appearance of free gas would be desirable in anything approaching a warning indicator by which divers might slow or reverse their ascent.

We are continuing work with the pulsed scanning system. A photograph of the apparatus

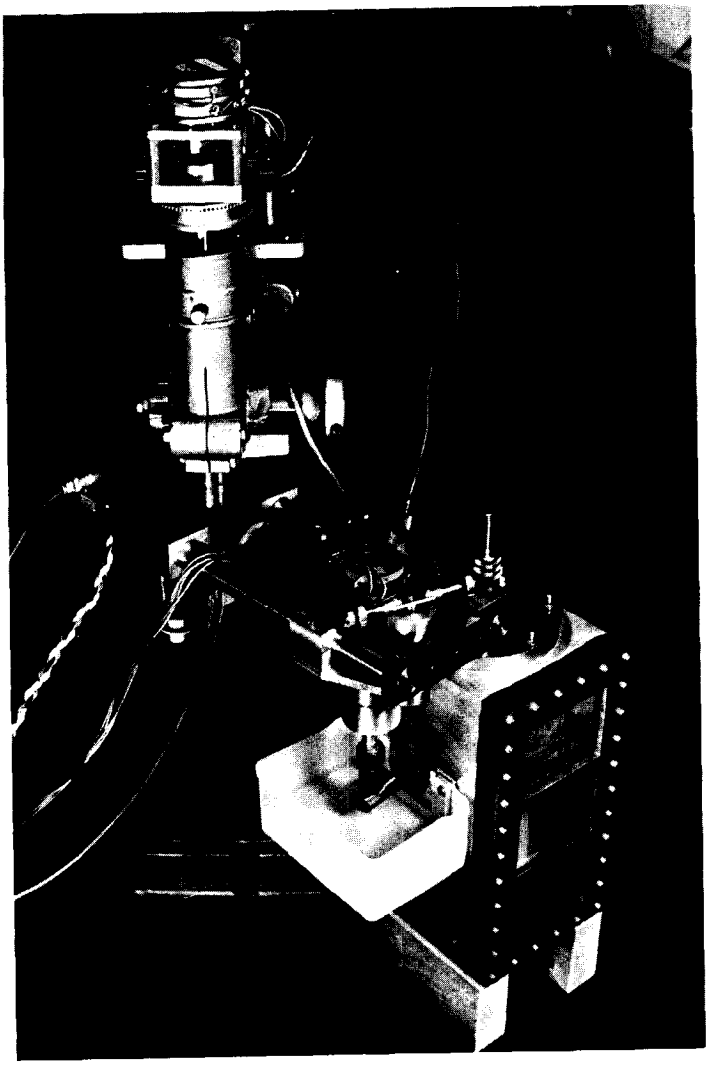


FIG. 5. Small rectangular compression chamber with Lucite front. In the side is the sound window, beside which is the transducer assembly immersed in a water trough. The scanner and pulse circuits are supported by a modified drill press for stability with ease of manipulation.

is given in Fig. 5. Here a simple scan is used at an acoustic window in the side of the small pressure chamber. The window is of 5-mil Mylar sheet clamped in a retaining ring after the edge has been melted into a rim with the help of a soldering iron. This window is $1\frac{1}{8}$ in. in diameter and readily withstands a pressure of 200 psig, while not distorting the image. The transducer oscillates through a full cycle 10 times per second without enough mechanical vibration to distort the image. The transducer can readily sustain pressure changes without problems, but in the present case, manipulation is made easier by placing it outside in degassed

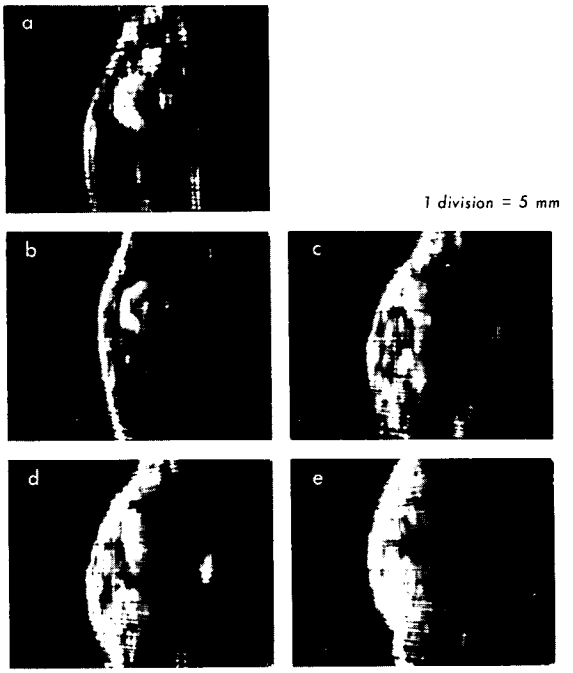


FIG. 6. Ultrasonic image through thigh of guinea pig. At top, with sound source to left, the near and far skin layers are seen at left and right, with the flattened bright spot being the bone. Compression was to 120 psig of 5% O_2 and 95% N_2 for 2 hr and 20 min. Linear ascent was 2 min to atmospheric pressure. The images are: (*a*) before compression; (*b*, *c*, *d*, and *e*) 1 min, 1 min and 30 sec, 1 min and 35 sec, and 1 min and 50 sec, respectively, after "surfacing." Note rapid formation of bubbles and obscuring of rear surface.

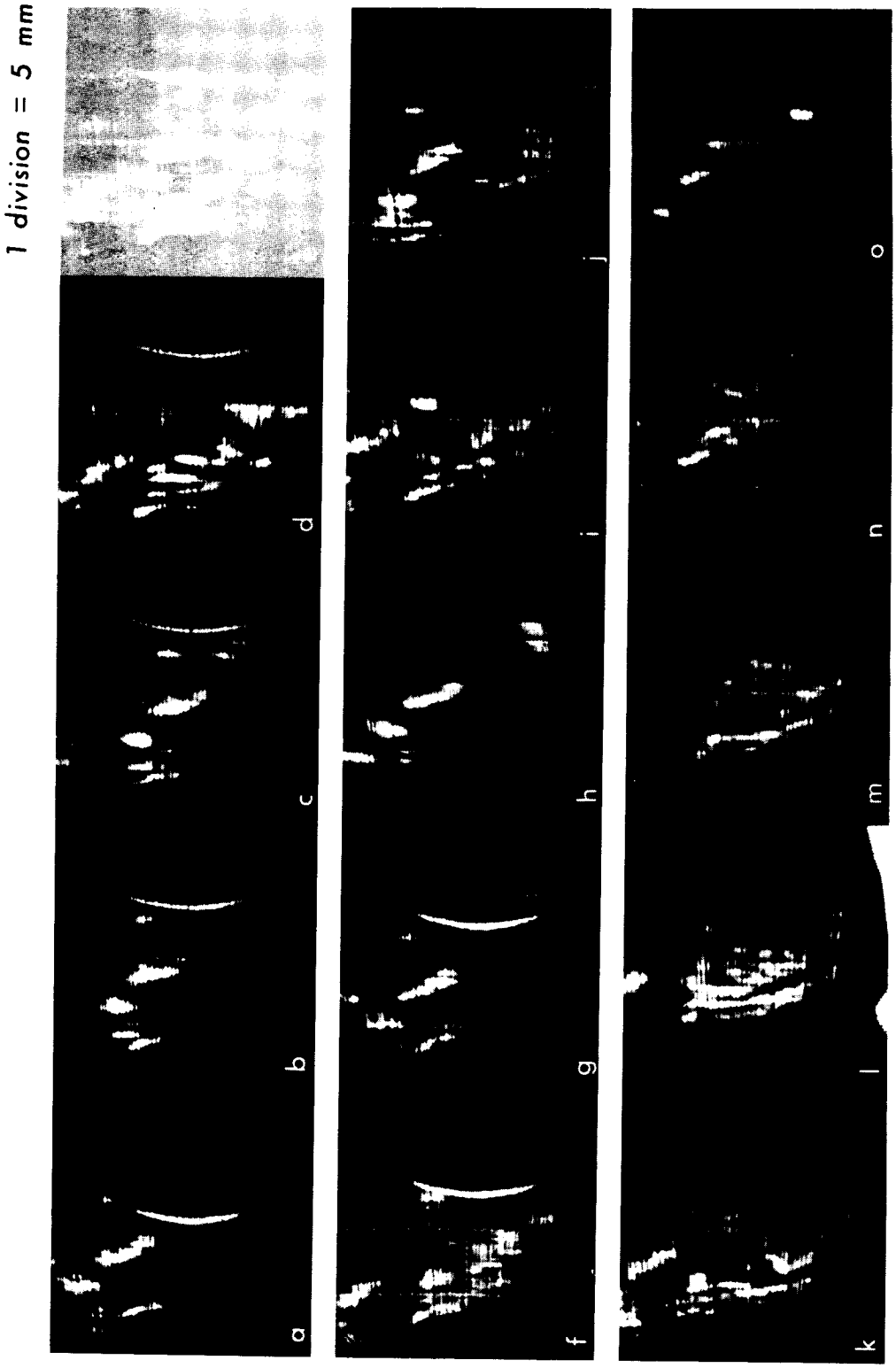
(boiled) water. The animal is held in a harness from the chamber top rod which can be rotated and translated through an "O ring" seal.

The electric pulse applied to the transducer 3000 times per second has a duration of 50 nsec and has a 500-V amplitude; this also appears at the input to the sensitive amplifier. We are presently using a 10-Mc transducer and lens that give a range resolution of 0.15 mm and an azimuthal resolution of 1.5 mm, the latter being the effective thickness of the slab observed in one scan.

Representative images are shown in Figs. 6–8 which depict cross sections of the upper right hind legs of a hamster and two Hartley guinea pigs. These last two involve different degrees of initial saturation. In each case, the animal was shaved and then a depilatory cream (Neet) applied for 4 min. The chamber was filled to over the sound window with physiological saline maintained at 33°C. The air was changed every 5 min.

Echoes probably due to bubbles appeared a few minutes after the animals decompressed to atmospheric pressure. Upon recompression, there was some immediate diminution in these echoes, and then gradual disappearance (at these amplifier gain settings) after approximately 10 min. Decompression more readily caused them to reappear the second time, suggesting that they were not totally removed.

If fast aspects of events are to be followed, the ultrasonic image converter tubes described


1 division = 5 mm
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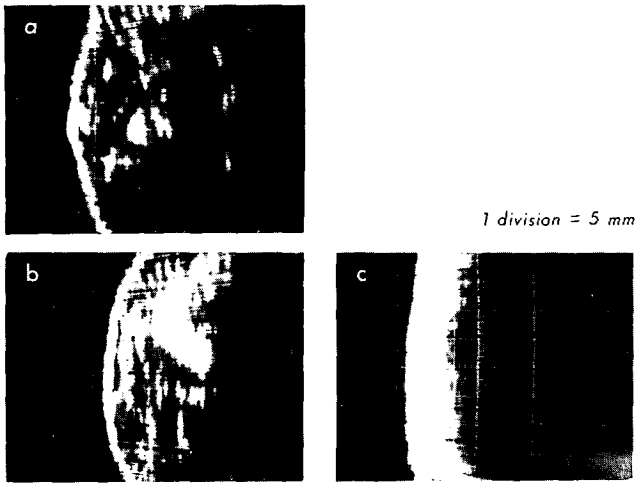

FIG. 8. Transverse ultrasonic image of hamster leg: (*a*) before compression; (*b* and *c*) after decompression. Compression was to 103 psig (8 atm) for 2 hr on 5% O_2 and 95% N_2. Images 1 min and 5 min after a 2-min linear ascent show vigorously increasing bubble activity which was fatal despite treatment.

by Jacobs, Smyth, and Turner [see MacKay (7)] could be important. In studying human subjects, it may be sufficient to extract blood, which has equilibrated with tissues, from the vena cava through a cuvette heated to 37°C. Most ultrasonic equipment can function effectively inside decompression chambers if this is desirable. It should be possible to rapidly check uncertain parts of any decompression table employing only a few persons in whom the ultrasonic criterion of bubble appearance would be used. Of interest would be the site of bubble appearance for failure at different parts of a decompression schedule, that is, do regions of low perfusion bubble during the last stages of decompression? Degree of supersaturation in a given body region can perhaps be measured by inducing bubbles in a known way with more intense ultrasound. The exact situation with "silent bubbles" still remains to be studied, as do the fundamental postulates of the onset of decompression sickness. In terms of diving practice, it may prove that the appearance of any bubble in a decompressing diver is cause for modification of the overall schedule. From long-term saturation dives, it may be possible to tell if altered blood flow patterns while asleep and awake during decompression demand periodically changed schedules. Successes in transmitting information through a watery medium (9)

FIG. 7. Cross section of crouching guinea pig leg monitored continuously throughout decompression, therapeutic recompression, and final stage decompression. A simple sector scan was used maximizing bubble reflections relative to normal tissue reflections. The crescent was a double reflection between the window and transducer. Compression was to 120 psig for 60 min with 5% O_2 and 95% N_2, while all subsequent procedures were on air. The scans are (*a*) before decompression; (*b–e*) bends following decompression, at 1, 5, 9, and 12 min, respectively, after surfacing by a 2-min linear ascent; and (*f–o*) recompression to 78 psig, and treatment.

Recompression over 2 min was initiated 13 min after surfacing. Reflection reduction is seen 3 min after recompression at *f*, while *g* at 10 min shows almost complete reduction with return of original appearance. The remaining scans during decompression over 10 hr show bubble reappearance with upward steps, which correlated with noticeable animal discomfort, and gradual return to normal.

suggest that this objective test for bubbles may be applied to free-swimming animals and humans in future experiments, and probably will settle the classic questions about whales and decompression sickness. In humans, it would be interesting to verify bubbles at the site of pain rather than elsewhere. Thus far, with a commercial transcutaneous flowmeter, we have not seen moving bubbles in human divers at the site sensation.

Even the eye can safely be studied, and similar methods should allow evaluation of changes in the elastic properties of the lens (9) for assessing long-term disturbance by O_2, etc.

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Part IV. FUNDAMENTALS OF INERT GAS EXCHANGE AND BUBBLE FORMATION

DISCUSSION

R. E. Forster, Chairman

Dr. Spencer: I wish to summarize our findings involving bubble detection using the Doppler ultrasonic flowmeter.

We find that the bubbles are detectable in the venous blood in the large veins before they are found in the large arteries. They occur in sheep before very severe signs and symptoms of decompression sickness occur. We also find some delay in the formation of the bubbles; that is, if we decompress the animal too rapidly, the bubbles will not be detected until the animal has such severe bends that the detection is not useful for preventive purposes. Therefore, slow decompression is necessary (apparently about 2 min is necessary at 60 ft/min decompression) to permit the bubbles to be detected in the large veins.

We have found these bubbles in the veins as long as 24 hr after decompression without any overt signs or symptoms of decompression sickness, and we find that recompression makes them disappear. Very recently we were able to detect bubbles in the brachial vein of a human subject who was decompressed after an exposure at 300 ft for 15 min on air. The exposure and decompression were for the purpose of checking equipment, but the subject developed skin lesion over the right biceps. Bubbles were clearly heard in the brachial vein between the lesion and the heart but were not present in the rest of the body, so it appears they are now definitely associated with minor symptoms in human subjects.

Dr. Lambertsen: It seems that there are two very tightly related matters under discussion. One concerns the concept of continuous multiple occurrence (and resolution) of bubble nuclei, and the other concerns detection of bubbles. We should now consider whether we can resolve the question of the occurrence of multiple nucleation by use of the Doppler and electromagnetic flowmeter techniques or whether the techniques adapt themselves only to such large bubbles that they are not going to be really of any use in studying the initial stages of bends. I interpret Dr. Spencer's findings as indicating that the methods are sensitive if aimed at venous blood.

Dr. Buckles: In response to that, I think that in all animal studies we are working at the present time with a response far more gross than we ever work with in humans, and we need a more subtle endpoint. There are three that I can think of. One is an absolute measure of the appearance or existence of a bubble. Another, from a physiological psychology point of view, would be a test of performance changes to determine when an animal has bends at a much more subtle level than we now use. The third would be the use of biochemical parameters of stress.

I do not know which of the three is going to give us the proper endpoint, but until we have a much more subtle endpoint than we now have, the results that we obtain from decompression studies on small animals just are incapable of extrapolation to any experience with man.

Dr. Hills: I would like to emphasize that this discussion has been mainly about bubbles, which represent a late phase of decompression sickness, but bends need not be initiated in the form of gross bubbles. Therefore, I think that the best experimental endpoints involve the occurrence or nonoccurrence of symptoms. This is the only way in which we can guarantee that we are dealing with the responsible tissue type.

Dr. Mackay: Then you are right back to the old question. One of the purposes of use of detectors is to find out if silent bubbles exist. Do bubbles (assuming that they are bubbles and there seem to be indications that they are) have to reach a certain size before they start exerting symptomatic effect?

One of the problems with the ultrasonic technique, and something that has to be considered very carefully, is the fact that all these nucleation phenomena are highly regenerative, of course, and the technique itself can act as a trigger. If you go to Yellowstone National Park and throw a bar of soap in the quiet pool, you have a geyser all of a sudden. Similarly, sound waves can trigger or start a very small aggregate to aggregating even faster. So those groups that have studied cloud chambers and bubble chambers have given some attention to what happens in sound fields, and it certainly is true that if the detection device is used improperly you may get misleading results.

I strongly suspect that if a deep-sea diver who is decompressing at the optimum rate (and the fastest way for him to decompress is to always be on the verge of decompression sickness) were hit by a sonar beam, he would suddenly get the bends.

Dr. Campbell: Does Dr. Mackay know of any experimental evidence where nucleation was generated by ultrasound in animals or human subjects? We have done a great deal of transcutaneous Doppler work in the chamber on human subjects as well as animal subjects and have never been able to correlate the appearance of bubbles with outward signs and symptoms of decompression sickness necessarily. Sometimes bubbles would appear when there were no signs and symptoms, and sometimes bubbles appeared when there were signs and symptoms.

Dr. Hills: There is one series of experiments which I did in which I titrated goats after 6 hr. They would not bend at 45 ft, nor at 50 ft, and then bent at 55 ft. Then I exposed them to 55 ft and decompressed them just on the brink of a dangerous state. I then put their legs into an ultrasonic bath but have never been able to induce symptoms by this means.

I think this is compatible with the phase equilibration concept as opposed to critical supersaturation where you would expect the excess energy would provide a little extra stimulus, or the ΔG of Buckles to induce another gas phase if it was supersaturated.

Dr. Mackay: With more intense ultrasound, at sound intensities less than the cavitation level, we have seen bubbles induced, but we haven't really pursued this far enough to know at what levels really it is happening.

Dr. Van Liew: The question is does sound increase the formation of bubbles. I would like to suggest a very simple experiment. Decompress rats from 6 atm on air for 2 hr as I did, and you'll get a 30% incidence of decompression sickness. Put some of them in a sound field and find out if the incidence increases.

Dr. Buckles: We have used ultrasound at two different frequency levels and for two different reasons. This goes back to the work of Goldman and others back in the mid-1950's, where they did determine the threshold for cavitation as a function of frequency of plasma and various tissues of the body. The observation was that at low frequencies, 20–50 kc, with very low sound energies cavitation indeed occurs very quickly, and I have used that to induce bubble formation in the cheek pouch of hamsters. It works very nicely. However, on going up to about 100 kc the energy requirement starts to increase logarithmically and the energy requirement at 1–10 Mc, which is the kind of range we all are working with, is enormous.

We have tried with Dopplers, using the energy we have available, to crank them up to maximum energy to see if we could induce cavitation in water, in supersaturated solutions of CO_2, and we have been unable with the currently available or commercially available Doppler to ever induce any cavitation. There is always the probability, but I consider it low.

Dr. Hempleman: As comment on Dr. Van Liew's suggestion, we have in fact done the experiment of an exposure of rats to a nonlethal concentration of inert gas, decompressed them and then put them in an ultrasonic field, and in fact they do bubble. But it is the acoustic coupling that is the critical factor. We find that if you put an ordinary furry rat into a water bath and attempt to radiate with ultrasound, the absorption of sound through the fur is so considerable that nothing happens, but if you shave the animal then it certainly cavitates.

Dr. Spencer: I hate to belabor that point on the causation of bubbles with ultrasound, but I think it behooves every investigator to investigate his own techniques. We did that by putting two transducers on the vein and the one that is upstream, when it is on or when it is off, does not generate more bubbles than that one that is downstream.

The second point that might be made is that if you find that the system you are using is indeed forming bubbles, it still could be a useful indication of a supersaturation state.

Dr. Doebbler: Dr. Van Liew, in your suggestion about possibilities for ways of decreasing decompression sickness in small animals, were you not suggesting really that in going from a rapidly diffusing gas to a slowly diffusing gas during a decompression that diffusion limitation is going to be the important factor? We have tried the reverse sort of thing with rats going from Ar to He and have found a marked improvement, where

from our perfusion-limited model calculations most of our compartments are increasing total gas tension during a period when bending symptoms are decreasing.

Dr. Van Liew: It depends on whether you have bubbles present. My explanation for the results of Keller and Bühlmann is that if you have a bubble there is going to be gas inside diffusing out, and if you change the gas outside you will change the gradient for that particular gas. You should put a gas on the outside that is going to diffuse into the bubble less rapidly than the gas that is inside is going to diffuse out.

We ran a model experiment of this by making a SF_6 gas pocket, and then let the rat breathe air. In that case N_2 comes in fast, SF_6 goes out slowly, and the gas compartment volume increases. And that is what led us to devise the experiment with N_2O which is the same principle. So if you just turn it around and put, say, N_2O on the outside, the N_2 will go out of the bubble faster than the N_2O will go in and this will cause a sort of step decrease in bubble size. Then from then on the N_2O will tend to go out slowly, but this big step may have gotten you out of the trouble zone into the place where the bubble will decrease very rapidly.

Dr. Doebbler: I misunderstood your suggestion originally because I was considering this in applying a change of gas without having preformed bubbles as the condition for decompression. You are presupposing bubbles already formed and are trying to control this situation once it occurs.

Dr. Van Liew: That is right. This is an important point; but the point is that if there are bubbles, then this is a way to get at them. If there are not bubbles, then changing of gases may change your rates of washout in other ways.

Dr. Doebbler: Dr. Hills, it would seem that composition changes would be more important or could be equally as important as multiple phase formation in a system in terms of affecting conductivity. Have you considered, at least theoretically, the various parameters that are going to contribute to conductivity changes and is it fortuitous that there is correlation to what should be multiple phases in your system?

Dr. Hills: The measurements were done upon excised tails, of course; the animal was dead. And the only effective change is in absolute pressure. There could be conductivity changes within the fluid, but I very much doubt that it came to the 10% level which was recorded in these experiments.

Dr. Lever: I think we worry a lot of people by the fact that we have observed decompression sickness in mice with SF_6 without the appearance of evident bubbles. I suspect, though, that bubbles are present because we have done very similar experiments to those Dr. Van Liew has done. We have recompressed the animals which had SF_6 decompression sickness, using rather low pressures of gases like He, N_2, and N_2O. These are pressures that would never cause bubbling in animals when used alone, or even in mixtures with SF_6. We find that on a subsequent decompression the animals are absolutely full of bubbles.

I think this is an important point because it does imply that with SF_6 at least there are bubbles in the animals which are causing gross signs of decompression sickness but which are very difficult to detect either optically or, I think, using ultrasonic techniques or techniques of this nature.

Part V. FACTORS IN DECOMPRESSION. THE INERT GASES

COMPARATIVE APPROACHES TO PROPHYLACTIC DECOMPRESSION

D. J. Kidd, R. A. Stubbs, and R. S. Weaver

The purpose of this paper is to examine the concepts used by various investigators in computing prophylactic decompression schedules. These concepts are discussed under three main headings: (*1*) body tissue gas transfer, (*2*) body tissue models, and (*3*) ascent criteria.

Under each of these headings a general model can be hypothesized. The actual methods of computation used by different groups of investigators can be shown to be adaptations of the general models.

Concepts

Body Tissue Gas Transfer

The salient features of gas transfer concepts are summarized in Table I.

The transfer of gas throughout the body's tissues has traditionally been considered a linear process in which the mass flow of gas is proportional to its partial pressure gradient (1). In a more simple physical system, however, mass flow of gas is nonlinear; the flow is proportional not only to the pressure gradient, but also to the mean absolute pressure of the gas. In such a physical system, mass flow approaches linearity when the pore size of the material through which the gas flows is small in comparison with the mean free path of the gas—that is, for gases of low molecular weight at low absolute pressures (19, 22). Thus the transfer of gas throughout the body's tissues is a linear process only under special conditions.

Nonlinearity in gas transfer causes a difference (asymmetry) between the rates of saturation and desaturation. Saturation of the tissues is faster than desaturation is under the same pressure gradient. This asymmetry is greater at higher absolute pressures. Hempleman (7) suggested that asymmetry might explain his findings with experimental animals.

Table II shows the degree of the asymmetry in a four-compartment series pneumatic analog computer (11). Asymmetry between saturation and desaturation is shown in differences between half-times when the gradient is reversed.

TABLE I

BODY TISSUE GAS TRANSFER CONCEPTS

		Proponents
Linear	Mass flow of gas or $\dot{P}_{tissue} \propto \Delta P$ —time constants are constant —saturation and desaturation equal (symmetrical) Mass flow of gas or $\dot{P}_{tissue} \propto \Delta P(A + \bar{P})$ —time constants decrease with increase in absolute pressure	All but Canada
Nonlinear	—saturation faster than desaturation (asymmetrical) —approaches linearity for small pore size, low gas molecular weight, and low absolute pressures	Canada

BODY TISSUE MODELS

The concepts of the body tissue models under consideration here are shown in Table III. The ideal model may be considered to be a continuous slab of tissue in which the tension of inert gas varies as a function of time and distance from a capillary. Hempleman (6) and Hills (9) are the main proponents of this model, a concept that might explain many natural phenomena.

Hempleman has been primarily concerned with the total gas flow into the tissue slab, whereas Hills has been more concerned with the peak gas tension within the slab. Although there is little difficulty in constructing a practical analog to deal with the two variables (gas flow and time) involved in the Hempleman concept, there is a major problem with the three-variable (gas tension, location, and time) Hills model, since inert gas tensions can be read out only at discrete distances from the capillary.

TABLE II

COMPARTMENT EFFECTIVE HALF-TIMES ($T_{1/2}$) FROM SATURATION FOR MK$\underline{V}$S PNEUMATIC ANALOG COMPUTER

Initial saturation depth (FSW abs)	Final depth (FSW abs)	Method of change to final depth	$T_{1/2}$ (min)					
			Single compartment	4 Identical compartments in series				Remarks
				1st	2nd	3rd	4th	
0	33	Step	27.7	39	168	262	304	Fastest method
33	0	Step	29.4	50	177	274	317	Fastest method
33	0	Continuous ascent	62	402	597	705	750	$R = 1.44$ $C = 0$
33	145	Step	18.1	26	100	170	200	
145	33	Step	20.8	37	129	192	222	Standard single compartment $T_{1/2}$ calibration

TABLE III

BODY TISSUE MODEL CONCEPTS[a]

		Proponents	
Distributed	Continuous tissue "slabs"		
	$P_{tissue} \propto f$ (time, distance from capillary)	Hempleman	
	In practice can only be read out discretely	Hills' Thermal Model	
Lumped	Discrete tissue "compartments"		
	$P_{tissue} \propto f$ (time)	Bühlmann	
	Parallel configuration	Haldane: 5[b]	USN: 4
	Each compartment independently communicates with gas source	Dieter: 2 to 3	Canada: 4
		Schreiner: 11+	Workman (M value): 9
	Series configuration		
	First compartment communicates with gas source, other compartments serially connected with first	Hills: 27	
	In the limit, large number of series compartments approximates a distributed system	Canada: 4	

[a] These model concepts were obtained from Hempleman (6), Hills (9), Bühlmann (3), Haldane (1), Dieter (4), U.S.N. (20), Canada (17, 18), Schreiner and Kelly (16), and Workman (M value) (23).

[b] Number after proponent indicates number of tissues considered in model.

This difficulty has been overcome by considering a lumped system of discrete compartments. Haldane's original hypothesis of parallel tissue compartments (1) has the advantage of possessing inherent analytical simplicity because of compartment independence. The Canadian approach (17) was initially based on the Haldane hypothesis, but evolved into a series configuration concept that is similar (18) to the continuous slab or distributed concepts (9). The series configuration concept has a practical advantage in that longer tissue half-times can be achieved simply.

ASCENT CRITERIA

Most of the concepts analyzed herein assume a linear relationship between the absolute pressure of a minimum safe ascent (P_{safe}) and the maximum tissue inert gas partial pressure ($\hat{P}_{tissue}$); that is,

$$P_{safe} \geq (\hat{P}_{tissue}/R) - C$$

in which R and C are constants.

Expressed in terms of the saturation ratio concept (K), this calculation becomes

$$K = \hat{P}_{tissue}/P_{safe} = R[1 + (C/P_{safe})]$$

or, when expressed in terms of the differential pressure concept (ΔP),

$$\Delta P = \hat{P}_{tissue} - P_{safe}$$
$$= P_{safe}(R - 1) + RC$$

Our classification of the concepts of various investigators is shown in Table IV.

TABLE IV

ASCENT CRITERIA FOR DECOMPRESSION[a]

	Constant K $C = 0$	Variable K $C \neq 0$
Constant ΔP $R = 1$ Must terminate decompression with jump to be practical	$K = 1 \; ; \Delta P = 0$ Canadian Modified Hills	$K = 1 + \frac{C}{P_{safe}} \; ; \Delta P = C$ Hills Rashbass
Variable ΔP $R \neq 1$	$K = R \; ; \Delta P = P_{safe}\,(R - 1)$ Canada: $R = 1.44$	$K = R\left(1 + \frac{C}{P_{safe}}\right) ; \Delta P = P_{safe}\,(R - 1) + RC$ Workman, Schreiner: $C = \frac{Mo}{A} - 33$, $R = A$ Hempleman: $C = 30$, $R = 0.8$

[a] The ascent criteria were obtained from Hills (9), Rashbass (13), Canada (11, 18, 19), Workman (23), Schreiner and Kelley (16), and Hempleman (8).

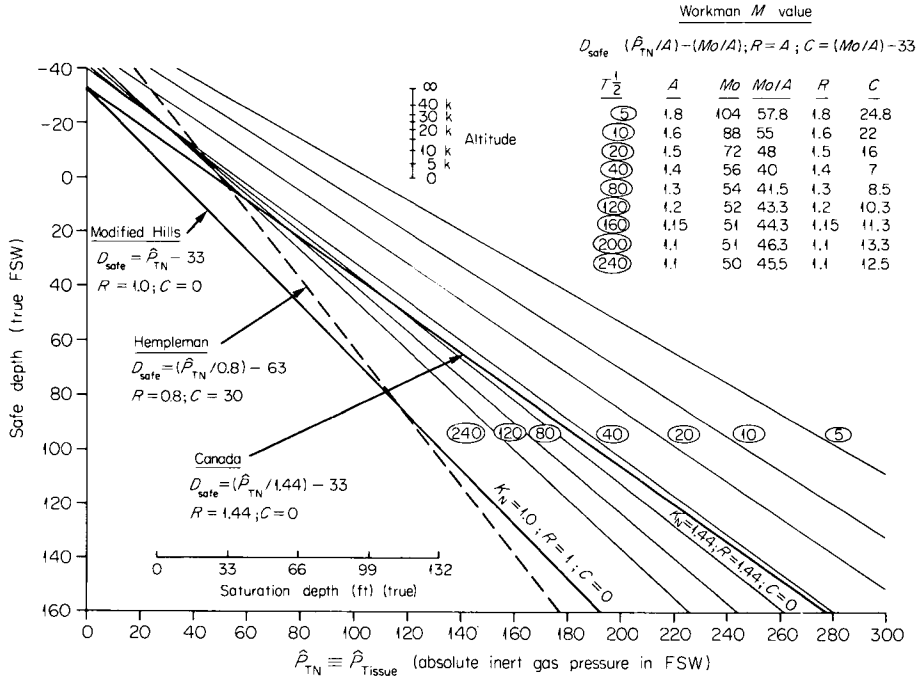

FIG. 1. Ascent criteria for air decompression plotted from equations set out in Table IV.

The most general of these concepts using a linear dependence for ascent from depth occurs for $C \neq 0$, in which K decreases and ΔP increases with depth. At altitude, K approaches an infinite value in those cases in which $C \neq 0$.

Certain ascent concepts are based on the assumption that a linear connection exists between P_{safe} and $\hat{P}_{tissue}$. It is possible, however, to postulate a nonlinear relationship between these pressures. Hempleman (8) in his decompression computations for caisson workers and Bühlmann (3) in his calculations of mixed gases to be breathed after deep dives appear to have used a nonlinear system. Both investigators state that their ascent criteria are valid only within a limited range of pressure.

The only practical ascent-from-depth concept that appears to provide a continuum through the pressure spectrum from depth to altitude is a linear one in which $C = 0$. Predictions based on several concepts are set out in Fig. 1, from which safe ascent from depth or to altitude can be calculated directly from $\hat{P}_{tissue}$ values.

Calculation of Decompression Profiles

Decompression profiles can be calculated through use of various combinations of gas transfer, body model, and ascent criteria. Such profiles can be computed by digital and analog methods in staged or continuous ascent form.

Pneumatic analog techniques permit computations based on any combination of the preceding factors to be carried out continuously in real time. Moreover, close approximations to specific linear models can be achieved with fewer parameters by a nonlinear gas transfer model

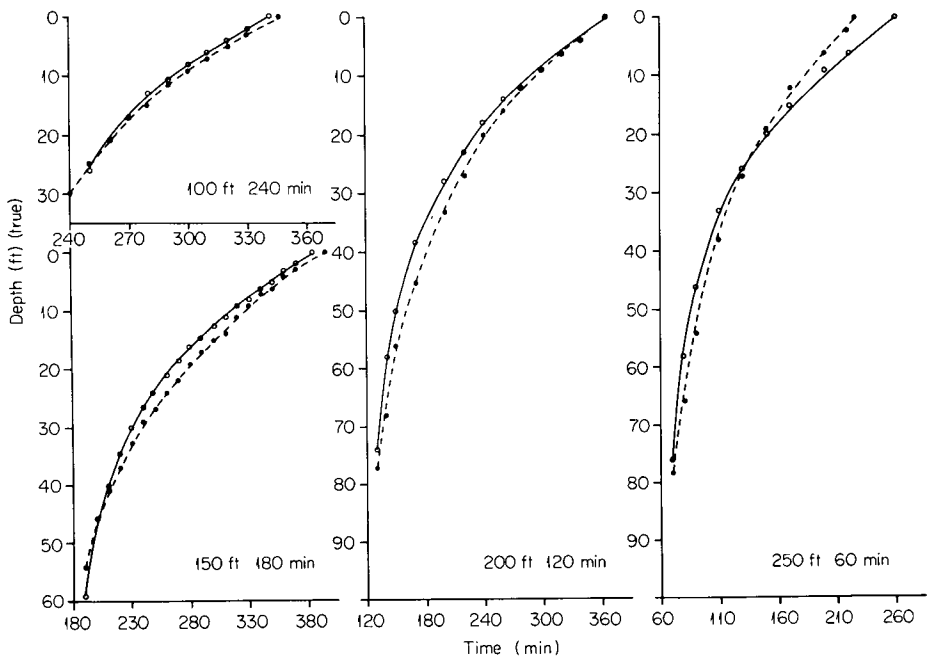

FIG. 2. Comparison of response of Workman M value model (●) (23) with MK$\bar{V}$S pneumatic computer model (18) as modified by Daniels (○) (private communication, 1969).

in series configuration. For example, the Workman M-value model (23) (based on nine parallel compartments with different half-times and on the most general ascent criterion) can be matched closely by a four-compartment nonlinear series system with a constant saturation ratio, as shown in Fig. 2. These preliminary comparisons were made by Daniels of SPAR Aerospace Products, Ltd. (private communication, 1969), who used only five parameters, compared with the 27 parameters used in the Workman M-value system. While such a comparison demonstrates the flexibility of the nonlinear series technique, it does not imply that we endorse the M-value system.

Experimental Evaluation

Practical Comparison of Decompression Profiles

Information is sparse regarding the incidence of decompression sickness arising from different decompression profiles that have been followed precisely. To provide ourselves with more meaningful comparative data, therefore, we carried out a series of exposures in which we arbitrarily selected the depths and times, and carefully controlled decompression according to various criteria. Sample decompression profiles based upon some of the foregoing concepts, together with the results of experiments involving human subjects, are shown in Figs. 3–5.

Our modification of the Hills concept uses a four-compartment nonlinear series system with an initial saturation ratio of $K = 1$. The half-times of all compartments are identical, equaling 52 min [derived from analysis of the Van der Aue no-decompression data (21)]. The ascent

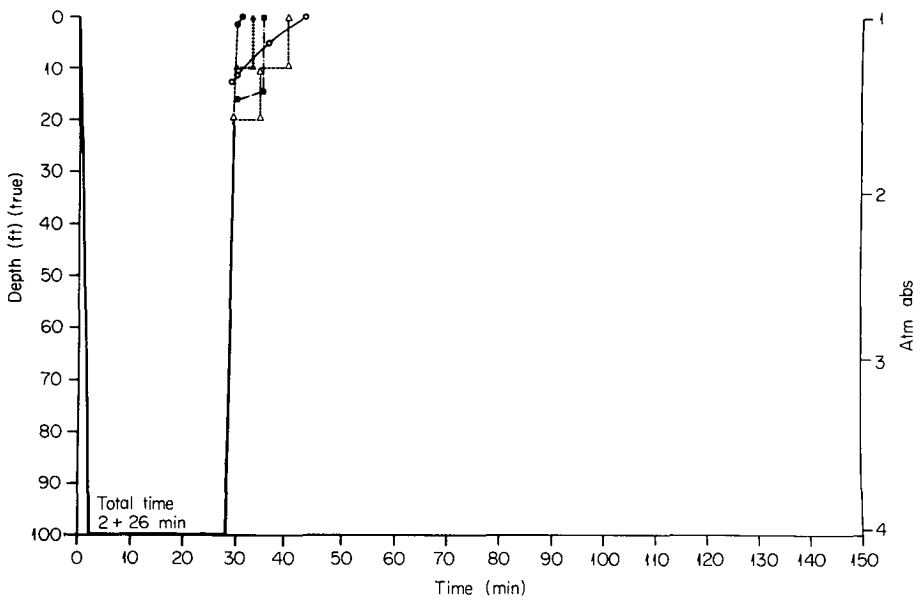

Fig. 3. Comparative decompression profiles for 100-ft dive, obtained from R.N. Table (15), U.S.N. Table (20), Workman M value (14, 23), and MK$\underline{\text{V}}$S computer (18).100 ft 2 + 26 min air: (■) Modified Hills; (△) RN table; (▲) USN table; (●) Workman $\bar{M}$ value (continuous); (○) $\underline{\text{V}}$S computer.

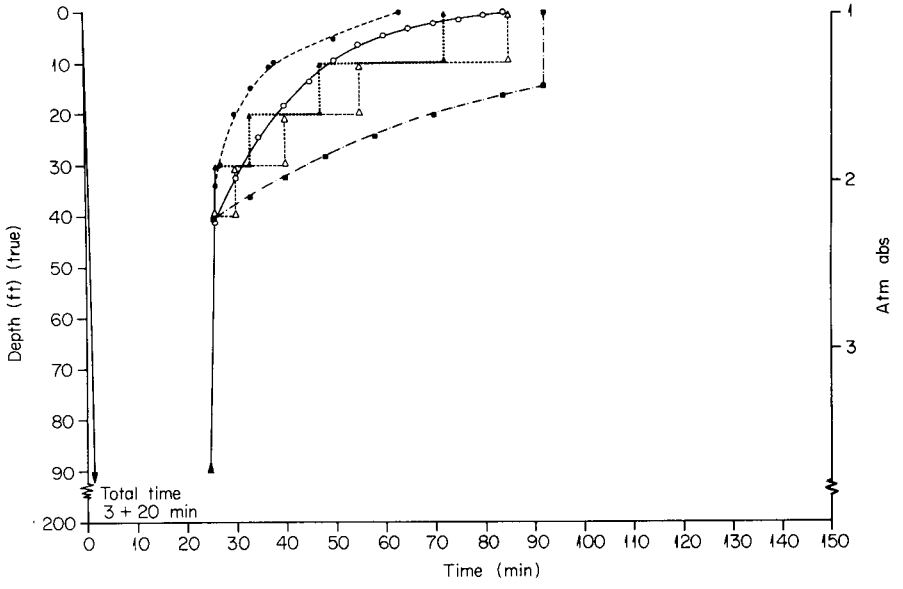

FIG. 4. Comparative decompression profiles for a 200-FSW dive.

200 ft 3 + 20 min air

Symbol	Profile	Precise dives	Bends II	Bends I
■	Modified Hills	28	—	2
△	RN table 1966	—	—	—
▲	USN table 1958	—	—	—
●	Workman M value (continuous)	8	—	3
○	VS computer	14	—	—

from depth proceeds until a depth of 14.5 FSW is reached, at which point the subject is brought directly to surface without further decompression procedures. The supersaturation ratio upon his surfacing corresponds to $K = 1.44$. The Workman M-value profiles were computed for continuous ascent by digital methods, based on the programs of Robertson and Moeller (14), and by analog methods similar to those of Buckles and Greenberg (2).

The dives of the first series—to 100 FSW with a descent time of 2 min and actual bottom time of 26 min (2 + 26) (Fig. 3)—produced no symptoms of decompression sickness in 61 dives in 15 subjects. In the next series of dives—to 200 ft for 3 + 20 min (Fig. 4) and to 300 ft for 5 + 25 min (Fig. 5)—differences in the effectiveness of the various profiles appeared. Although the number of exposures was small, the high incidence of decompression sickness indicating insufficient decompression seems conclusive.

It is interesting that the subjects decompressed according to the modified Hills decompression profiles had no symptoms of decompression sickness until the final increment of ascent to surface, shortly after which most of them complained of moderate to severe pruritus.

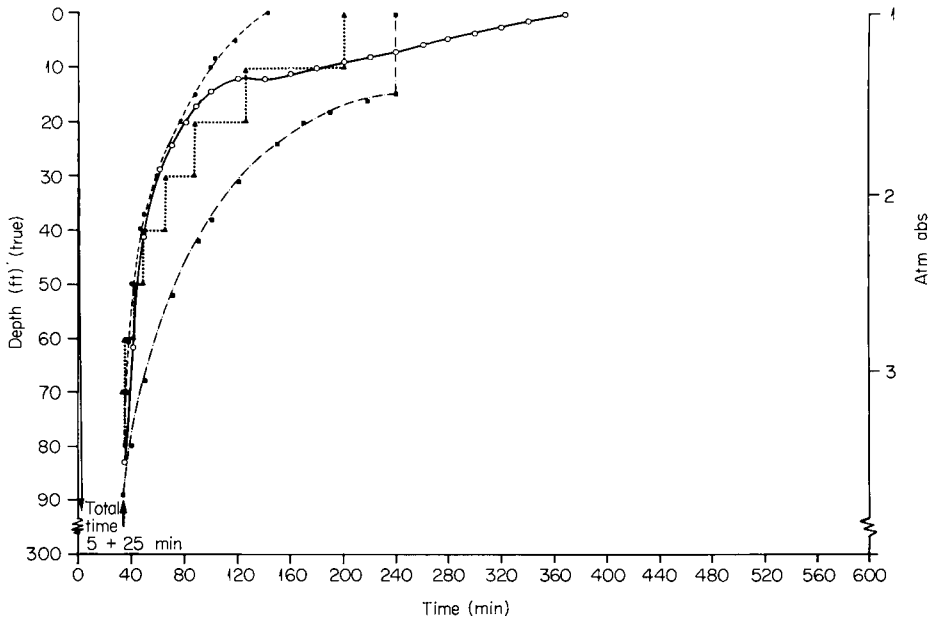

FIG. 5. Comparative decompression profiles for a 300-FSW dive.

300 ft 5 + 25 min air

Symbol	Profile	Precise dives	Bends II	Bends I
■	Modified Hills	5	—	4
▲	USN table 1958	6	1	2
●	Workman *M* value (continuous)	3	—	3
○	$\underline{\bar{V}}$S computer	10	—	2

Discussion

The results of these few dives emphasize the fact that prophylactic decompression still presents a problem in dives with an air atmosphere to depths much greater than 200 ft. Although a variety of decompression profiles was tested, none was completely effective; this fact underscores the lack of understanding of the fundamental etiological processes involved in decompression sickness.

Solving this problem requires careful investigation into at least four areas:

1. Are gas transfer processes *in vivo* linear or nonlinear?

2. What are the criteria for equilibrium between gases in solution and asymptomatic cavitation and what is the effect of such cavitation on gas elimination?

3. How does saturation ratio vary with depth?

4. What effect does the presence of other gases have on transfer of the individual gas constituents in the body? What is the influence of total and partial pressure gradients?

With respect to the third question, we have conducted 24-hr exposures at depth in order to reduce the effect of tissue time constant. The subjects then ascended according to a selected ratio K and stayed at the new depth long enough (i.e., 11 hr) to reveal any latent symptoms of decompression sickness. Surfacing directly from 33 FSW (K = 1.6) resulted in type I (pain only) bends (5, 10) in six of nine subjects, casting doubt on the validity of the long-duration pressure exposure data of Van der Aue *et al.* (21).

Surfacing directly from 26.4 FSW (K = 1.44) resulted in only one mild attack of type I bends among 14 subjects. We tested the validity of this ratio in exposures at 66 FSW. No symptoms were observed in four subjects during an 11-hr stay at 22 FSW. We intend to continue this experimental program at discrete increments of depth until the ratio becomes invalid.

With respect to the fourth item above, we conducted a simple experiment (see Fig. 6). A chamber containing air at 14.4 psia was separated by a porous membrane from another chamber containing 100% O_2 at 7.2 psia. A total pressure gradient of 7.2 psi was thereby opposed by a P_{O_2} gradient of 4.2 psi. The total gas pressure in the first chamber (P_T) and the P_{O_2} were measured continuously.

The P_{O_2} response was biphasic (first a fall, then a rise), indicating that the gas flows due to the total pressure gradient and the partial pressure gradients were interacting. The time

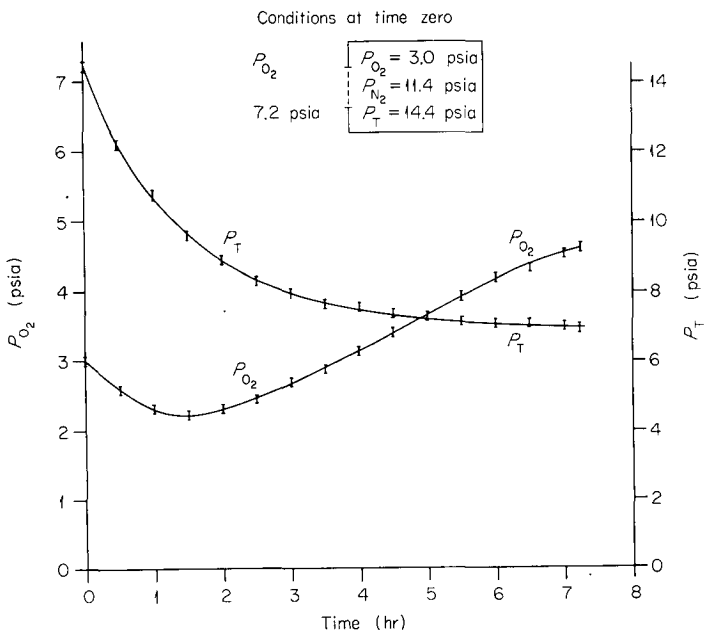

FIG. 6. Gas transfer slip flow and diffusion. Effect of interference between total and partial pressure gradients on gas transfer.

constant associated with the gas flow due to the total pressure gradient is shorter than that associated with the P_{O_2} gradient. Calculations using the P_T and P_{O_2} values indicate that the P_{N_2} in the first chamber initially fell at a slower rate than it would have if 7.2 psia of air had been the final condition. When the P_{O_2} gradient is less than the P_T gradient, the P_{O_2} will fall until these gradients are equal. Thereafter, as the P_{O_2} gradient exceeds the P_T gradient, the P_{O_2} will rise, and the rate of N_2 elimination will increase. Thus gas transfer cannot be calculated as though the gas constituents exist independently. If this effect occurs *in vivo*, the result of a transient fall in P_{O_2} might be critical. Similarly, this interaction must be considered in decompression schedules incorporating alternation of inert gases or changes in mole fractions of O_2 (12).

Clearly, basic research to answer all the above questions is an immediate necessity.

Conclusions

Some evidence exists to show that gas transfer in the tissues is nonlinear under hyperbaric conditions. The many linear models and systems discussed in this paper are special or simplified adaptations of a more complex general model. A pneumatic analog computer, which computes (in real time) directly from the breathing mixture, can be programmed for any of the decompression concepts described. Such a computer (11, 19)—programmed for nonlinear gas transfer with four equal time constant compartments in series and with a constant ratio ascent criterion—continues to offer a very acceptable real time solution to the problem of decompression sickness resulting from diving in an air atmosphere.

The computer's efficacy has been verified for exposures ranging from 24 hr at 26.4 FSW to 1 hr at 250 FSW, and for single and repetitive exposures of any shape. Our experimental diving program using a 20% O_2–80% He mixture has to date been restricted by the limitations of our facilities to half-hour exposures at 300 FSW. Experience with the same computer parameters suggests equal reliability, however, at this depth for longer periods of exposure.

The chief obstacle in the attempt to assess current decompression models and diving tables is the lack of published validated data. It is urged that a standard system for reporting the results of experimental diving and decompression table validation be devised for the mutual benefit of countries, organizations, and individuals concerned with diving.

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CALIBRATION OF INERT GAS EXCHANGE IN THE MOUSE

Edward T. Flynn, Jr. and C. J. Lambertsen

Although the advent of deep-diving systems has increased the relative comfort and safety of diving operations, the problem of providing safe and efficient decompression schedules continues to limit manned undersea activity. Of the many proposed methods of improving decompression tolerance, few have as yet been systematically investigated (7, 8). Time and logistic support requirements, as well as small population sizes, have limited these investigations in man. The studies to be described in this paper were conducted in an effort to assess the usefulness of the common laboratory mouse as an experimental animal in decompression studies. Although quantitatively the mouse is vastly different from man on an absolute basis, the relative effects of gas mixtures, decompression profiles, and drugs in the mouse might be quite similar to those in man. If so, experimentation with the mouse could provide a system in which the relative efficacy of many decompression techniques could be evaluated in sufficiently large numbers to permit statistical evaluation.

Method

Male albino mice weighing 18–22 gm were selected for investigation. Exposures were carried out in a small cylindrical chamber in which the temperature was controlled between 29 and 31°C. During compression and while at pressure, the O_2 tension was maintained at 0.3 atm. At 30–60 sec prior to decompression, the chamber was further compressed with pure O_2 to a depth sufficient to raise the O_2 concentration to 17.5%. Subsequent decompression was carried out maintaining this constant fraction of O_2. Carbon dioxide was absorbed by a centrally placed canister containing Baralyme.

Signs of decompression sickness usually appeared either during the decompression or within the first 5–7 min thereafter. Initial signs consisted of a rapidly developing monoplegia or paraplegia involving the lower extremities. In more severe cases, paraplegia was followed by the development of severe dyspnea, cyanosis, and prostration and by the onset of one or more convulsive seizures. Death followed in many cases. Recovery from all apparent signs of decompression sickness was the rule in almost all animals not killed acutely by the decompression. Disappearance of symptoms was often complete in as little as 15 min following their onset. Of the animals who apparently recovered, however, 13 and 30% (N_2 and He exposures,

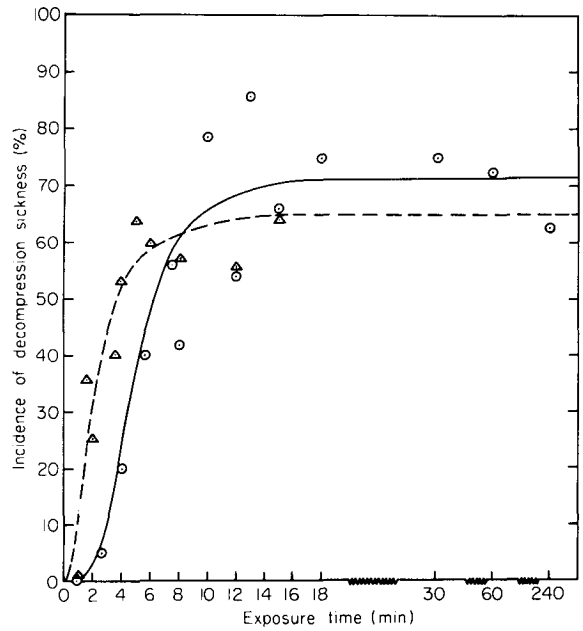

FIG. 1. Relationship of bends incidence to exposure time at 200 psig. Mice were exposed to 200 psig on N_2–O_2 and He–O_2 mixtures for varying periods of time, and then decompressed in 6–9 sec. The data points indicate the mean incidence of decompression sickness observed at each exposure time. (⊙) N_2–O_2; (△) He–O_2.

respectively) demonstrated progressive weight loss postdecompression and ultimately died within the 7-day observation period.

In general, death immediately following decompression could be attributed to cardiovascular collapse. In all cases, autopsy revealed massive gas embolization of the venae cavae and the right side of the heart. In more than 90% of the cases, bubbles were also present in the subcutaneous, mesenteric, epigastric, pelvic, and limb veins as well as the dorsal aorta. In contrast to these vessels, the pulmonary veins were often spared. Bubbles were seen in these veins in less than 30% of the animals autopsied.

Inert Gas Exchange

Initial studies were directed toward an analysis of inert gas exchange in the mouse. Groups of animals on N_2–O_2 and He–O_2 mixtures were subjected to progressively increasing exposure times at 200 psig, followed by rapid decompression to the surface in 6–9 sec. The exposure times, which included both the compression time and actual "on-bottom" time, ranged from 16 sec to 240 min. Compression times were rapid relative to the total exposure time in order to maximize the exposure at 200 psig. The most rapid compression time, 15 sec, appeared to be tolerated without difficulty.

Figure 1 shows the results of decompression from these exposures. The incidence of decompression sickness increased markedly as the longer exposures increased the absorption of inert gas, but then plateaued as saturation was approached. With both N_2 and He, no

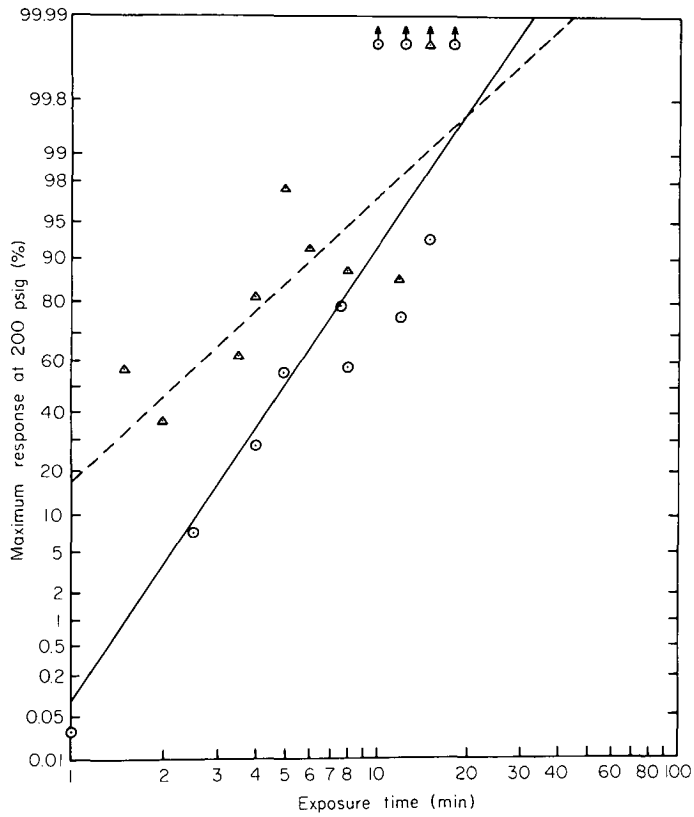

FIG. 2. Log-probability relationship of exposure time and response at 200 psig. The mean incidence of decompression sickness following 30, 60, and 240 min of exposure to N_2 and the mean incidence following 15 min of exposure to He (Fig. 1) were selected to represent the maximum response expected at 200 psig following an infinitely long exposure. The actual incidence at each exposure time (Fig. 1) was then plotted as a fraction of this maximum response. ED_{50} (effective dose producing decompression sickness in 50% of the animals tested) values for N_2 and He are 4.9 and 2.2 min, respectively. ED_{99} values are 16.1 and 14.5 min, respectively. (△) He–O_2; (⊙) N_2–O_2.

further increase in incidence was apparent beyond approximately 15 min of exposure. Figure 2 shows the transformation of these data to log-probability coordinates. The combined N_2 means at 30, 60, and 240 min (71%) and the He mean at 15 min (65.2%) were considered to represent the maximum attainable bends incidence on decompression from 200 psig. The observed incidence values were then plotted as a fraction of this response. Curves were fitted to the data points using the technique described by Finney (6). The N_2 and He bends incidence curves converge toward a common maximum incidence time, suggesting that whole-body saturation occurs at or near the same time for both gases. For the purpose of this study, saturation was assumed to be essentially complete at the 99% incidence level. Helium required 14.5 min to reach this level, N_2, 16.1 min. These times correspond to the 99% saturation times of 2.2- and 2.4-min "half-time tissues," respectively, and should represent the slowest rates of inert gas exchange in the major tissues of the mouse.

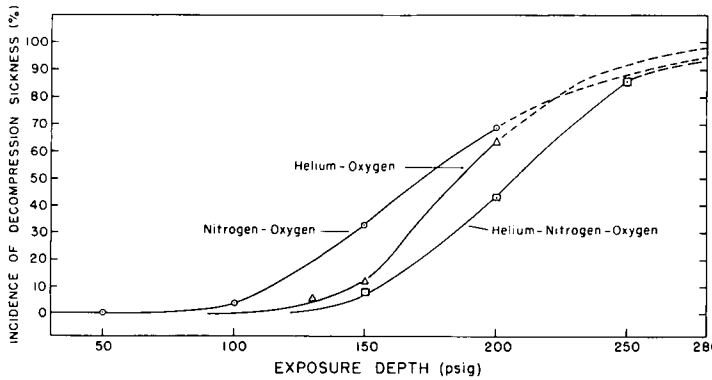

FIG. 3. Relationship of bends incidence to the depth of saturation exposure. Mice were allowed to saturate fully with N_2–O_2, He–O_2, and He–N_2–O_2 mixtures at exposure pressures ranging from 50 to 250 psig, and then decompressed in 2 to 10 sec. The data points indicate the mean incidence of decompression sickness observed at each exposure depth tested. Dotted lines represent projections to higher pressures based on information derived from Fig. 4. (⊙) N_2–O_2; (△) He–O_2.

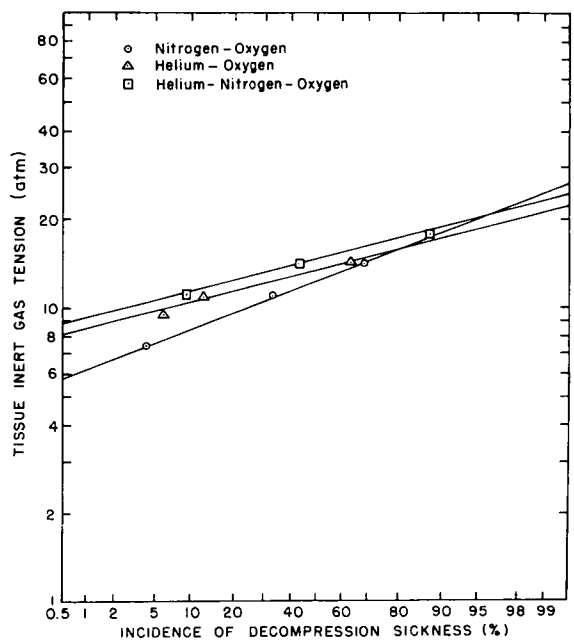

FIG. 4. Log-probability relationship of bends incidence to tissue inert gas tension following saturation exposures. The mean incidence of decompression sickness following saturation exposures at various pressures (Fig. 3), and the total tissue inert gas tension at these respective pressures are plotted on log-probability coordinates. ED_{50} values for N_2, He, and the multiple inert gas mixtures are 12.2, 13.4, and 14.6 atm, respectively. (△) N_2–O_2; (⊙) He–O_2.

With these derived outside limits for the tissue half-time spectrum defined, a second series of exposures was conducted to assess the individual contributions of tissues within this spectrum. Groups of mice breathing N_2–O_2 and He–O_2 mixtures were saturated at depths ranging from 50–200 psig, and then decompressed in 2–10 sec. The incidence of decompression sickness with each gas (Fig. 3) increased as a function of exposure depth along an S-shaped curve. At all depths tested, He produced less decompression sickness than N_2. When the data were transformed to log-probability coordinates (Fig. 4), a linear relationship between bends incidence and the tissue inert gas tension following saturation at a given depth was apparent for both gases.

Since the inert gas tension following saturation is uniform throughout the tissues regardless of the time courses of their approach to saturation, the curves of Fig. 3 provide a reference against which to compare the nonsaturation exposures in which the inert gas uptake by a tissue is a function of both the exposure time and the tissue-inert gas exchange time constant. Figure 5 compares the data for N_2. The regression line for the saturation exposures is identical to that shown in Fig. 4. On the same coordinates are plotted the incidence rates for four arbitrarily selected points on the 200 psig nonsaturation exposure curve (Fig. 1). The corresponding N_2 tensions were calculated from the exposure time at each point using the empirically estimated slowest tissue half-time of 2.4 min. There is a close correlation between the saturation and nonsaturation exposures, indicating that the observed behavior of the animal may be explained solely on the basis of changes in the 2.4-min tissue. If tissues having half-times faster than 2.4 min contributed significantly to the incidence of N_2 bends, there would be a systematic deviation of the data points of the nonsaturation exposures below and to the right of the regression line of the saturation exposures.

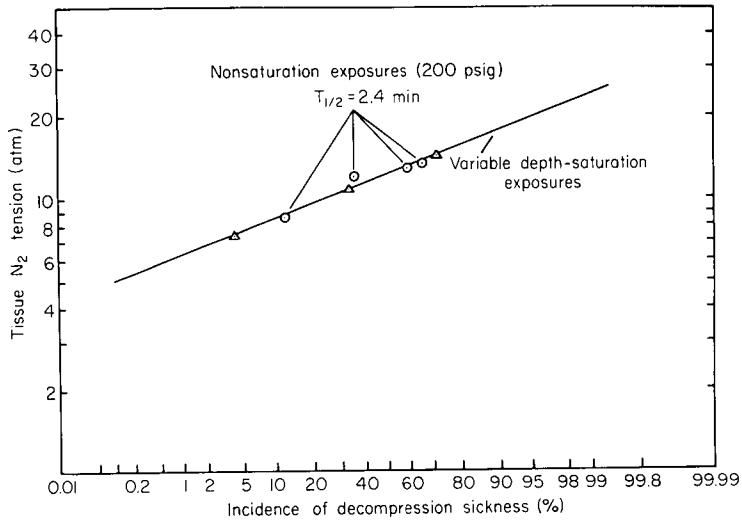

FIG. 5. Comparison of bends incidence and calculated tissue N_2 pressures in mice rapidly decompressed after saturation and nonsaturation exposures to N_2–O_2 mixtures. Triangular data points connected by a straight line represent the data of variable depth-saturation exposures (Fig. 4). The other data points represent arbitrarily selected incidence levels from the 200 psig nonsaturation exposure curve (Fig. 1). Tissue N_2 tensions for these points were calculated from their respective exposure times using a $T_{1/2}$ of 2.4 min.

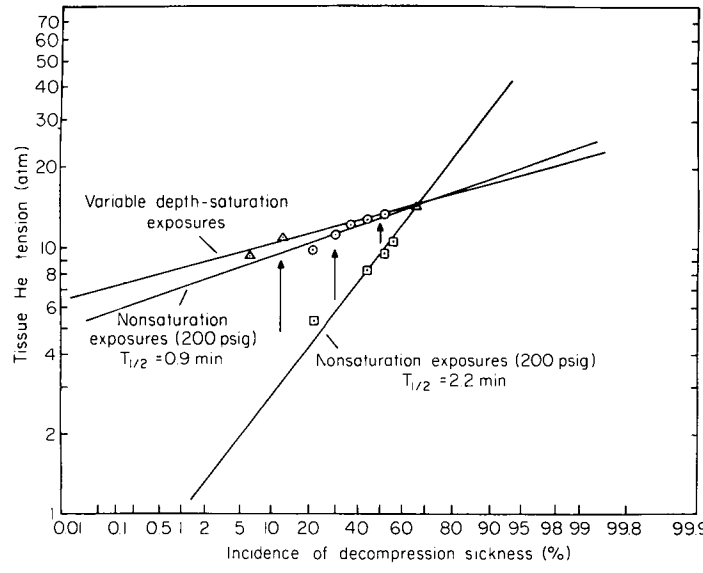

FIG. 6. Comparison of saturation and nonsaturation exposures in rapidly decompressed mice on He. Triangular data points connected by a straight line represent the data of variable depth-saturation exposures (Fig. 4). The other data points represent arbitrarily selected incidence levels from the 200 psig nonsaturation exposure curve (Fig. 1). Tissue He tensions for these points were calculated from their respective exposure times using $T_{1/2}$ of either 2.2 min or 0.9 min. The data point at 65% is common to all three curves.

Figure 6 compares the data for He. The regression line for the saturation exposures again is identical to that shown in Fig. 4. On the same coordinates are plotted the incidence rates for several arbitrarily selected points on the 200 psig nonsaturation exposure curve (Fig. 1). A very poor correlation between the data points is obtained when the estimated slowest tissue half-time of 2.2 min is used to calculate the He uptake during the nonsaturation exposures, indicating that tissues faster than this contribute significantly to the incidence of He bends. This is not an unexpected finding in that He reached 50% of its maximum response at 200 psig in slightly less than half the time required by N_2. The correlation is greatly improved when the incidence rates are related to the He tensions in a 0.9-min tissue.

The close fit of the data points obtained with a half-time of 0.9 min not only suggests that the bulk of the He tissues exchange at or near 0.9 min but also leads one to question the existence and/or importance of a tissue longer than this. Statistically, the lower limit of the 95% confidence interval for the 99% He response time (Fig. 2), 8.6 min, is longer than the 99% saturation time of a 0.9-min tissue. Accepting the probability of error denoted by a 95% confidence interval, it is possible to state that longer tissues do exist. However, the relative contribution of these tissues to the development of actual signs of decompression sickness is difficult to assess with certainty. The comparative analysis described above (Fig. 6) and visual inspection of the data (Fig. 2) suggest that the contribution is small, accounting only for somewhat more than 10% of the total response at 200 psig.

It is interesting to speculate why a change to a $He–O_2$ atmosphere causes at least two half-time tissues to appear (0.9 and 2.2 min), while only one tissue is discernible with a $N_2–O_2$ atmosphere. One possible explanation is that the apparent single 2.4 min N_2 tissue actually

represents a combination of a poorly perfused "aqueous" tissue and a rather briskly perfused "lipid" tissue. The N_2/He exchange ratios in these two tissue types would be expected to be close to 1 and 2.66, respectively, and the corresponding He half-times, therefore, 2.4 and 0.9 min. These half-times are quite close to the observed values.

In part, the usefulness of the mouse in decompression studies designed to obtain information directly pertinent to man will depend on how the relative tissue gas exchange rates for the various inert gases compare in mouse and man (e.g., N_2 vs He exchange rates in the same compartment). In spite of the gross difference in actual inert gas exchange rates for mouse and human tissue, the N_2–He exchange ratio describing the bulk of the murine tissues

$$\frac{\text{2.4-min half-time for } N_2}{\text{0.9-min half-time for He}} = 2.7$$

appears to be nearly identical to the N_2/He exchange ratio of 2.6 described by Bühlmann *et al.* (2) for the most slowly exchanging human tissues. The small fraction of He tissues in the mouse having a half-time of 2.2 min and, therefore, a N_2/He exchange ratio of 1.1

$$\frac{\text{2.4-min half-time for } N_2}{\text{2.2-min half-time for He}}$$

will exert their influence primarily in slow, low bends incidence decompressions, in which the faster 0.9 min tissue has had ample time to eliminate its excess He. The mouse therefore may lose its value for predicting the relative incidence of decompression sickness with He–O_2 and N_2–O_2 mixtures in man at these low bends incidence levels unless it is eventually found that a similar pattern of nearly equal inert gas exchange rates exists for N_2 and He in the slowest tissues of man. Such a tissue would consist of an extremely slowly perfused mass with inert gas solubilities similar to those of blood.

The finding of rapid tissue exchange times in the mouse was not unexpected. A high cardiac output and rate of capillary perfusion accompany the relatively high metabolic rate of the mouse and undoubtedly account in large part for the rapidity of gas exchange. While no data exist concerning the actual cardiac output of a mouse, an approximate value may be calculated from estimates of O_2 consumption, 3500 mm^3/gm/hr (9), and an assumed arterio-mixed venous O_2 difference of 4.5 ml/100 ml of whole blood. The cardiac output thus calculated for a 20-gm animal is 25.8 ml/min, or approximately 18 times the resting cardiac output per gram of tissue in man. If this high cardiac output is assumed to flow to a single tissue and the blood and the tissue inert gas solubilities are considered equivalent to those in water and olive oil, respectively, the resultant tissue half-times for N_2 and He, given by

$$T_{1/2} = \frac{0.693}{\dot{Q}(\alpha_B/\alpha_T)}$$

where

$\dot{Q}$ = tissue blood flow
α_B = inert gas solubility in blood
α_T = inert gas solubility in tissue

are 2.52 and 0.94 min, respectively. These values agree closely with those determined experimentally.

Due to the extremely rapid time constants for inert gas exchange in the mouse, studies of the decompression advantages of alternation of inert gases and/or O_2 will be technically difficult. The apparently bimodal distribution of inert gas exchange time constants in the mouse makes impractical a direct comparison of bends incidence in men and mice following nonsaturation exposures, since in man the distribution of gas exchange time constants appears to be continuous (10).

However, the existence of a single limiting tissue for N_2 may afford the opportunity to assess experimentally the influence of various vasoactive agents on tissue gas exchange. Changes in gas elimination half-time induced by such agents could be identified through comparison of saturation and nonsaturation exposures. If, however, as the data presented above suggest, the 2.4-min tissue actually represents two tissues characterized by different perfusion rates and blood–tissue partition coefficients, analysis of this type would be complicated by the possibility that changes in tissue blood flow induced by a drug might not be equal in these two or, in fact, any tissues.

Relationship of Small Animals to Man

It has been a common observation since the early experiments of Haldane (1) that small animals tolerate greater degrees of inert gas loading than do larger animals and man. Figure 7 presents a comparative analysis of the susceptibility to decompression sickness of men,

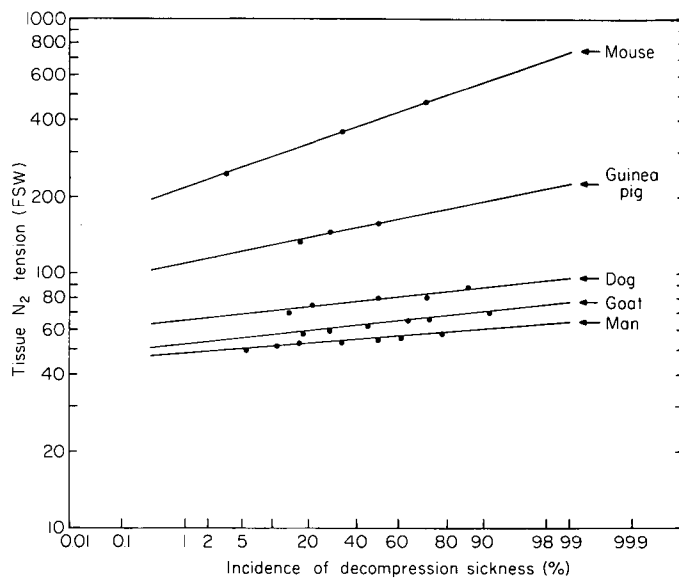

FIG. 7. Relative susceptibility to decompression sickness in animals and man. Following rapid decompression from saturation or near-saturation exposures on N_2–O_2 mixtures, the susceptibility to decompression sickness in man (3, 5), goat (4), dog (Reeves, personal communication, 1969), guinea pig (8), and mouse is shown as a function of the N_2 tension to which they were exposed. The data points for the mouse and guinea pig represent actual incidence values, while the data points for man, goat, and dog represent the cumulative incidence of individual bends in bends threshold determinations for the individuals in the series.

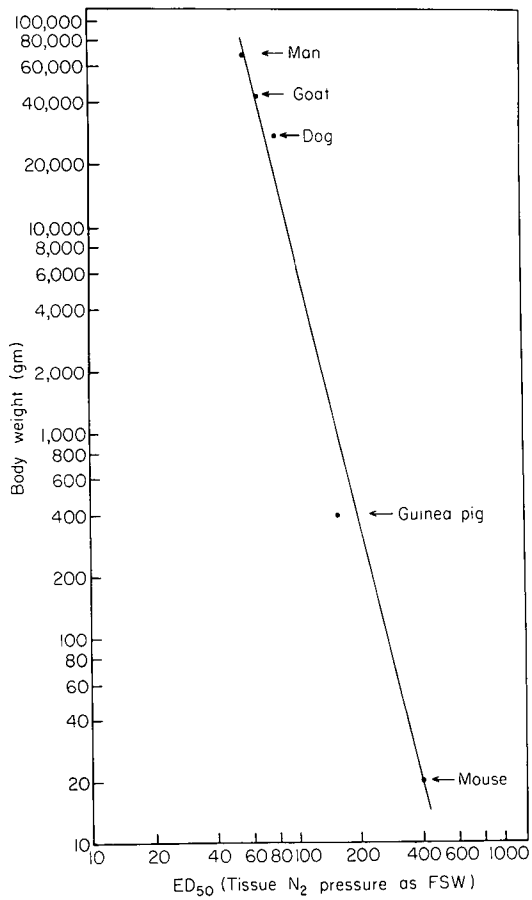

FIG. 8. Susceptibility to decompression sickness as a function of body weight. The dose of N_2 required to produce decompression sickness in 50% of the animals tested (Fig. 7) is plotted as a function of the body weight of each species.

goats, dogs, guinea pigs, and mice following saturation or near saturation exposures at various depths on N_2–O_2 mixtures (3–5, 8; Reeves, personal communication, 1969). Decompression in each case was directly to 1 atm and was rapid relative to the slowest inert gas exchange rates of the animal. In Fig. 7, exposure depth has been converted to the equivalent N_2 tension in the tissues following saturation with N_2 at that depth. The data points for the guinea pig and mouse represent the actual bends incidence values observed, while the data points for man, goat, and dog represent the cumulative frequency of bends in individual bends threshold determinations for the subjects or animals in the series.

The susceptibility to decompression sickness in each species is a linear function of the tissue N_2 tension on log-probability coordinates. Both the slopes of these dose–response curves and the tissue N_2 tensions required to produce a given bends incidence increase as the animal size decreases. A linear relationship also exists between the log of the ED_{50} for each animal and the log of its body weight (Fig. 8). A similar linear log–log relationship between the slopes

of these curves and body weight was suggested by the data, but the correlation was not as good. These species differences in decompression tolerance are undoubtedly related both to differences in inert gas exchange and to tolerance to the presence of gas separated as bubbles in tissue, but the interactions of such factors are far from clear. The clear relationship of body weight to decompression tolerance presented in Fig. 8 indicates, however, that these species differences reflect the degree of susceptibility to decompression sickness rather than a fundamental difference in the nature of the decompression syndrome. This relationship cannot be used to project quantitatively to man data obtained in small animals since small errors in the estimation of the slope of the projection will lead to unacceptable errors in the estimation of human decompression tolerance.

Decompression from Saturation Exposures to Multiple Inert Gases

In addition to the saturation exposures with N_2–O_2 and He–O_2 mixtures described above, mice were saturated at 150, 200, and 250 psig with a mixture containing equal fractions of N_2 and He. Decompression was performed in 7 to 10 sec. Figure 3 compares the findings for all three types of gas mixtures. Helium alone produced less decompression sickness than did N_2 at each depth tested. The multiple inert gas mixture produced less decompression sickness than did either He or N_2 alone. When the data were transformed to log-probability coordinates (Fig. 4), a linear relationship between bends incidence and the tissue inert gas pressure existed for each of the inert gas–O_2 mixtures. ED_{50} values for these curves are given in Table I.

The relative propensity of these gas mixtures for producing decompression sickness is qualitatively similar in mice and men (5, 10). On the basis of this finding, it may be expected that studies in mice with other single and multiple inert gas–O_2 mixtures will permit similar qualitative ranking of the relative decompression hazard of these gases in man. Such studies should also permit definition of the optimum mixture of two or more inert gases with O_2, for use during human exposures in which the subsequent decompression will be controlled by the relatively rapidly exchanging tissue compartments. Whether the quantitative relationships between two inert gas mixtures in the mouse can be transferred directly to man as well will depend on how closely the ratio of the ED_{50}'s for the gas mixtures and the ratio of the slopes of the two dose–response (tissue inert gas–bends incidence) curves in the mouse approximate those ratios in the comparable dose–response curves for humans. Although perfect agreement between the two species for all gases is not likely, evidence is available which indicates that the relative susceptibility of normal men to He and N_2 bends at the lowest detectable incidence level is quantitatively the same as that shown in Fig. 4 for mice. Duffner and Snider (5) deter-

TABLE I
VARIABLE DEPTH–SATURATION EXPOSURES

Gas mixture	ED_{50} (atm of inert gas)	95% Confidence limits (atm)
N_2–O_2	12.2	11.3–12.9
He–O_2	13.4	12.9–14.3
He–N_2–O_2	14.6	13.0–15.6

mined the minimum (threshold) depth required to produce decompression sickness in each of five divers following 12-hr exposures to air or to an 80% He–20% O_2 mixture. In each case, the subjects were decompressed directly to the surface at a rate of 25 ft/min. The threshold depth required to produce decompression sickness may also be expressed as a threshold inert gas pressure difference (Δp), where Δp is defined as the difference between the alveolar inert gas tension at the threshold depth and the ambient hydrostatic pressure at the surface. The mean threshold Δp for helium in these five subjects was 27.0 FSW, whereas the mean threshold Δp for N_2 was 20.4 FSW. The ratio of these two threshold Δp's is 1.32. A nearly identical Δp ratio of 1.33 is obtained in mice by projecting the dose–response curves of Fig. 4 to the "threshold" (1% incidence) level.

Slow Decompression in Mice

In addition to the experiments involving rapid decompression, mice were used in two studies in which the decompression was slow relative to the estimated rates of tissue gas exchange. In the first, eight groups of eight mice were saturated on a N_2–O_2 mixture at 200 psig and then decompressed exponentially at progressively decreasing rates. Total decompression times ranged from 9 to 607 sec. The incidence of decompression sickness decreased as decompression time increased (Fig. 9).

In a second study, five groups of eight mice were saturated on a N_2–O_2 mixture at 400 psig and then decompressed in a manner calculated to maintain the Δp in a theoretical 2.0-min tissue constant at values of 3, 5, 8, 10, or 12 atm. Δp was defined as the difference between the tissue N_2 tension at a given moment and the ambient hydrostatic pressure at the same moment. After an initial rapid pressure drop (2–6 sec) to establish the desired Δp, the remaining decompression was carried out in a series of 10-sec stages. The incidence of decompression sickness increased as an S-shaped function of the Δp used in the decompression (Fig.

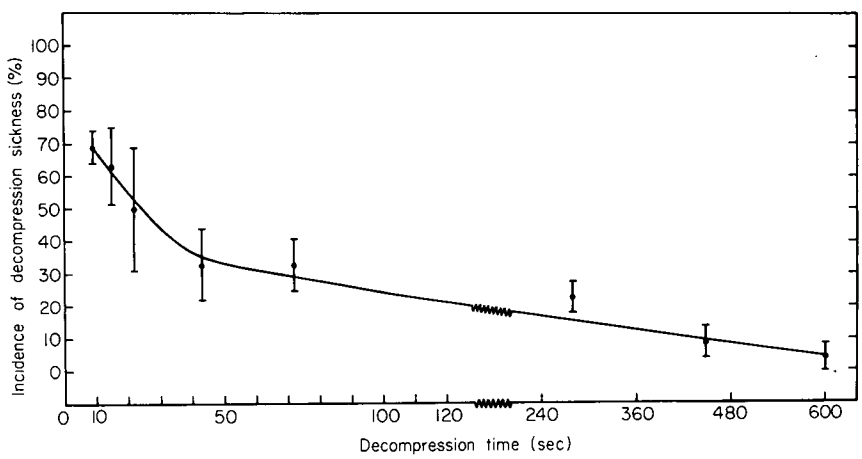

FIG. 9. Relationship of bends incidence to the time of exponential decompression from 200 psig. After saturation exposures at 200 psig on a N_2–O_2 mixture, mice were decompressed in exponential fashion to the surface. Total decompression times are indicated on the abscissa. The data points indicate the mean incidence of decompression sickness at each decompression time. Vertical brackets enclose ±1 standard error of the mean.

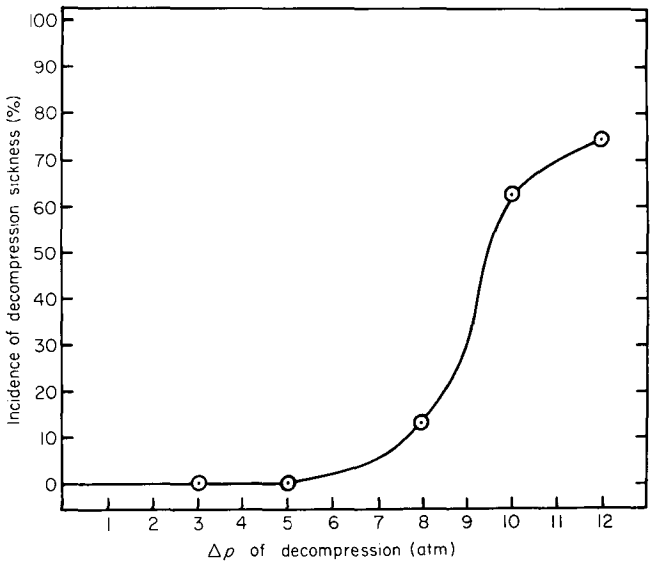

FIG. 10. Relationship of bends incidence to the Δ_p of constant Δ_p decompression. Following saturation exposures to a N_2–O_2 mixture at 400 psig, mice were decompressed on schedules calculated to make the quantity Δ_p in an arbitrarily selected 2-min half-time tissue constant. The Δ_p's of these decompressions are indicated on the abscissa. The data points indicate the mean incidence of decompression sickness observed at each Δ_p.

10). A similar, but inverted curve was obtained when the decompression time was substituted for Δp on the abscissa.

Although decompressions were carried out from different depths in these two studies, the results indicate that the use of mice will permit a comparative evaluation of different decompression profiles from the standpoint of either (*1*) relative bends incidence for a given decompression time, or (*2*) relative decompression times required to produce a given bends incidence. The direct application of these findings to man will depend on the relationships of the tissue inert gas–bends incidence curves obtained in men and mice. Such studies in the mouse should at least provide a means of predicting the rank order of efficiency of various decompression profiles in man, even though absolute values for decompression requirements are grossly different.

ACKNOWLEDGMENTS

This work was supported in part by grants (HE-08184 and HE-08899) from the National Institutes of Health and by a contract (NONR 551) with the Office of Naval Research.

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GAS NUCLEATION CONCEPT APPLIED TO DECOMPRESSION

G. Albano and M. Columba

Experiments on animals provide an excellent starting point for studying gas nucleation in man. Tests carried out on 156 guinea pigs affected by decompression sickness (3) allowed us to make the following observations:

1. Only major errors in decompression give rise to gross bubble formation and to death. In these experiments postmortem histological findings show great and confluent gas bubbles in almost all tissues without manifest connections with blood vessels (Fig. 1).

2. Localized damage ensues after precise and always reproducible patterns of exposure and

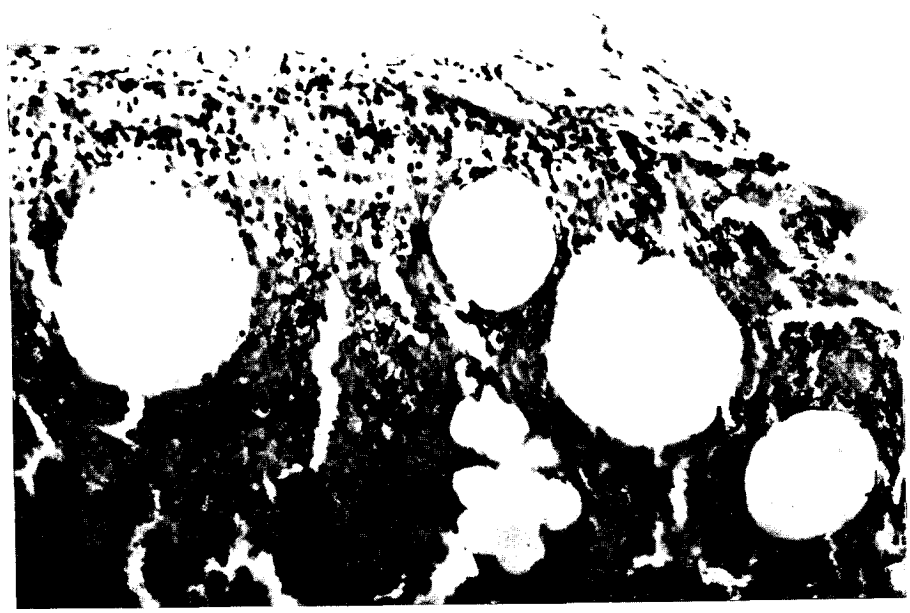

FIG. 1. Adrenal cortex of a guinea pig, exposed to air at 6 atm abs for 4 hr, who died 15 min after surfacing.

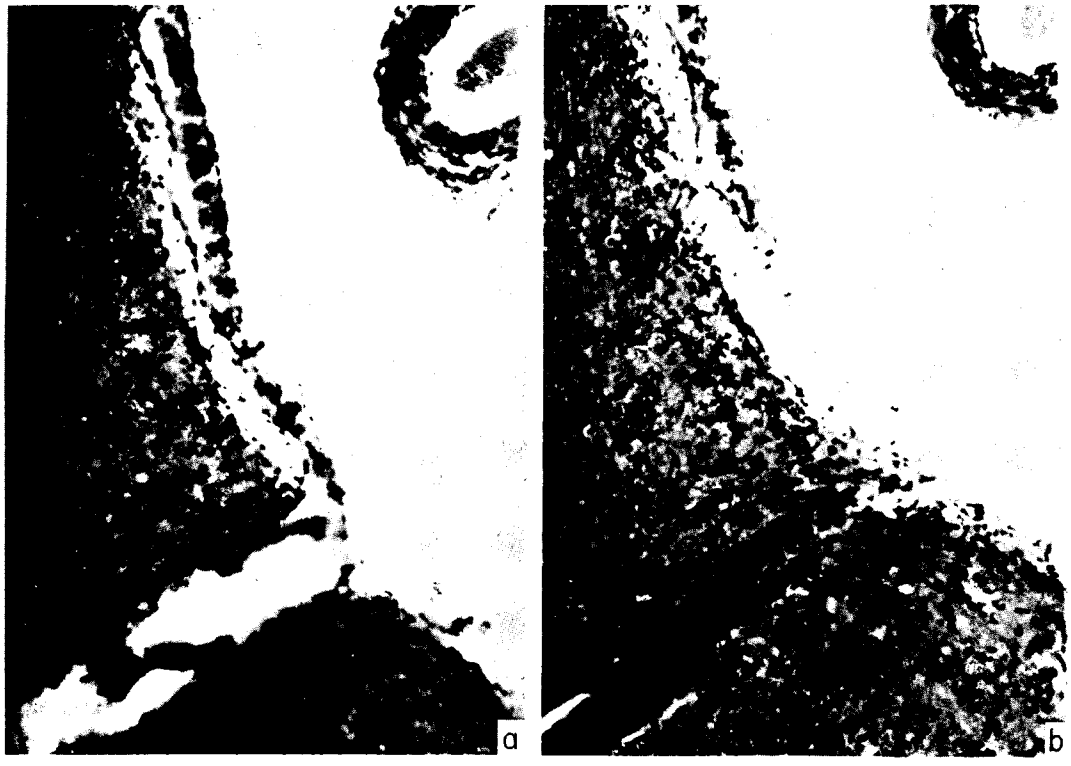

FIG. 2. Two slices of brain stem of a guinea pig killed 2 hr after occurrence of decerebrate rigidity with opisthotonus. The guinea pig developed the disease 15 min after surfacing from exposure to air at 8.5 atm abs.

decompression. This damage has special characteristics according to the specific tissue and animal species involved; some effects are also related to the sex of the animal, its body mass, and the degree of adaptation. Thus, in a selected group of animals it is possible to produce at will damage of the bones, the mesenteries, the spinal cord, and the brain stem.

3. If an animal is killed some minutes after localized damage has occurred, there are indications of an ischemic infarct due to obstruction of vessels on the venous side by gas emboli with secondary thrombi on the arterial side (Fig. 2).

4. However, if the animal is killed at the initial development of localized damage, one can often detect penetration into the vessels of small gas bubbles originating in the adjacent tissue mass (Fig. 3).

We believe that these four observations can also be applied to man. However, questions then naturally arise: what is the origin of gas bubbles, and what is the cause of their growth inside the blood vessels? The following discussion will attempt to answer these questions. The symbols used in the mathematical equations are defined in Table I. Equation Table 1 gives the basic formulas from which equations describing decompression under various conditions may be derived.

In 1944 Newton Harvey and co-workers (7) were able to ascertain that the pressure gradi-

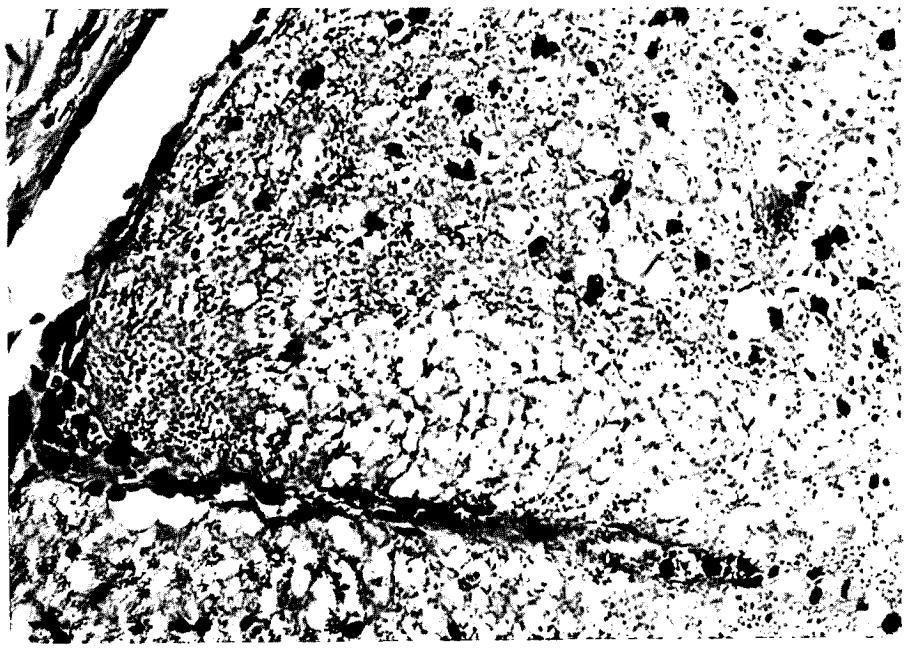

FIG. 3. Spinal cord (lumbar enlargement) of a guinea pig killed while developing hind quarter palsy due to decompression from 20-min air exposure at 8.5 atm abs.

ents, attainable by the decompression of man and animals, cannot cause the creation of *de novo* bubbles in blood and tissues. However, supersaturation by inert gas can produce growth of preexisting gas nuclei if the balance defined by Eq. (1) is exceeded (see Equation Table 1).

At about the same time Aggazzotti and Ligabue (1) tested the compressibility of dog tissues and were able to demonstrate that permanent gas nuclei not only exist in the solid tissues (although the nuclei are of different sizes), but also are subject to volume changes due to changes in environmental pressure (Boyle's law). Equation (2) shows that the whole tissue volume, Vt, is the sum of a gas-free fraction, Vf, and gas fraction, VG.

The main point of Harvey's theory (7) on gas nucleation is that the balance between the inside pressure of the gas nucleus, pi, and the pressure of gases dissolved in the tissue, pt, can be maintained [see Eq. (1)] only if the entire transfer of gas across the contact surface is accompanied by an appropriate change of the contact surface radius, r.

Applying these fundamentals to the gas nuclei adhering to a capillary wall, one sees that decompression (a decrease of the environmental pressure, H) will be unable to cause damage if the increase of the nucleus volume [Eq. (2)] creates inside the capillary a projection smaller than a hemisphere. Indeed, if the salient part of a gas nucleus is greater than a hemisphere, the transfer of gas to the inside of the nucleus will be accompanied by an increase in the radius and therefore by a further decrease of the pressure in the gas nucleus. The nucleus then progressively grows larger due to the presence of gas in supersaturation, originating a gas embolus.

It is known that the minimum decompression that produces bends of the long bones in subjects equilibrated at sea level is ascent to a simulated altitude of 9000 m (10). At this level

TABLE I

DEFINITION OF SYMBOLS

Symbol	Definition
k	Specific time constant for inert gas saturation in tissue
kp	Specific critical constant of gradient Δpi
m	Fraction of total gas pressure due to inert gas
p	Partial pressure of inert gas dissolved in tissue
pG	Sum of partial pressures of the gases dissolved in tissue, except the inert gas
pi	Pressure inside the gas nucleus or the bubble
pt	Sum of partial pressures of all gases dissolved in tissue
r	Radius of contact surface of the bubble
s	Surface tension (for the blood = 0.0352 mmHg)
t	Time in minutes
$t/2$	Half-life
u, w	Constants for calculation of pG, with breathing mixtures at constant percentage of inert gas
C	Specific time constant for mass exchanges in the gas nucleus at maximum depth
D	Time constant for mass exchanges in the gas nuclei during a decompression step
E	Constant of integration
H	Environmental pressure
P	Partial pressure of inspired (tracheal) inert gas
S	Safe plus value of gradient Δpi
V	Volume of the gas nucleus
Vf	Volume of the gas-free tissue
VG	Gas volume contained in the tissue at free state
Vt	Whole tissue volume
Δpi	Pressure gradient of gas nucleus
PI_{O_2}	Partial pressure of inspired (tracheal) oxygen
p_{H_2O}	Water vapor pressure (at 37° C = 47 mmHg)
pv_{O_2}	Pressure of oxygen in the venous blood
pv_{CO_2}	Pressure of carbon dioxide in the venous blood
e	Base of the natural logarithms
ln	Natural logarithm
π	Mathematical constant = 3.1416
$'$	Indication of the depth of first ascent
n	Indication of the nth decompression step
b	Indication of the bottom

Equation Table 1. Basic Formulas Describing Decompression

$$pi = H + (2s/r) \simeq pt \tag{1}$$

$$Vt' = Vf + VG(H°/H') \tag{2}$$

$$pt = p + pv_{O_2} + pv_{CO_2} + p_{H_2O} \tag{3}$$

$$\Delta pi^3 = [pt - (H' + 10)]^3 \simeq \frac{kp^3}{(H/H') - 1} \tag{4}$$

$$kp = 2s(2\pi/3V_0)^{1/3} \tag{5}$$

$$(pt - H' - 10 + S)^3[(H/H') - 1] = kp^3 \tag{6}$$

the hydrostatic pressure inside the venous capillaries is 10 mmHg greater than the environmental pressure (230 mmHg). At sea level the value of *pi*, equal to *pt*, results in a pressure of 698 mmHg [Eq. (3)]. Then the radius of critical gas nucleus surface [Eq. (1)] is 1.53 μ and the volume of the hemisphere is 7.6 μ^3. Consequently, the volume of the original cavity left outside the vessel amounts to 3.3 μ^3, according to principles established by Aggazzotti and Ligabue (1).

In the same way, since the minimal environmental saturation pressure able to give rise to bends at surfacing is 2.1 atm abs (4), the volume of the critical hemisphere has to be 3.63 μ^3, corresponding to a volume for the original gas nucleus of 3.3 μ^3 as in the preceding case. This leads to the unavoidable conclusion that *at saturation the gas nuclei, which give rise to the same decompression damage, have the same volume, regardless of the saturation pressure and, therefore, contain the same amount of gas.*

Therefore, with reference to the critical state for growth of nuclei, Eq. (1) can be written in the form of Eq. (4), where the critical constant kp is defined by Eq. (5). Solving Eq. (5) for the bends of long bones, one finds the kp value to be 605 mmHg. Finally, introducing into Eq. (4) the additional value S, useful for the calculation of a safe decompression, one obtains Eq. (6).

From this fundamental expression formulas applicable to different conditions of underwater and altitude decompression can be derived.

Examples of Decompression under Various Conditions

Equation Table 2 shows the equations which define calculation of the first ascent after saturation with a mixture at constant PI_{O_2}; Eq. (11) allows this computation. Table II gives the

Equation Table 2. First Decrease of Environmental Pressure after Saturation with Mixtures at Constant PI_{O_2}

$$P = H - p_{H_2O} - PI_{O_2} \tag{7}$$

$$pG = pv_{O_2} + pv_{CO_2} + p_{H_2O} = \text{constant} \tag{8}$$

$$pc = 10 + p_{H_2O} + PI_{O_2} - pG - S = \text{constant} \tag{9}$$

$$H_b - pc = H' + \left[\frac{kp^3}{(H_b/H') - 1}\right]^{1/3} \tag{10}$$

where $z = H'/H_b$

$$z^4 - \left(1 + 3\frac{H_b - pc}{H_b}\right)z^3 + 3\left[\left(\frac{H_b - pc}{H_b}\right)^2 + \frac{H_b - pc}{H_b}\right]z^2 + \left[\left(\frac{kp}{H_b}\right)^3 + 3\left(\frac{H_b - pc}{H_b}\right)^2 + \left(\frac{H_b - pc}{H_b}\right)^3\right]z + \left(\frac{H_b - pc}{H_b}\right)^3 = 0 \tag{11}$$

$$H' = zH_b \tag{12}$$

$$\frac{H' - 760}{75.485} = \text{depth in meters of seawater} \tag{13}$$

TABLE II

DECOMPRESSION AFTER SATURATION DIVES–BREATHING MIXTURE: HELIUM WITH CONSTANT PI_{O_2} OF 0.3 ATM, BETWEEN 60 AND 350 METERS OF SEAWATER[a]

Bottom		First ascent			Total ascent time with uniform speed	
Depth (m)	H_b (mmHg)	Depth (m)	H′ (mmHg)	Constant $k \cdot F'$ for continuous ascent	hr	min
60	5,289	45.97	4,230	3.0595	18	55
70	6,044	55.45	4,945	3.1724	22	00
80	6,799	64.98	5,665	3.2747	24	58
90	7,554	74.56	6,388	3.3661	27	52
100	8,309	84.16	7,113	3.4529	30	40
110	9,063	93.80	7,841	3.5310	33	26
120	9,818	103.48	8,571	3.6011	36	10
130	10,573	113.15	9,301	3.6734	38	46
140	11,328	122.85	10,033	3.7393	41	20
150	12,083	132.55	10,766	3.8036	43	51
160	12,838	142.26	11,499	3.8669	46	17
170	13,592	152.05	12,237	3.9137	48	53
180	14,347	161.85	12,977	3.9570	51	28
190	15,102	171.59	13,713	4.0126	53	48
200	15,857	181.31	14,446	4.0744	55	59
210	16,612	191.07	15,183	4.1259	58	16
220	17,367	200.90	15,925	4.1629	60	43
230	18,122	210.68	16,663	4.2130	62	55
240	18,876	220.52	17,406	4.2467	65	20
250	19,631	230.36	18,149	4.2805	67	43
260	20,386	240.15	18,888	4.3273	69	50
270	21,141	250.00	19,631	4.3600	72	09
280	21,896	259.85	20,375	4.3927	74	26
290	22,651	269.74	21,122	4.4155	76	52
300	23,406	279.58	21,864	4.4516	79	00
310	24,160	289.38	22,604	4.4952	81	00
320	24,915	299.20	23,345	4.5347	83	01
330	25,670	309.12	24,094	4.5519	85	27
340	26,425	319.00	24,839	4.5789	87	39
350	27,180	328.76	25,576	4.6306	89	20

[a] Equations 11–13 and 22: assumed $S = 55$ mmHg and $t/2 = 240$ min (5).

solutions to this equation after saturation at depths between 60 and 350 m of seawater with mixtures of He and 0.3 atm O_2.

Equation Table 3 shows the mathematical treatment for saturation with mixtures at constant percentages of inert gas. There, the sum of partial pressures of other gases dissolved in tissues, pG, is not constant, since it is dependent on the PI_{O_2} and on hemoglobin desaturation. Table III shows that, if the environmental pressure changes within narrow boundaries, the term pG can be considered a linear function of the environmental pressure H, according to

Equation Table 3. First Decrease of Environmental Pressure after Saturation with Mixtures at Constant Percentage of Inert Gas (m = constant)

$$P = m(H - p_{H_2O}) \tag{7a}$$

$$PI_{O_2} = (1 - m)(H - p_{H_2O})$$

$$pG = f(H) = u + wH \tag{14}$$

$$a = u + S - 10 - 47m \tag{15}$$

$$z^4 - \left[1 + 3\frac{H_b(m + w) + a}{H_b}\right]z^3 + 3\left\{\frac{H_b(m + w) + a}{H_b} + \left[\frac{H_b(m + w) + a}{H_b}\right]^2\right\}z^2 - \left\{3\left[\frac{H_b(m + w) + a}{H_b}\right]^2 + \left[\frac{H_b(m + w) + a}{H_b}\right]^3 + \frac{kp^3}{H_b^3}\right\}z + \left[\frac{H_b(m + w) + a}{H_b}\right]^3 = 0 \tag{11a}$$

Eq. (14). Then Eq. (11a) is used to compute the first ascent. Table IV shows the solutions of this equation after saturation with air at depths between 10 and 60 m of seawater.

We believe that the bends threshold curve established by Albano (2) in 1960 is of fundamental importance in the analysis of gas nucleation in unsaturated dives. This curve combines data on the minimal exposures at different depths in the open sea that can produce bends in man at surfacing. In Fig. 4 Albano's curve is compared to the curve obtained by Hempleman (9) on goats and that obtained by Hawkins and co-workers (8) on men in a wet tank.

Before analyzing the curve data, it is necessary to know the saturation rate of N_2 in the tissues at the ends of long bones when the exposure time is less than that required for saturation. To do this we may neglect the diffusion of N_2 from the nearby vessel-free cartilages (9).

TABLE III

DETERMINATION OF (pG) IN MIXED VENOUS BLOOD–BREATHING MIXTURE:AIR AT ENVIRONMENTAL PRESSURES BETWEEN 1 AND 7 ATM ABS[a]

Environ pressure (atm abs)	Percentage of O_2			P_{O_2} (mmHg)	Calculated pG (mmHg)	
	Linked to hemoglobin	Physically dissolved	Total		$p_{H_2O} + pv_{CO_2} + pv_{O_2}$	$126.27 + 0.0025 \cdot H$
1	13.188	0.112	13.302	34.0	127.0	128.1
2	14.045	0.122	14.167	37.0	130.0	130.0
3	14.500	0.130	14.630	39.5	132.5	132.0
4	14.856	0.136	15.092	41.4	134.4	133.7
5	15.415	0.140	15.555	42.6	135.6	135.6
6	15.873	0.144	16.017	43.8	136.8	137.5
7	16.329	0.151	16.480	45.9	138.9	139.6

[a] Assumed O_2 uptake of 6.605% and RQ = 0.8.

TABLE IV

DECOMPRESSION AFTER SATURATION DIVES. BREATHING MIXTURE:AIR BETWEEN 10 AND 60 METERS OF SEA WATER

Bottom		First ascent		Constants for continuous ascent		Total ascent time at decelerated speed	
Depth (m)	H_b (mmHg)	Depth (m)	H' (mmHg)	M'	θ	hr	min
10	1515	−0.27	739	540.38	2222.37	—	—
11	1590	0.43	792	547.58	2257.06	—	36
12	1666	1.12	844	555.10	2293.30	1	41
15	1892	3.24	1004	574.61	2387.30	4	31
20	2270	6.84	1276	602.17	2520.09	8	50
25	2647	10.51	1553	624.25	2626.48	12	41
30	3025	14.23	1834	642.97	2716.68	16	10
35	3402	17.98	2117	658.48	2791.41	19	26
40	3779	21.76	2402	671.83	2855.73	22	29
45	4157	25.56	2689	683.49	2911.91	26	38
50	4534	29.38	2978	693.77	2961.44	28	04
55	4912	33.22	3268	703.30	3007.36	30	39
60	5289	37.07	3558	711.92	3048.90	33	07

[a] Equations 11a–13 and 22a; S = 55 mmHg; assumed $t/2$ = 520 min (11); ϵ = 0.00028.

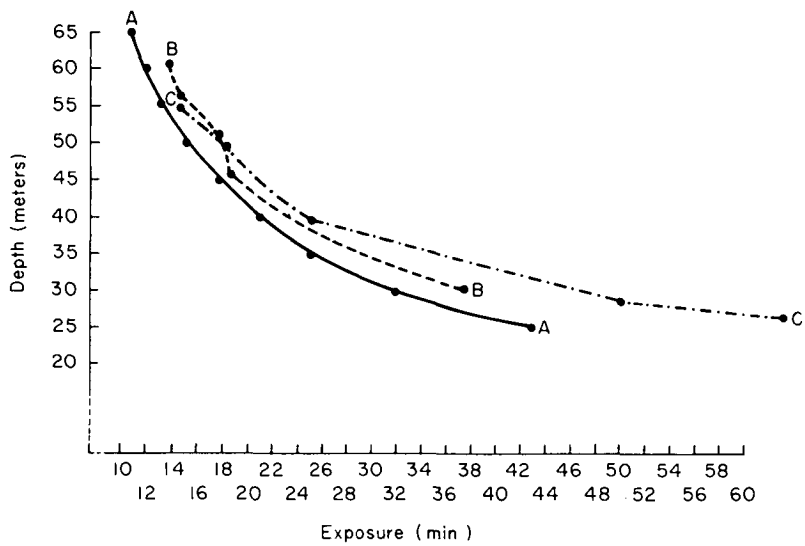

FIG. 4. Bends threshold curves for (A) men in open sea [from Albano (2)]; (B) goats in dry chamber [from Hempleman (9)]; (C) men in wet tank [from Hawkins *et al.* (8)].

TABLE V

JOINTS INVOLVED IN BENDS OF LONG BONES AFTER EXPOSURES AT 4.5 ATM ABS FOLLOWED BY DECOMPRESSION TO 1.635 ATM ABS IN 2 MIN

Subjects	Bends due to bottom times of				
	60 min	61 min	62 min	63 min	64 min
A. Gaspare	—	—	—	—	—
C. Calogero	—	—	—	—	Right hip
C. Ubaldo	—	—	—	—	—
D. T. Costantino	—	—	—	—	Both knees
M. Giovanni	—	—	—	—	Right knee
P. Andrea	—	—	—	—	—
P. Vincenzo	—	—	—	—	—
V. Giacomo	—	—	—	—	—

For this purpose eight subjects were exposed to air at 4.5 atm abs for different periods of time, followed by direct ascent to 1.635 atm abs (Table V). The results show that one can assume a half-life of 63.5 min for N_2 in long bones. A Haldanian (6) constant, k, of 0.0109 is useful for solving Eq. (16) in Equation Table 4. The fourth column of Table VI shows that Eq. (4) is not directly applicable to the bends curve. This was to be expected, since some time must elapse before the gas nuclei have returned to their original volume by absorption of gas during saturation (1, 7). Therefore, the coefficient $[(H/H') - 1]$ may be used in unsaturated dives only if one applies a corrective factor related to the saturation time. This factor can be expressed in an exponential form, similiar to the Haldanian saturation law with a time constant, C. Experimental and theoretical values of C are tabulated respectively in the fifth and sixth columns of Table VI. We can, therefore, compute C using Eq. (17).

We can then describe the calculation of the first ascent after unsaturated dives with mixtures at constant percentage of inert gases by equations in Equation Table 4. Of course, Eq.

Equation Table 4. First Decrease of Environmental Pressure after Unsaturated Exposures with Mixtures at Constant Percentage of Inert Gas ($m \equiv$ constant)

$$p_b = P_b - (P_b - p_0)e^{-kt_b} \tag{16}$$

$$C = k\left[1 + \frac{1}{5}\left(\frac{H_b}{H_0}\right)^2\right] \tag{17}$$

$$z^4 - \left[1 + 3\frac{H_b(m+w)+a}{H_b}\right]z^3 + 3\left\{\frac{H_b(m+w)+a}{H_b} + \left[\frac{H_b + (m+w)+a}{H_b}\right]^2\right\}z^2$$
$$- \left\{3\left[\frac{H_b(m+w)+a}{H_b}\right]^2 + \left[\frac{H_b(m+w)+a}{H_b}\right]^3 + \frac{kp^3}{(1-e^{-Ct_b})H_b^3}\right\}z + \left[\frac{H_b(m+w)+a}{H_b}\right]^3 = 0 \tag{11b}$$

TABLE VI

EXPERIMENTAL BENDS DUE TO MINIMAL EXPOSURES IN OPEN SEA FOLLOWED BY SURFACING[a]

Depth (m)	Exposure (min)	Δpi (mmHg)	$\left[\Delta pi^3\left(\frac{H}{H'}-1\right)\right]^{1/3}$	Determination of C	
				Experimental	$k\left[1+\frac{1}{5}\left(\frac{H_b}{H_0}\right)^2\right]$
25	43.0	488.4	661	0.0338	0.0373
30	32.0	458.1	659	0.0463	0.0454
35	25.0	432.6	655	0.0618	0.0546
40	21.0	423.9	671	0.0627	0.0648
45	17.5	402.1	662	0.0820	0.0761
50	15.0	385.8	658	0.0998	0.0885
55	13.0	372.7	656	0.1175	0.1019
60	12.0	378.2	680	0.0968	0.1165
65	10.5	361.3	673	0.1238	0.1321

[a] From Albano (2).

(11b) must be solved four times, once for each of the main (four) target tissues, using the constants of Table VII, discussed elsewhere (3). The higher value of z is of importance here.

At this point in calculating a decompression, a choice must be made between using a continuous or stage decompression. Equation Tables 5 and 6 describe the mathematical treatment of continuous ascent; Equation Table 7 shows the equations for stage decompression.

Continuous ascent is feasible when one breathes either a mixture with constant P_{O_2} or a mixture with constant percentage of inspired O_2. The gas projection inside the venous capillary will remain at the attained mechanical balance until its inside pressure, pi, is the same or greater than the sum of pressures of dissolved gases pt. This can be accomplished by decreasing the environmental pressure by an amount equal to the decrement of dissolved gas pressure according to Eq. (18) in Equation Table 5.

TABLE VII

VALUE OF SOME TISSUE CONSTANTS

Tissues	kp (mmHg)	S (mmHg)	For nitrogen and short exposures only	
			k	$t/2$ (min)
Bone	605	55	0.0109	63.59
Mesentery	1020	93	0.0158	43.87
Spinal cord	1035	94	0.0169	41.01
Brain stem	1270	115	0.0204	33.98

Equation Table 5. Continuous Ascent with Mixtures at Constant PI_{O_2}

$$pi - (H + 10) = pt_b - (H' + 10) = M' = \text{constant} \tag{18}$$

$$PI_{O_2} + p_{H_2O} = cp_0 = \text{constant}$$

$$pG = \text{constant}$$

$$p = (H - cp_0) - (H - cp_0 - p)e^{-kt} \tag{19}$$

$$p - P = pt - pG - H + cp_0 = M' + 10 - pG + cp_0 = F' = \text{constant} \tag{20}$$

$$\frac{dH}{dt} = -kF' \tag{21}$$

$$\Delta t = \frac{\Delta H}{kF'} \tag{22}$$

Then a continuous ascent with breathing mixtures at constant PI_{O_2} can be expressed by the general Eq. (21). The solution is simple [Eq. (22)]. In Table II (sixth and seventh columns) are shown the times together with the appropriate conditions. Such short ascent times are possible due to the large pressure head allowed by the first ascent.

If one breathes a mixture with constant percentage of O_2, the mathematical treatment is more complicated, as shown in Equation Table 6. The solution of the general equation is not linear, but exponential. This means that decompression must be carried out at decelerating speed, solving Eq. (22a) for closely spaced environmental pressures. The last two columns of Table IV show the overall ascent times after saturation with air at depths between 10 and 60 m of seawater.

The constant balance law imposed for continuous ascent by Eq. (18) is not applicable for stage decompression, since every step will give rise to a new gas projection into the venous

Equation Table 6. Continuous Ascent with Mixtures at Constant Percentage of Inert Gas (m = constant)

$$-\frac{dpG}{dt}\cdot\frac{dH}{dt} + \frac{dH}{dt} = -k[M' + pG + (1 - m)H + (10 - 47m)] \tag{21a}$$

$$\epsilon = k[(1 - m - w)/(1 - w)]$$

$$\xi = k[(M' - u + 10 + 47m)/(1 - w)]$$

$$\frac{dH}{dt} = \epsilon H + \xi$$

$$H = E\cdot e^{-\epsilon t} - (\xi/\epsilon)$$

$$E = H' + (\xi/\epsilon)$$

$$\vartheta = \xi/\epsilon = (M' - u + 10 + 47m)/(1 - m - w)$$

$$H = (H' + \vartheta)e^{-\epsilon t} - \vartheta$$

$$t = 1/\epsilon \ln[(H' + \vartheta)/(H' + \vartheta)] \tag{22a}$$

Equation Table 7. Stage Decompression

$$(pl_n - H_{n+1} - 10 + S)^3\left(\frac{H_n}{H_{n+1}} - 1 + B_n\right) = 0 \tag{6a}$$

$$B_n = A_n \cdot e^{-kD_n t_n} \tag{23}$$

$$A_n = \left(\frac{H_b}{H_n} - 1\right)(1 - e^{-Ct_b}) - \left(\frac{H_n}{H_{n+1}} - 1\right) + B_{n-1} - A_{n-1} \tag{24}$$

$$D_n = 1 + 2(H_{n-1}/H_n)^2 \tag{25}$$

$$a_n = P_n + pG_n - H_{n+1} - 10 + S \tag{26}$$

$$\beta_n = p_{n-1} - P_n \tag{27}$$

$$\gamma_n = \frac{H_n}{H_{n-1}} \tag{28}$$

$$(a_n + \beta_n e^{-kt_n})^3\left(\frac{\gamma_n}{A_n} + e^{-kD_n t_n}\right) - \frac{kp^3}{A_n} = 0 \tag{29}$$

capillary, while the effects of the preceding step are still present. Safe conditions for stage decompression can be expressed by Eq. (6a). The coefficient D has been approximated by experiments on man which will be reported elsewhere; Eq. (25) gives its empirical expression. The calculation of stage decompression is given by Eq. (29). This transcendental equation can be solved by trial and error. It is necessary to remember that the trial time, t_n), to be assumed depends on the constants k and kp selected according to the current concept of leading tissue (Table VII).

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Schreiner + Kelley

A PRAGMATIC VIEW OF DECOMPRESSION

H. R. Schreiner and P. L. Kelley

> "The whole meaning of a conception expresses itself in practical consequences, either in the shape of conduct to be recommended or in that of experience to be expected if the conception is true."
>
> William James, 1842–1910 (20)

Pragmatic Criteria of a Mathematical Decompression Model

Until the mechanisms of inert gas transport in the human body, of gas phase separation in the tissues and bubble growth therein, and of the pathophysiology of decompression sickness are fully understood, we have no choice but to develop decompression schedules by methods of trial and error. At the present state of our quantitative knowledge of the physiological and physical processes that are affected by a reduction in ambient pressure, this approach is basically sound. It becomes practical as well if it is guided by a systematic and well-controlled concept that will hold to a minimum the number of trials necessary and the number of errors that will be made in the generation of satisfactory decompression schedules through manned diving experiments.

To serve as useful guidelines, mathematical models of decompression must lead to practical consequences, in particular to decompression regimes that permit the most rapid rate of reduction of ambient pressure consistent with an acceptable level of risk of decompression sickness. Equally important pragmatic demands that must be met by a mathematical decompression model include an ability (*1*) to be tested experimentally, (*2*) to reflect fundamental physiological realities, (*3*) to account adequately for past decompression experience, (*4*) to predict with statistical accuracy the probable outcome of future decompression experiences, no matter how protracted or complex, and (*5*) to cope with a multiplicity of inert gases that may be breathed either simultaneously or sequentially.

Properly modified, the basic Haldanian model (3) of parallel tissue compartments meets every one of these demands. For the time being, it still provides the simplest and most reliable frame of reference for decompression experimentation and, in one form or another, has been used for the development of decompression schedules by the majority of workers in the diving field (4, 8, 12, 13, 22, 23, 25, 27–30, 33, 35, 37, 38).

To be sure, other basic mathematical decompression models have been advanced in recent years, some more elegant and perhaps more descriptive of the physiological processes involved in decompression (14–19), others more complex and speculative (5). We make no claims that the model which we are about to describe is truthful in the sense that it accurately depicts inert gas transport as it occurs in the human body. What we do claim is that this model is a useful and to us, at this time, an indispensable tool for the development of satisfactory decompression schedules.

Derivation of the Decompression Model*

We are basing our model on the frankly unproven assumption that the transport of inert gas to and from the body tissues is limited by the rate at which these tissues are perfused with blood, and not by the rate at which inert gas is being transported between the capillaries and the surrounding tissue. That is to say that we assume that on passage through the capillaries an equilibrium is attained between inert gas dissolved in the blood and inert gas dissolved in the surrounding tissue. In other words, the partial pressure of inert gas dissolved in blood leaving a capillary is identical with that dissolved in the tissue region surrounding the capillary.

We also assume complete equilibration of inert gas partial pressure between blood and the pulmonary alveoli so that, in this simple view, inert gas transport in the human body can be represented schematically as shown in Fig. 1.

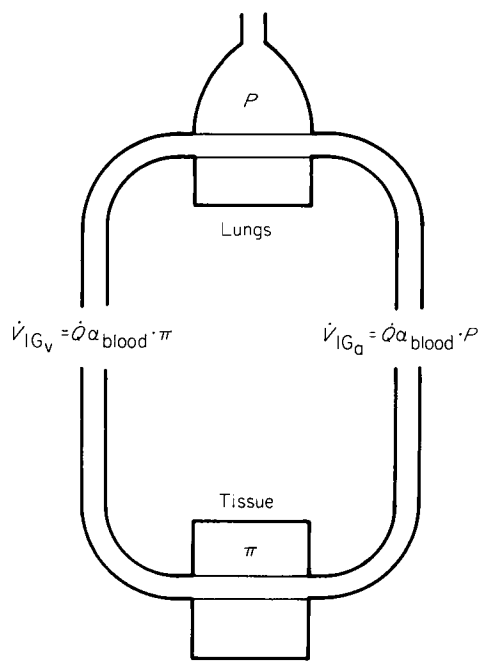

FIG. 1. Schematic presentation of the basic premises of the decompression model.

* Throughout this paper, pressure is expressed in terms of millimeters of mercury. One mmHg is defined as 1333.22 dynes/cm².

Given an alveolar inert gas partial pressure P and a tissue inert gas partial pressure π, the quantity of inert gas entering a tissue region of volume R per unit of time is given by

$$\dot{V}_{IG_a} = \dot{Q}\alpha_{blood} \cdot P \tag{1}$$

where*

$\dot{Q}$ = Rate of tissue perfusion (l^3t^{-1})
α = Inert gas solubility ($m^{-1}lt^2$)
P = Alveolar partial pressure of inert gas ($ml^{-1}t^{-2}$)

The quantity of inert gas leaving this tissue region per unit of time is given by

$$\dot{V}_{IG_v} = \dot{Q}\alpha_{blood} \cdot \pi \tag{2}$$

where

π = Partial pressure of inert gas dissolved in the tissue ($ml^{-1}t^{-2}$)

The quantity of inert gas dissolved in the tissue region at any given time is given by

$$V_{IG_{ti}} = \alpha_{tissue} \cdot R \cdot \pi \tag{3}$$

where

R = Volume of the tissue region (l^3).

The rate at which this quantity of dissolved inert gas changes with time is given by

$$dV_{IG_{ti}}/dt = \alpha_{tissue} \cdot R \cdot (d\pi/dt) \tag{4}$$

This rate is also equal to the difference in the rates of inert gas entering and leaving the tissue region under consideration, i.e.,

$$dV_{IG_{ti}}/dt = \dot{V}_{IG_a} - \dot{V}_{IG_v} \tag{5}$$

or

$$dV_{IG_{ti}}/dt = \dot{Q}\alpha_{blood} \cdot P - \dot{Q}\alpha_{blood} \cdot \pi \tag{6}$$

Rearranging Eq. (6) and combining it with Eq. (4) yields Eq. (7):

$$\frac{d\pi}{dt} = \frac{\dot{Q}}{R} \cdot \frac{\alpha_{blood}}{\alpha_{tissue}} (P - \pi) \tag{7}$$

which states that for a particular rate of blood flow and tissue composition (which determines the solubility of inert gas in that tissue), the rate of change of partial pressure of inert gas dissolved in a given tissue is at all times proportional to the difference between alveolar and tissue inert gas partial pressures, i.e.,

$$\frac{d\pi}{dt} = k(P - \pi) \tag{8}$$

The proportionality constant

$$k = \frac{\dot{Q}}{R} \cdot \frac{\alpha_{blood}}{\alpha_{tissue}} \tag{9}$$

* m = mass, l = distance, t = time.

represents the specific time constant of inert gas transport. It relates to the half-time of a particular inert gas exchange unit in the human body according to

$$k = 0.693/t_{1/2} \tag{9a}$$

The differential Eq. (8) is the basic inert gas transport equation which we discussed at the last symposium on underwater physiology (33). It implies, among other things, that inert gas exchange in the body tissues is symmetrical: for a particular tissue gas uptake proceeds at the same rate as gas loss under the influence of a given "driving force," i.e., partial pressure difference between that tissue and the alveoli. The general solution of Eq. (8) is given by

$$\pi = e^{-\int k dt}\left[\int P_e e^{\int k dt}\, dt\right] + C_1 e^{-\int k dt} \tag{10}$$

To solve this equation numerically for π, it is necessary to define the functional relationship between the alveolar inert gas partial pressure, P, and time. If the rate of change of P with time is constant (including zero), a numerical solution

$$\pi = P_0 + c(t - k^{-1}) - [P_0 - \pi_0 - (c/k)]\, e^{-kt} \tag{11}$$

where $c = dP/dt$, may be simply obtained. With the aid of Eq. (11) and a knowledge of the initial values of $P(P_0)$ and $\pi(\pi_0)$, it is therefore possible to compute the inert gas partial pressure π at any time during linear or stepwise descent or ascent for any tissue for which the value of k can be computed.

Computation of Specific Time Constants of Inert Gas Transport

Assuming that body tissues, in terms of their capacity to dissolve inert gas, can be represented by a variable mixture of fat and water, and further assuming that the solubility of inert gases in blood equals that in water, the specific time constant k for a particular gas can be derived from the relative solubility of an inert gas in fat and water, and from the fat fraction x of a given tissue, as long as the specific rate of tissue perfusion $\dot{Q}/R$ is known. Thus,

$$k = \frac{\dot{Q}}{R} \cdot \frac{\alpha_{\text{water}}}{x \cdot \alpha_{\text{fat}} + (1 - x)\alpha_{\text{water}}} \tag{12}$$

or

$$k = \frac{\dot{Q}}{R} \cdot \frac{1}{1 + x([\alpha_{\text{fat}}/\alpha_{\text{water}}] - 1)} \tag{13}$$

It is obviously impossible to compute values of k for every pair of tissue fat fraction and perfusion rate values in the human body. However, a fairly realistic representation of the infinite number of combinations possible can be obtained by limiting one's attention to the combination of but a few selected values of both parameters. By arbitrarily selecting four possible values of fat fraction x (0, 0.3, 0.7, and 1.0, denoting, respectively, 0, 30, 70, or 100% fat content) and four values of specific rate of tissue perfusion $\dot{Q}/R$ (0.3, 0.1, 0.03, and 0.0085

Specific rate of tissue perfusion ($\dot{Q}/R$)	Tissue fat fraction (X)			
min^{-1}	0	0.3	0.7	1.0
0.3	0	1	2	3
0.1	4	5	6	7
0.03	8	9	10	11
0.0085	12	13	14	15

FIG. 2. Derivation of inert gas exchange compartments by the arbitrary pairing of four specific rates of tissue perfusion and four levels of tissue fat fraction. The resulting compartments are numbered 0 to 15 as shown.

min^{-1})* in solving Eq. (13), one obtains a total of 16 different values of k representing 16 inert gas exchange *units* or *compartments*. These entities are not necessarily identifiable anatomical substructures of the body but rather represent assemblages of those regions within the human body that happen to be characterized by one and the same specific time constant of inert gas transport. These 16 inert gas exchange *compartments* (numbered 0 to 15 for ease of reference) are shown schematically in Fig. 2. It is immediately clear that any other arbitrary array of $\dot{Q}/R$ and x may be employed to derive gas exchange *compartments* as long as representative and minimal rates of the specific rate of tissue perfusion and extreme values of fat fraction are included.

For example, the traditional half-times for He of 5, 10, 20, 40, 80, 120, 160, 200, and 240 min employed by the U.S. Navy (37) can be obtained by selecting appropriate combinations of $\dot{Q}/R$ and x as shown in Fig. 3.

For this or any other array, the specific time constant k or half-time $t_{1/2}$ of inert gas transport can be calculated for each inert gas for which the coefficient of distribution between fat and water ($\alpha_{fat}/\alpha_{water}$) is known. The half-times for the 15-*compartment*† array which we are using have been calculated for a number of inert gases. Values for He ($\alpha_{fat}/\alpha_{water} = 1.7$), Ne (2.1), N_2 (5.1), and Ar (5.3) are shown in Fig. 4.

* Specific rates of tissue perfusion in the human body range from about 0.01 to 5 min^{-1} (21). Rates greater than about 0.3 min^{-1} give rise to extremely fast inert gas exchange *compartments* that need not be considered in decompression computations. A minimum value of $\dot{Q}/R$ of 0.0085 min^{-1} was selected to enhance the probability that the array chosen will under most circumstances include the slowest inert gas exchange *compartments* of the human body.

† For most inert gases of interest in diving, *compartment* 0 yields half-times so small that they need not be considered in decompression calculations.

Specific rate of tissue perfusion ($\dot{Q}/P$) min^{-1}	Tissue fat fraction (X) 0	0.189	0.595	1.0
0.1386	5			
0.0693	10			
0.0347	20			
0.01964		40		
0.00982		80		120
0.00491		160	200	240

FIG. 3. Derivation of conventional half-times of He transport by the pairing of appropriate specific rates of tissue perfusion and levels of tissue fat fraction. Each compartment could have been derived alternately by an infinity of other combinations of these two parameters.

Specific rate of tissue perfusion min^{-1}	Tissue fat fraction (X) 0	0.3	0.7	1.0
0.3	$\frac{He}{N_2}\,\frac{Ne}{Ar}$	$\frac{3}{5}\,\frac{3}{5}$	$\frac{3}{9}\,\frac{4}{9}$	$\frac{4}{12}\,\frac{5}{12}$
0.1	$\frac{7}{7}\,\frac{7}{7}$	$\frac{8}{15}\,\frac{9}{16}$	$\frac{10}{27}\,\frac{12}{28}$	$\frac{12}{35}\,\frac{15}{37}$
0.03	$\frac{23}{23}\,\frac{23}{23}$	$\frac{28}{52}\,\frac{31}{53}$	$\frac{34}{89}\,\frac{41}{93}$	$\frac{39}{118}\,\frac{49}{122}$
0.0085	$\frac{81}{81}\,\frac{81}{81}$	$\frac{99}{182}\,\frac{108}{187}$	$\frac{122}{315}\,\frac{145}{327}$	$\frac{139}{416}\,\frac{171}{432}$

$$t_{1/2} = \frac{\ln 2}{k} = 0.693 \cdot \frac{P}{\dot{Q}}\left(1 + X\,\frac{\alpha_{fat}}{\alpha_{water}} - X\right)$$

FIG. 4. Half-times in min of transport of He, Ne, N_2, and Ar in the 15 compartments of the decompression model.

It is readily seen that the "bloodlike" *compartments* 4, 8, and 12, for which x is assumed to be zero, exchange inert gases at a rate that does not depend on the distribution coefficient $\alpha_{fat}/\alpha_{water}$ of a given inert gas. For these inert gas exchange *compartments all* inert gases give rise to the same half-time of inert gas transport. For these compartments, in fact, the specific time constant of inert gas transport equals the specific rate of tissue perfusion. As the fat fraction x increases, the computed half-times of inert gas transport become more and more sensitive to the magnitude of the distribution coefficient $\alpha_{fat}/\alpha_{water}$. Inert gases showing very great solubility in fat relative to that in water would therefore tend to be exchanged at very slow rates in fatlike body tissues. For example, we would expect the half-time for Xe ($\alpha_{fat}/\alpha_{water} = 20$) transport in *compartment* 15 to be 1400 min, or roughly 1 day; the equivalent half-time for SF_6 ($\alpha_{fat}/\alpha_{water} = 50$) would be 3500 min.

When more than one inert gas is present in the human body—which, incidentally, is almost invariably the case in all O_2–He dives or altitude flights, since in most instances neither the length of exposure nor environmental conditions make possible the complete removal of N_2 from the body—we would expect each gas to be transported in a manner that is independent of the presence of any other inert gas or gases. Thus the total inert gas pressure in a particular inert gas exchange *compartment* would be the sum of the partial pressures of all inert gases present, even though they may differ in the specific time constant of their transport.

In view of the fact that the U.S. Navy is traditionally employing a half-time of 240 min to characterize the slowest transport of N_2 in the body, the use in our model of a compartment (No. 15) with a N_2 half-time of 416 min (cf. Fig. 4) could well be questioned. In washout studies on human subjects breathing O_2 at sea level, Lundin (24) identified a slow tissue compartment with a half-time of 102–200 (mean: 138) min, a finding which did not take into account uptake of N_2 by these subjects through the skin. Groom and Farhi (9) have recently shown that the half-time of the slowest N_2 washout compartment in the dog increases by a factor of 2.5 when cutaneous diffusion of atmospheric N_2 is eliminated during N_2 washout. Applying this observation to Lundin's finding would yield a corrected slowest half-time for N_2 transport of approximately 255–500 (mean: 345) min. The results of recent studies by Bühlmann *et al.* (4) are consistent with the assumption that the slowest half-time for nitrogen in the human body is in the order of 420–480 min. The selection of our compartment No. 15 therefore seems to enjoy some support from the experimental evidence available.

Computation of Alveolar Partial Pressure of Inert Gas at Low Ambient Pressure

For the computation of inert gas tissue tensions at the ambient pressures encountered in diving, no significant error is introduced by the assumption that the alveolar partial pressure of an inert gas is equal to its inspired partial pressure. In decompression to altitude, the effect of alveolar CO_2 and water vapor must be considered in computing the alveolar partial pressure of inert gas.

The PA_{N_2} is related to the inspired fraction of nitrogen, FI_{N_2}, PA_{CO_2}, PA_{H_2O}, respiratory quotient, R_Q, and total ambient pressure, P_B, by the alveolar N_2 equation (26)

$$PA_{N_2} = FI_{N_2}\left(\frac{PA_{CO_2}(1 - R_Q)}{R} + P_B - PA_{H_2O}\right) \tag{14}$$

By substituting standard values of R_Q(0.8), PA_{CO_2} (40 mmHg) and PA_{H_2O} (47 mmHg),

Eq. (14) reduces to

$$PA_{N_2} = FI_{N_2}(P_B - 37) \tag{15}$$

Assuming that the alveolar N_2 equation can be applied to inert gases in general ($FI_{N_2} = FI_{IG}$), the value P in Eqs. (1, 6, 7, 8, 10, and 11), correctly termed PA_{IG}, is given by

$$PA_{IG} = FI_{IG}(P_B - 37) \tag{16}$$

Ascent-Limiting Values of Partial Pressures of Dissolved Inert Gases

General deep-diving experience (30) does not invalidate the assumption that inert gases will remain in solution in the tissues of the body even if the ambient pressure is reduced to a point where it is lower than the partial pressure of the dissolved inert gas. The extent of this supersaturation that can apparently be maintained without inflicting injury seems to increase with increasing ambient pressure, and with increasing specific time constant (decreasing half-time) of inert gas transport. It also seems to vary with the nature of the inert gas present (1, 2, 6, 11, 32). Workman (37, 38) has systematized the concept of permissible levels of supersaturation by suggesting for different levels of ambient pressure, and for two inert gases, He and N_2, maximum (or M) values of π that can apparently be tolerated in each of several inert gas exchange compartments of the human body without injury. Figure 5 shows Workman's M values for He adapted by graphic interpolation to the inert gas exchange compartments of our decompression model.

Specific rate of tissue perfusion ($\dot{Q}/P$)	min^{-1}	Tissue fat fraction (X) 0	0.3	0.7	1.0
	0.3		2119 (+1.55ΔP_B)	2119 (+1.55ΔP_B)	2045 (+1.53ΔP_B)
	0.1	1856 (+1.46ΔP_B)	1796 (+1.43ΔP_B)	1704 (+1.40ΔP_B)	1642 (+1.38ΔP_B)
	0.03	1488 (+1.28ΔP_B)	1446 (+1.24ΔP_B)	1409 (+1.22ΔP_B)	1382 (+1.20ΔP_B)
	0.0085	1290 (+1.20ΔP_B)	1267 (+1.20ΔP_B)	1244 (+1.20ΔP_B)	1244 (+1.15ΔP_B)

FIG. 5. Ascent-limiting values of partial pressure of He in the 15 compartments of the decompression model. These values were adopted from Workman's M values by graphic interpolation and represent partial pressures of dissolved He limiting ascent to a total pressure of 760 mmHg. With increases in total ambient pressure, the ascent-limiting values of π_{He} increase by increments shown in parentheses. ΔP_B indicates increase in ambient pressure above 760 mmHg.

Ascent-limiting values of tissue inert gas tensions may also be expressed as ratios of the partial pressure of dissolved inert gas to total ambient pressure. For example, in Fig. 5 the M value of 2,119 mmHg for compartment 2 for an ambient pressure of 760 mmHg may alternately be stated as a ratio of 2,119/760 or 2.79. M values increase with increasing ambient pressure. Workman suggests that the rate of this increase is linear, and that it is greatest for the fastest inert gas exchange compartments. These rates are indicated in Fig. 5 as incremental additions to the M value for 760 mmHg ambient pressure. For compartment 6, for example, the M value of 1704 mmHg must be increased by 140 mmHg for every 100 mmHg increase in ambient pressure; thus the M value for this compartment increases at a rate that is 1.4 times that of the increase in ambient pressure.

Experimental Testing of the Decompression Model

By reducing the ambient pressure in a manner so as not to exceed at any time prescribed maximum values of π in any one of the several inert gas exchange compartments of the decompression model, it is possible to test suggested M values experimentally.

The procedure generally followed is to select a particular set of M values, compute decompression schedules that permit ascent without exceeding them at any time, and conduct decompressions accordingly. If an incident of decompression sickness occurs during the reduction in ambient pressure, the computed values of π are analyzed up to the moment the first symptoms are reported in an effort to pinpoint partial pressures of dissolved inert gas that may have been too large to permit safe ascent. Most suspect are values of π that are close to ascent-limiting M values. The appropriate M values are then reduced, and a new schedule is computed and tested. With repeated and accurately controlled decompression experiments, the mathematical decompression model is refined by feeding back into it experimentally sustained M values.

Analysis of Past Decompression Experience

All adequately monitored decompression experiments, not only those conducted specifically for the purpose, can be utilized to test and refine the decompression model. For example, in the now classical chamber dive performed by Professor Bühlmann in December 1967 (personal communication), two divers were decompressed after being exposed to a total pressure of 22,433 mmHg (29.5 atm) for 2 hr. On reaching a total pressure of 12,136 mmHg, one of the subjects complained of hearing loss and nausea, and was promptly and successfully given recompression therapy.

By utilizing the basic gas transport equation, it is possible to calculate the values of partial pressure of dissolved inert gas (essentially He) sustained during this particular decompression in the several compartments of the model. Using Workman's M values as a basis for comparison, one finds that the computed values of π at the moment signs of decompression sickness were noted were smaller in each of the 15 compartments than the corresponding M values for He extrapolated to the ambient pressure of 12,136 mmHg. This observation, which is recorded in Fig. 6, may be interpreted in two ways: either Workman's M values for the ambient pressure under consideration are too large, especially for compartments 11, 12, and

Specific rate of tissue perfusion ($\dot{Q}/P$)	Tissue fat fraction (X)			
min^{-1}	0	0.3	0.7	1.0
0.3		+7947	+8539	+8413
0.1	+6114	+6408	+6176	+5695
0.03	+2330	+1628	+970	+433
0.0085	+219	+71	+2052	+2178

FIG. 6. Values of $M - \pi$ (Workman's ascent-limiting M value minus partial pressure of dissolved He) on arrival at a total pressure of 12,136 mmHg during decompression from a 2-hr exposure to 22,433 mmHg (29.5 atm).

13, or values of π sustained during earlier stages of this particular decompression may have been too large. Further analysis shows that values of π_{11} did exceed Workman's M values on arrival at two previous decompression stops, 13,754 and 13,018 mmHg. This is shown in Fig. 7. Even though many of Workman's M values have not been experimentally verified, they are based on past diving experience and have proven to be very useful in our hands as general guidelines for the testing of our decompression model. It would therefore be our first recommen-

Total pressure (P_B)	M_{11}-π_{11} on arrival at P_B
15446	+252
14710	+ 40
13754	- 143
13018	- 95
12136	+433

FIG. 7. Values of $M - \pi$ (Workman's ascent-limiting M value minus partial pressure of dissolved He) in compartment No. 11 of the decompression model on arrival at five different stops during decompression from a 2-hr exposure to 22,433 mmHg (29.5 atm).

dation to reduce the rate of ascent in this particular dive in such a way as to arrive both at 13,754 mmHg and at 13,018 mmHg with values of π_{11} that are equal to or smaller than the corresponding Workman M values. Only if this course of action failed would we recommend reducing Workman's M values for the ascent-limiting inert gas exchange compartments.

The calculation of partial pressures of dissolved inert gas or gases in several inert gas exchange compartments during the time course of a given decompression is most efficiently carried out by digital computer. If ascent-limiting values of π are to be derived by the analysis of past decompression experience, the use of a high-speed digital computer becomes mandatory. In this manner it is possible to analyze large numbers of well-documented decompression experiences, compute values of inert gas partial pressures sustained during decompression in the several compartments of our model, and to store this information together with relevant information describing both the subject and the decompression experience. As a significant body of information accumulates in such a data bank, it becomes possible to ascertain the probability of risk of decompression sickness associated with sustaining a particular value of π in a given inert gas exchange compartment at a given total pressure. With such data at hand, further direct testing of the decompression model can then be limited to the elucidation of safe maximum values of π for compartments and total pressures for which no or insufficient past decompression experience is available.

Physiological Variables of Concern

Up to this point we have tacitly assumed that inert gas transport and the metastable aspects of supersaturation can be quantitatively described by our decompression model. This, of course, is not true. The rate of tissue perfusion, a critical factor in our model, is not likely to remain constant during a particular decompression experience. Many factors, such as increased tensions of O_2 or CO_2, exercise, temperature, and psychogenic stimuli, to mention but a few, have an effect on the state of vasodilatation and hence on the specific rate of tissue perfusion (31). It would be unrealistic to expect any mathematical model to cope quantitatively with these effects. However, qualitative estimates of changes in tissue perfusion can be made and they can be reflected in the values of $\dot{Q}/R$ that are used in computing inert gas tissue tensions. Professor Bühlmann (personal communication), for example, extends the half-times of selected tissues to take into account nocturnal reduction in the rate of perfusion in decompressing subjects. Dr. Workman (personal communication) employs arbitrarily increased alveolar inert gas partial pressures in calculating decompression schedules, to cope with the problem of vasoconstriction caused by the breathing of O_2 at high partial pressures. In either instance the operative change is a decrease in the values of $\dot{Q}/R$ for several or all inert gas exchange compartments.

Differences in individual susceptibility to decompression sickness and acclimatization to decompression are well known in tunnel workers (15) and divers (7). These, too, can be accommodated by our decompression model by the selection of particularly conservative (low) M values for the computation of decompression schedules for individuals showing a high susceptibility to decompression sickness, and conversely, the employment of exceptionally high M values may be appropriate to develop decompression schedules for individuals who have demonstrated unusual resistance to decompression sickness. It is also possible, and with high-speed electronic computers quite simple and inexpensive, to develop entire families of

decompression schedules based on increasingly demanding sets of M values that may be used progressively by divers as they become acclimated to decompression.

Computation of Decompression Schedules

Once a particular set of M values has been selected as the basis of computation of a decompression schedule, Eqs. (11) and (13) are combined and solved for π for each value of k for each continuous segment of pressure increase. This operation yields a value of π for each of the 15 compartments of our model at the beginning of decompression. Immediate reduction in pressure can then commence to that total pressure where the first of these 15 values becomes identical to the M value prescribed for the total pressure and compartment in question. The gas transport equation is then solved for t for compartments with a half-time equal to or greater than that of the ascent-limiting compartment, by selecting for the value of π in the inert gas transport equation the appropriate M value prescribed for the total pressure associated with the next ascent step. The greatest value for t so calculated determines the length of the decompression stop that is required before ascent to the target pressure can be safely attempted. This process is repeated until the final target pressure of ascent is reached. In this manner it is possible to compute decompression schedules for all time-depth profiles (including dives performed at altitude, ascent to altitude after diving, repetitive dives, saturation and saturation–excursion dives, etc.), always providing that the rate of change in alveolar inert gas parial pressure, dP/dt, remains a constant.

Schedules based on experimentally verified M values have been computed for the commercial purposes of Ocean Systems, Inc., for O_2–He dives to depths ranging from 200 to 450 FSW (total pressure: 5366–11,124 mmHg or 7.06–14.64 atm). After more than 200 manned exposures in the open sea we are experiencing an overall rate of decompression sickness of 2.3% on these schedules. The repetitive saturation–excursion decompression schedules for Operation Ludion II (10) are another example of the pragmatic utility of our approach. In this particular experiment two divers performed a total of 12 consecutive 2-hr dives each from a total pressure of 7205 mmHg (9.48 atm) to 9960 mmHg (13.1 atm) without incidence.

Predicting the Probable Outcome of Future Decompression Experience

The reduction in ambient pressure which occurs in flights to altitude generally follows a protracted period of residence at ground level, and hence represents the decompression of tissues saturated with nitrogen at a partial pressure of about 580 mmHg. Saturation decompressions are limited by the extent of supersaturation that can be safely sustained in the slowest inert gas exchange compartment of the body. The human body can therefore be treated as a single inert gas exchange compartment (compartment 15 in our model) and it therefore becomes relatively easy to accumulate information from which to predict the probable outcome of future decompression experience. In one recent analysis of 315 records of altitude decompression of subjects exposed to N_2–O_2 mixtures (34, 36) we were able to relate the risk of decompression sickness to the value of π_{15} for N_2 on arrival at three target pressures. This is shown in Fig. 8. At this early stage of analysis no differentiation has been made between incidents of decompression sickness of different degrees of severity such as paresthesia, vasomotor

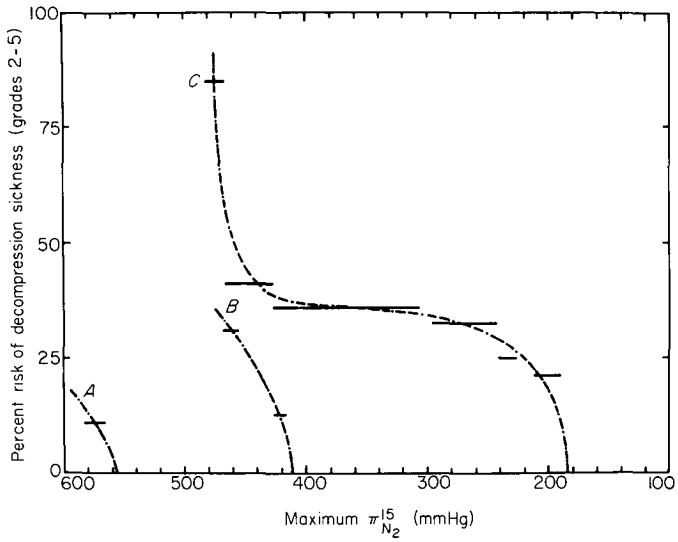

FIG. 8. Risk of decompression sickness as a function of computed tissue N_2 tension in the slowest (saturation ascent-limiting) compartment (No. 15) of the decompression model, and target pressure. Target pressure range (mmHg): (*A*) 350–400; (*B*) 250–300; (*C*) 150–200.

abnormalities, skin rash, blurred vision, and similar mild manifestations that are likely to be signs of decompression sickness (grade 2) on the one hand and severe pain (grade 5) on the other. Nevertheless, it is possible to predict from this information the probability of incurring some symptoms of decompression sickness in future decompressions to altitude if, on arrival at a particular target pressure, a given value of π_{15} prevails. For example, application of the gas transport equation for compartment 15 shows that decompression to a total pressure of 181 mmHg following 3 hours of preoxygenation at ground level produces a value of π_{15} of 426 mmHg. As can be seen from Fig. 8, this value of π_{15} on arrival at 181 mmHg is associated with a 35% risk of incurring signs of decompression sickness.

Similar predictions of the probable outcome of future decompression experiments can be made in all cases where a data bank has collected sufficient information from past decompression experiences to make a statistical treatment of the data at hand meaningful.

Conclusions

We have prescribed a pragmatic model of decompression that lives up to all expectations that one can reasonably have of such a concept. It yields decompression regimes that permit the most rapid rate of reduction of ambient pressure consistent with an acceptable level of risk of decompression sickness; it can be tested and refined experimentally; it reflects fundamental physiological realities such as the importance of tissue perfusion and individual differences in susceptibility to decompression sickness; it accounts adequately for past decompression experience; it predicts with statistical accuracy the probable outcome of future decompression experience and can cope with a multiplicity of inert gases that may be breathed simultaneously or sequentially.

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DECOMPRESSION IN SATURATION DIVING

A. A. Bühlmann

We started our deep-diving experiments, as amateurs, with the concept that inert gas half saturation times are related to the perfusion of the tissues with blood; that the half-time for a given tissue and the rate of perfusion are determined by inert gas diffusion; and that diffusion depends upon the physical properties of the particular gas, especially its molecular weight.

Despite the fact that some of our original concepts were erroneous, the experiments conducted with H. Keller between 1960 and 1962 were successful (3). In these experiments, pressures up to 1000 FSW were used, physical work was performed in a wet chamber, and short swimming excursions were made in the sea. The extremely short decompression times used—for instance, 270 min after an exposure of 5 min at 1000 ft—were the result of a fast descent, a high O_2 pressure in the range of 2–2.5 atm during the whole dive, and switching from He to N_2 breathing mixtures during the decompression.

Between 1964 and 1966 (2) we were able to confirm the differences between the saturation rates of He and N_2 by dives at 4.0 atm abs. The longest tissue half-time seems to be 480 min for N_2 and 180 for He, as estimated by sufficient decompression to avoid an attack of decompression sickness and by a supersaturation ratio of 1.5 to 1.6:1 (Fig. 1).

After bottom times of 20–72 hr at 4 atm abs while 20% O_2–80% He was breathed, decompressions were made without an incidence of bends by eight subjects breathing 100% O_2, by two subjects breathing 60% O_2–40% He, and by six subjects breathing 30% O_2–70% N_2. After diving exposures on air for 42–48 hr, decompressions were made by eight subjects breathing 70% O_2–30% N_2 (2).

The differences in the saturation and desaturation speed for a constant blood flow in the slowest fatty tissue can be explained by the difference in the oil–water solubility ratio of N_2 and He. The difference in the pressure equilibration rates in watery tissues seems to be determined by the molecular weight of the particular gas, similar to the diffusion-limiting concept. To date, our decompression schedules for mixed-gas decompression have been calculated with a ratio of 2.645:1, N_2 to He, which corresponds to the ratio of the square roots of the molecular weights of N_2 and He.

When there are changes in the activity of divers during long decompressions after deep diving (such as sleeping, resting, and work), the total and regional blood flows vary consid-

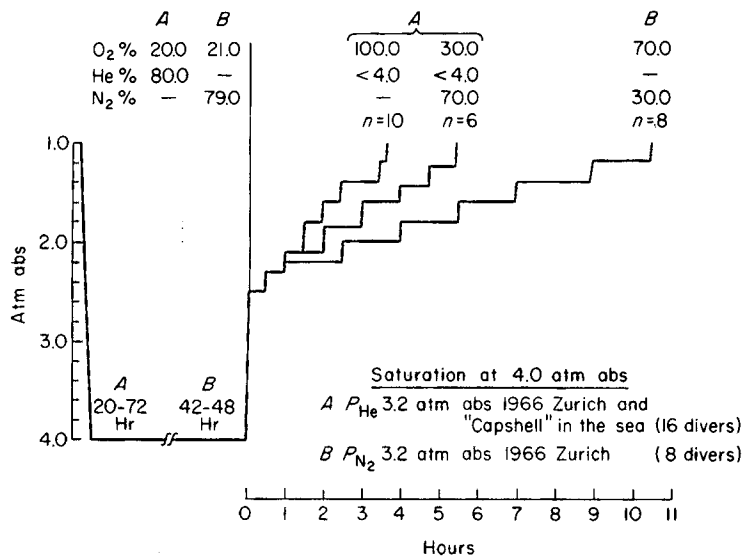

FIG. 1. Saturation at 4.0 atm abs (1 atm abs = 1 kg/cm² = 735 mmHg).
A. 20–72 hr with 20% O_2–80% He. Decompression time: 215 min with 100% O_2 or 60% O_2–40% He, followed by 330 min with 30% O_2–70% N_2.
B. 42–48 hr with air. Decompression time: 630 min with 70% O_2–30% N_2.

erably. Our calculations are therefore based on 240 min as being the longest He tissue half-time, and 640 min the longest N_2 half-time (1).

It has been determined through preliminary experiments with bottom times of up to 6 hr at 23 atm abs that the supersaturation ratio of 1.6:1 suggested by Haldane is too large for deep dives, and that the same ratio is not applicable to all tissues. The supersaturation ratio has to be drastically reduced, especially for the slow tissues that control the whole decompression schedule following saturation dives (5, 6). Our present experience in tolerated supersaturation ratios, gathered from saturation dives made between 1967 and 1969 at 23 and 31 atm abs with excursions to 36 atm abs, is set out in Fig. 2.

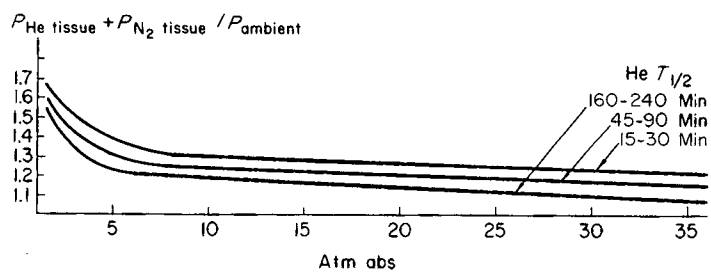

FIG. 2. Tolerated supersaturation factor (ordinate) for fast tissues with He half-times to 30 min; for slower tissues, from 45 to 90 min; and for the slowest tissues, from 160 to 240 min, correlated with the total inert gas pressures in the respective tissues (abscissa). The stages of decompression were calculated according to these supersaturation factors.

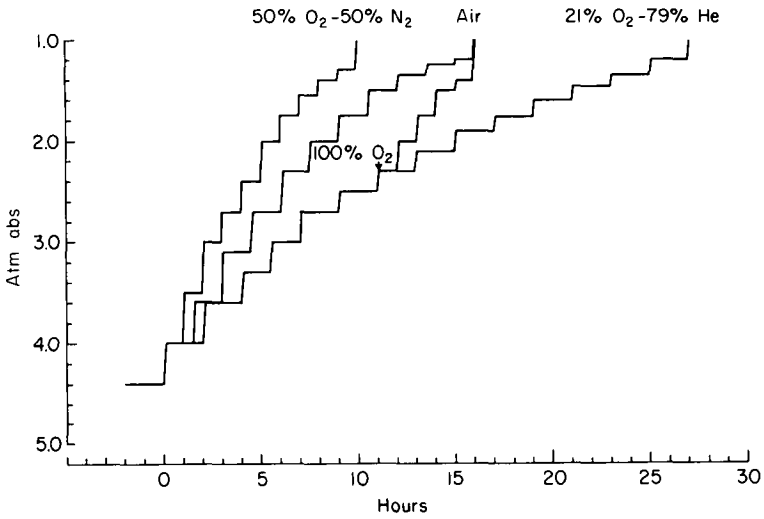

FIG. 3. Final decompression below 4.0 atm abs after saturation diving with O_2–He mixtures. 27 hr with 21% O_2–79% He (not performed); 16 hr with 21% O_2–79% He and with 100% O_2 below 2.4 atm abs (not performed); 16 hr with air below 4.0 atm abs; 10 hr with 50% O_2–50% N_2 below 3.3–4.0 atm abs.

So-called "vertigo bends" can occur if the limits—45–90 min—shown on the middle curve of Fig. 2 are exceeded. The lower curve represents the slow tissues, such as those about the joints, in which the more typical attack of bends occurs. To determine the supersaturation ratios, the inert gas partial pressures of He and N_2 have to be added together. In all our experiments, final decompression was calculated on an approximate ratio of 1.5:1.

After deep diving with exposures lasting some hours to saturation, decompression was simplified by putting O_2, He, and air directly into the pressure chamber. This method eliminated the need for preliminary preparation of gas mixtures and the need for breathing apparatus. The decompression procedures are identical once the same inert gas pressure of He and N_2 is reached in the tissues with a He half-time of 180–240 min. The P_{O_2} is below an average of 1 atm with a short and transient increase above 2 atm when a mixture of 50% O_2–50% N_2 is used. In all our chamber experiments, we maintained a comfortable temperature and a relative humidity of 70–90%, and eliminated practically all CO_2 from the chamber.

We performed two versions of the final decompression below 4.0 atm abs, first with a breathing mixture of 50% O_2–50% N_2, and second with air. The time difference is only 4–6 hr. We now prefer to avoid the risk of O_2 poisoning by using air in the final stage of decompression, and we accept He contamination of up to 5% in the chamber atmospehre (Fig. 3).

Based on the same supersaturation ratios, calculations indicate that the final stage of decompression would take 27 hr when the breathing mixture is 21% O_2–79% He, as opposed to 16 hr when air is breathed. However, we have never performed a total decompression using only O_2–He with a low O_2 concentration. The frequency of minor attacks of bends, some of which disappeared without treatment, was not high, but shows that we have neared the limit in shortening decompression time.

We have performed three saturation dives at 23.0 atm abs without decompression troubles.

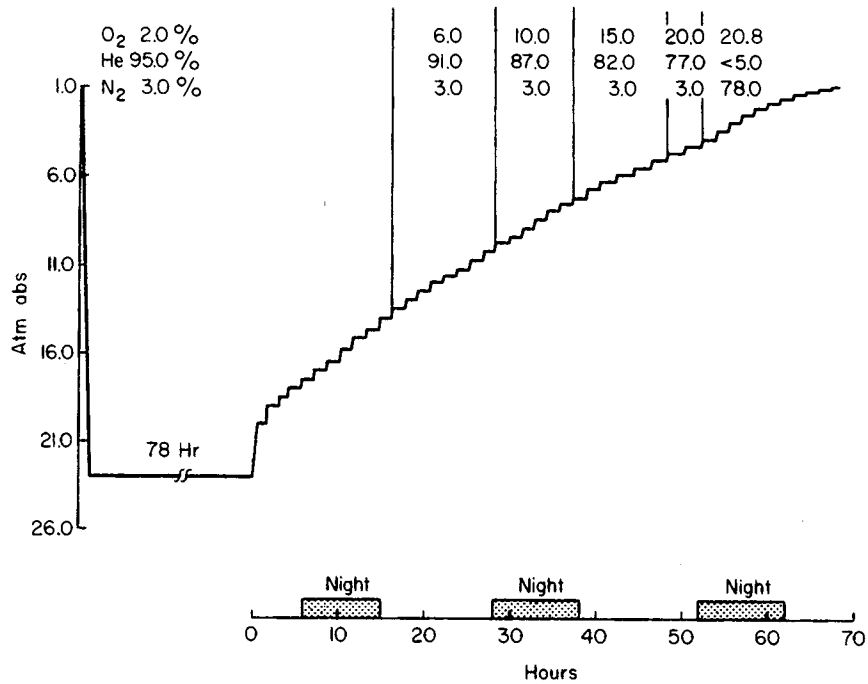

FIG. 4. Decompression after saturation at 23.0 atm abs with 93–95% He. Stepwise increase of O_2 concentration and inert gas change from He to N_2 during the final phase below 4.0 atm abs. Total decompression time: 62–64 hr if 50% O_2–50% N_2 is breathed below 4.0 atm abs, and 68 hr if air is breathed below 4.0 atm abs. 78 hr 23 atm abs (P_{He} 22.0 atm abs): R. Gamba, age 47; E. Landolt, age 22; A. Kaelin, age 21; H. Molnar, age 22; J. P. Beltrami, age 27. 68 hr decompression time: June 18–24, 1968, Deep Trials Unit, Alverstoke. 64-hr decompression time: May 5–11, 1967, Zürich, with 50% O_2–50% N_2 less than 4.0 atm abs. 62 hr decompression time: July 17–20, 1967, Zürich, with 50% O_2–50% N_2 less than 4.0 atm abs.

Two experiments were done involving four different subjects in our dry chamber in Zürich; and one experiment was done with three divers of the Deep Trials Unit of the Royal Naval Physiological Laboratory at Alverstoke, England. The latter divers made swimming excursions and did other physical work in the wet compartment of the pressure chamber (Fig. 4) (6).

The decompressions were not carried out continuously but in steps lasting 60–120 min. Beginning with 50% O_2 at 4.0 atm abs, two subjects showed mild symptoms of O_2 toxicity, such as paresthesia of the fingertips and twitching of facial muscles. The successful final decompression with normal air in England confirmed to us the validity of our concepts and calculations derived from the experiments in Zürich.

"Vertigo bends" occurring in our three Zürich experiments, which lasted to 4 hr at 31 atm abs and involved six different subjects, gave us some information on the handling of supersaturation factors in the pressure range of 31 atm abs. We made a faster initial decompression, and each time one diver had trouble. Since the divers had to be recompressed for treatment, a longer total decompression time was obviously required. Figure 5 shows the corrected decompression schedule after a bottom time of 4 hr at 31 atm abs.

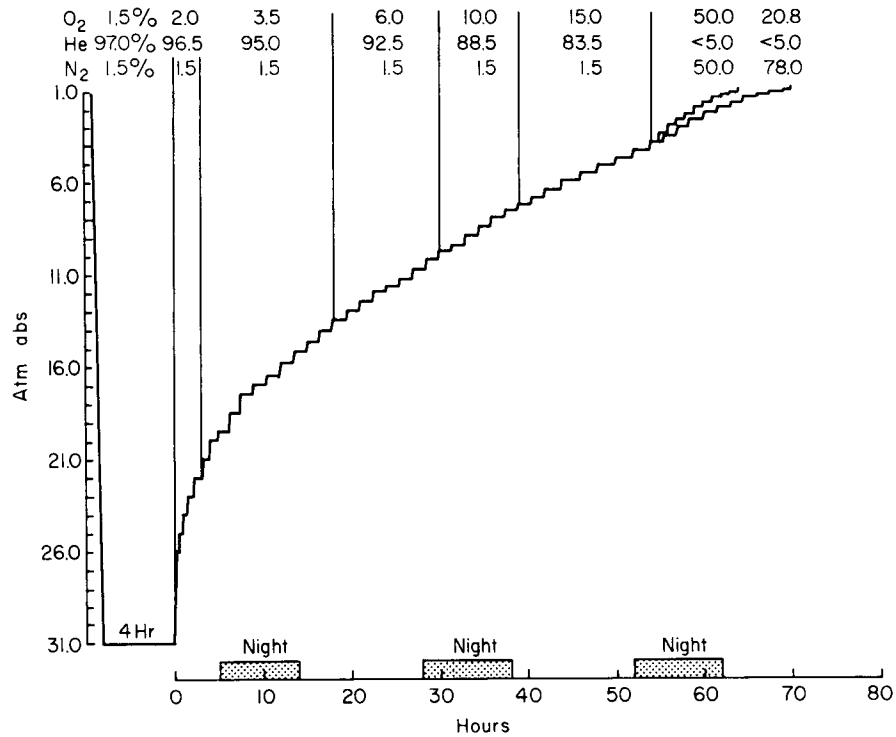

FIG. 5. Bottom time 4 hr at 31.0 atm abs with 96–97% He; compression time of 30 min not included in bottom time. Stepwise increase of O_2 concentration and inert gas change from He to N_2 during the final phase below 4.0 atm abs. Total decompression time: 64 hr if 50% O_2–50% N_2 is breathed below 4.0 atm abs. 4 hr 31 atm abs (P_{He} 30.0 atm abs): R. Gamba, age 46; J. P. Beltrami, age 27. 64 hr decompression time breathing 50% O_2–50% N_2 at 4.0 atm abs, May 14–18, 1968. (70-hr decompression time breathing air at 4.0 atm abs.)

Another saturation dive at 31 atm abs was performed in February, 1969, at the Royal Navy's facilities in Alverstoke. On the first day, three divers made an excursion from 31 to 36 atm abs for 1 hr. During the second and third days, they made excursions lasting 2 hr to 36 atm abs, and engaged in swimming and other physical activity in the water (Fig. 6). We performed an extensive program of tests and medical measurements, and it seems to me important to

TABLE I

TYPES OF DIVES PERFORMED AT ZÜRICH AND ALVERSTOKE, FOLLOWED BY MULTIPLE INERT GAS DECOMPRESSION

Final decompression stage after
1. Repetitive dives during 24 hr with a total bottom time of 8–12 hr at 23.0 atm abs
2. Saturation at 23.0 atm abs
3. Bottom time of 2–4 hr at 31.0 atm abs
4. Saturation at 31.0 atm abs

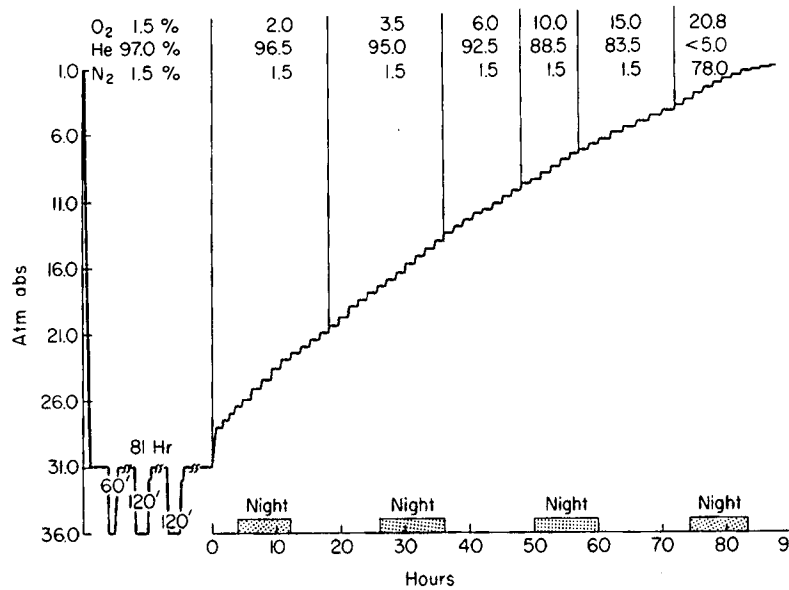

FIG. 6. Decompression after saturation at 31.0 atm abs with 97% He. During the first, second, and third days, excursions were made to 36.0 atm abs utilizing the same breathing mixture. Total decompression time: 88 hr if air is breathed below 4.0 atm abs. Procedure is similar to that of Fig. 5, except that the rapid decompression from depth has not been used. 81 hr 31 atm abs (P_{He} 30.0 atm abs): E. Landolt, age 23; A. Kaelin, age 22; H. Molnar, age 24. 88-hr decompression time: Feb. 3–10, 1969, Deep Trials Unit, Alverstoke.

stress that we did not find any real reduction in motor activity, motor coordination, and attention span at 31 or 36 atm abs during any of these dives, except during the first hour after initial compression from 1 to 31 atm abs (which took 30 min in Zürich and 70 min in Alverstoke). Decompressions in the dives from 36 to 31 atm abs took 20 min for the 1-hr excursion and 30 min for the excursions lasting 2 hr. Total decompression, scheduled to last 88 hr, was trouble-free, except for one diver who had pains in his knees during the last hours, and was separated for additional decompression lasting 4½ hr. It might be mentioned that this diver,

TABLE II

INCIDENCE of MINOR BENDS FOLLOWING THE INERT GAS DECOMPRESSIONS SHOWN IN TABLE I

Breathing mixtures	No. of decompressions	No. of minor bends	No. of subjects
50% O_2–50% N_2 below 3.3–4.0 atm abs	16	1	8
21% O_2–79% N_2 below 4.0 atm abs	6	1	4
Total	22	2	8[a]

[a] 8 different subjects.

in contrast to the other two, who had made recent dives, had made his last dive 12 months before the saturation experiment (4). A tabulation of the types of dives performed and the breathing mixtures used during decompression, in addition to the number of subjects who developed decompression sickness, is shown in Tables I and II.

If we compare the results of our decompression procedures with those of American, British, and French investigators who have used only O_2–He mixtures after short and saturation dives, we can conclude that the method of mixed-gas decompression described in this paper is not less safe than the other, and is certainly more economical in time.

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PART V. FACTORS IN DECOMPRESSION. THE INERT GASES

DISCUSSION

H. V. Hempleman, Chairman

Dr. Glauser: The discussion of gas nucleation phenomena in thermodynamics in this morning's session must be considered in relation to our discussion of species variability. Thermodynamics is not species-dependent and therefore I question why nucleation apparently is species specific.

Dr. Flynn: We really do not know what the relevant variables are that give rise to species differences. One can only surmise that they are related to the rapid gas exchange times and perhaps increase in severity of the decompression syndrome. In other words, you may have to evolve more gas as bubbles in order to reach a given endpoint in a mouse than you do in man. So the thermodynamics of separation of gas may be the same, but the symptomatic tolerance of the animals to this amount of separated gas may be rather different.

Dr. Harel: Does the panel have an explanation of the well-known fact that the divers who are diving regularly are less at risk as far as decompression sickness is concerned than those who dive occasionally or who dive after a long interval?

Chairman Hempleman: We have accumulated a lot of data on caisson work, as in tunnelers, which is unequivocal in this respect; and the data on divers are, in my opinion, really not statistically valid. However, it seems to follow the same pattern when we test experienced men versus inexperienced men. This is one of the difficulties of estimating the parameters for decompression.

Mr. Feld: How do you cope with more than one inert gas at a time when you use the M-value concept?

Dr. Schreiner: That is an excellent question. How you arrive at M values for the gases is a really frightening question because to do it experimentally you would have to test M values for all conceivable combinations, at any given time, of inert gas in particular structures, which is just not possible to do. For the interim the best approach that we have used and that Dr. Bühlmann and others are using is to try to be as conservative as possible for projected M values, as for He and N_2 in a mixture of the two.

We may, however, not be taking full advantage of these two gases because if they are present simultaneously, the M value may well be higher than the one we assume. But I see no good way of direct testing of all conceivable combinations here.

Dr. Hills: Dr. Schreiner, you have presented an empirical model with no specific theoretical basis, or at least you left it open to alternative theoretical bases. The only problem with these empirical time constants and decompression ratios is that these are very good as long as there is supersaturation, but the great problem is if a gas phase is formed then you cannot even use the exponential function which relates them.

Dr. Schreiner: I had this model described to me by Dr. Hempleman yesterday as a black box. On one side you push in 200 divers who go down in the sea in He to 300–450 ft and on the other side you get a bends incidence of 2.3%. That describes the model.

Dr. Stubbs: There is extreme difficulty in a physical system, let alone the physiological, keeping track of the inert gases that you transfer. The one thing we have observed in our oxyhelium dives using the pneumatic computer, starting initially as man does usually on air, is that when we expose the man to a given schedule on oxyhelium, his ascent profile generated by the computer, which is capable of being analyzed mathematically, did not always come out exactly on the final surfacing schedule as we had thought.

The reason for this, we ascertained, was that if we prewashed the computer with the oxyhelium used

* *Panelists:* R. A. Stubbs, E. T. Flynn, G. Albano, H. R. Schreiner, A. A. Buehlmann, P. O. Edel, J. A. Kylstra.

during the breathing, then it did follow the mathematical rules. In other words, we were holding up final decompression in the pneumatic model by the fact that it had N_2 in it.

Dr. Miller: For those of you who believe diffusion is important, it is not generally recognized, I think, that diffusion coefficients of gases in liquids do not seem to be related to the square root of molecular weight. They seem to be instead related to molecular size. This has been shown convincingly by Hildebrand and his co-workers at Berkeley.

Dr. Bühlmann: It is only a factor to calculate; I have no idea if it is a reality, but we calculate with this factor.

Dr. Linaweaver: Dr. Bühlmann, I noted your statement describing rapid compression to 31 atm, in terms of minutes. Would you discuss the incidence and severity of compression arthralgia, which has been plaguing us in our chamber saturation dives?

Dr. Bühlmann: We made the very rapid compression to 31 atm with only three people, and we trained them to avoid hyperventilation during compression. They had no He tremors. Later for the long-lasting exposure, we used relatively slow compression of 30 or 40 min and we had no tremors. We had sometimes had some feeling in the shoulder or in the arms. I am not sure what it is. It is important for us to avoid hyperventilation during compression, and to hold the temperature normal.

Dr. Buckles: I was interested in the report by the Canadians that they were able to take the Workman *M* values in dive profiles and reduce the decompression to a simple four-step serial model. For different lengths of dives or within limited depth and time, we do the same thing. The nine tissues do not always come into play, and maybe only three or four are important in a given dive. I wonder over what range of depth and time you were able to make that correlation.

Dr. Stubbs: We have studied this. An example is to 250 ft for, I believe, 2 hr with 1 atm of O_2. The agreement is within about 8 ft at the maximum discrepancy. Our best agreement, of course, is at the shallower depths, for example, at 100 ft for about 6 hr and at 150 ft for about 5 hr. The agreement is extremely good within a couple of feet throughout the range.

Incidentally, this was using the series system reading out only one compartment.

Dr. Van Liew: Things are complicated during a staged decompression or various kinds of compressions, and the only way to keep track of what is going on is to describe tissue half-times and supersaturation ratios. I try to keep remembering that this is only a model.

A specific example of another kind of interpretation is that decompression sickness can be related to the total amount of inert gas that the animal has in him, independent of the time of exposure to a particular gas or the solubility of various kinds of gases.

Mr. Krasberg: I have a partial answer to a previous question concerning acclimatization. In our saturation dives where work was done at the saturation depth, we have had a 12% incidence of bends. Where we have excursions to a greater depth, this incidence is less than 2%.

Chairman Hempleman: One point which interests me is that in employing any of these pragmatic methods, the predictions used by various schools for decompression from saturation diving are orders of magnitude apart. For instance, there is the Bühlmann group and others who seem to come from depths of the order 800 to 1000-ft saturation dives in times like 80–90 hr. In contrast to this, there are others (like the Navy–Duke University dive) who use extremely long periods of time for decompression.

It seems to me there are two difficulties here for both groups to consider. For those who decompress rather rapidly to consider, why do the others who decompress extremely conservatively sometimes run into trouble at reasonably deep depths such as 150 ft? On the other hand, for those who are pioneering the slower, linear decompressions, why isn't the individual who is rapidly decompressed dead halfway through the decompression?

Will Professor Bühlmann say what his views are on individuals who decompress much more slowly than his subjects do but still develop bends?

Dr. Bühlmann: I cannot explain this phenomenon, but I have the impression that the final decompression, according to the American schedule, is too fast. The total decompression is very long, but the final decompression—let us say between 6 and 7 atm—is a little too fast for our calculations. But it is very difficult to recalculate all the dives. We have no computer. I have to make it by myself.

Dr. Schreiner: Generally speaking, the decompression sickness that has been encountered in some 90 or more saturation dives I know of has, as a rule, been, as Dr. Bühlmann says, in the last 150 ft or the last 6 atm of the dive. Dr. Buckles has emphasized that there is a very significant time element in sustaining supersaturation; that is to say, even if supersaturation is relatively small and you expose a tissue to it for a very

long period of time, you will court an accident. The system may break down while you are still in the chamber. In very slow linear decompressions, with tissue in a state of supersaturation for days, you should not be surprised if an accident occurs after some time. On the other hand, the subjects who come up very fast are avoiding this difficulty. They are capitalizing, I believe, on the fact that the tissues at depth can take much more supersaturation than we have believed, but they are cautious and are slowing as they come into the end zone and we are not. So I believe there may be an explanation for the discrepancy.

Dr. Strauss: We know some of the things that make an individual or an animal more susceptible to decompression sickness, such as obesity, the gas mixtures, or the pressure. Do you have any clues to what would make an animal more resistant to decompression sickness, such as fluids or repeated decompression?

Dr. Schreiner: The question is a very complex and large one. I would say that basically anything that would tend to minimize gas uptake during the gas uptake phase and anything that would enhance gas elimination during the gas elimination phase would tend to improve the chances of a successful decompression. And there are many ways in which this can be accomplished. There are many ways in which the circulation can be controlled and thus in which gas uptake can be controlled.

Chairman Hempleman: Dr. Edel, do you have any techniques that will make your men more resistant or less susceptible to decompression problems in your work?

Dr. Edel: Other than preselection?

Chairman Hempleman: That is one, yes. How do you preselect? Do you just bend them to find out?

Dr. Edel: This is the best way in the long run: We try to make guesses beforehand as to which men are more susceptible. This does not work out all the time, but in some cases it does when you base it on some factors such as body build and age. There are always the exceptions to the rule, but you can more or less make a fair guess in advance.

Chairman Hempleman: What sort of limits do you set? Men over 30 or 35 or 50?

Dr. Edel: No specific limit, but in general we can expect a greater susceptibility to decompression sickness as age increases. So although there are no limits, we can say that on the average we find that men of late 40's or 50's are by and large going to be the ones that we can expect to bend. And this usually holds pretty true.

Chairman Hempleman: Do you find at all that some men may be good at short-term diving and some at long-term diving? Have you made any observations of this sort?

Dr. Edel: Where some can tolerate supersaturation of tissues, for example? One of the things that we were looking at was this. Normally we thought obesity would be a disadvantage in the longer dives where the slowest tissues were saturated, but actually it might turn out to be an advantage in the shorter dives, acting as a sort of buffer for the gas that is in the faster tissues.

Dr. Fife: The question of formalized physical conditioning should be raised. Mr. Edel and others have pointed out that when they take a crew of divers out, they initially have an increased incidence of bends until they get more exercise and then it decreases. I wonder if we should not have formalized physical conditioning before we use men as models.

Dr. Bühlmann: I have the impression that physical condition is a very important factor in assuring a good decompression. We had one severe decompression accident in a man diving 40 days after influenza, with a reduced blood volume and tired and not absolutely fit. He had a severe accident. His partner had absolutely nothing. Now we always look for good physical condition.

Dr. Behnke: I think a remarkable finding is that these dives to deep depths and saturation dives to 1000 ft have been made. The depths and the times have been extended and individuals have gotten along reasonably well. And this has all been accomplished despite these complicated mathematical models. All of this has been accomplished without a single measurement of inert gas exchange, without any quantitative work whatsoever. We have a beautiful, heavy overlay of mathematical work, but no measurement. Now, I think rational diving would be that in which the decompressed individual is using alternation of gases, which was used in the Experimental Diving Unit about 30 years ago, and at no time permits the inert gas in the tissue to exceed the ambient pressure. And a specific test, which we fail to do, is, at any given depth—if we really understood this matter of gas transport—to be able to alternate gases (N_2, He, and H_2 too) and bring individuals up from saturation depth without any decompression at all.

Chairman Hempleman: Would Dr. Kylstra like to comment on that, that we are overmathematicizing everything?

Dr. Kylstra: I have been delighted to see Dr. Stubbs' trial and error with all these various decompression schedules based on a variety of very ingenious models, and my impression was really that it does not make

that much difference what particular model you use or what particular schedule you use. You are going to be bent if you go deep and if you stay long.

The other conclusion was that perhaps this may be due to the fact that we may be overlooking a very basic principle in decompression and decompression sickness. I would suggest that perhaps we might consider not only the possibility that the gases in solution act as gases which can form bubbles, but also perhaps we should consider the osmotic effects of dissolved gas. We know that in the tissues, partial pressure gradients of inert gases do exist during a diving or decompression situation, and we could therefore anticipate that if these partial pressures of dissolved gas have, as we have shown, an osmotic pressure, that therefore these gradients would also cause osmotic gradients and water shifts which might at the capillary level seriously impair capillary circulation and washout of inert gas.

Part VI. FACTORS IN DECOMPRESSION. THE CIRCULATION AND THE CIRCULATING BLOOD

BLOOD AGGLUTINATION IN DECOMPRESSION SICKNESS

Edgar End

Growing interest in blood agglutination and a recent publication (11) concerning its relation to decompression sickness bring to mind the fact that Swindle (19) in 1937 and End (9, 10) in 1938 and 1939 first suggested agglutination as a cause of decompression sickness. Substantial evidence in support of this theory has accumulated in the past three decades.

Following Boyle's discovery in 1670 (5, 6) of bubbles in the bodies of rapidly decompressed animals, work by many investigators, particularly that of Paul Bert (3), served to establish the bubble theory of decompression sickness. This theory has tended to preoccupy investigators to the exclusion of considering other possible causative factors.

The bubble theory is based on the behavior of gases, chiefly the diluents of O_2 in air or in breathing mixtures supplied under pressure. These gases dissolve in increased amounts when the body is under pressure and tend to come out of solution in the form of bubbles during too rapid decompression. The laws of physics pertaining to gases should be sufficient to explain the phenomena of decompression sickness, but they are not.

Probably the most striking disregard for physical laws relates to the long interval that elapses between surfacing after a dive and the onset of symptoms of decompression sickness. In a representative series of cases reported by Shilling *et al.* (17), the average time lapse between the subjects' surfacing and the onset of symptoms was 59 min, and the maximum time was 7 hr. The logical time for symptoms to appear, however, is following a subject's return to atmospheric pressure, when pressures of gases in solution are at their highest.

Human blood is considered remarkable for the constancy of its physical characteristics, yet no two isomorphous individuals have the same resistance to decompression sickness. Can this difference be explained on the grounds that one diver's body is able to sustain a higher state of inert gas supersaturation than that of his companion? And what of the man who suddenly develops decompression sickness while working under conditions of pressure that are no different from those he has worked under for weeks? Surely his blood cannot suddenly have rebelled against physical laws! These discrepancies were mentioned by End (9) in 1938 and by Shilling (16) in 1941 as serious contradictions to a decompression sickness theory based entirely on gas bubble formation in the body.

Other observations refuting the theory that inert gas bubbles play an exclusive role in decompression sickness are as follows.

It is usually difficult, if not impossible, to demonstrate gas bubbles, unless the diver or experimental animal has been subjected to almost explosive decompression.

Bubbles discovered in postmortem examinations may not have been there before death, and vice versa.

The body appears to be capable of absorbing without ill effects large amounts of air accidentally injected intravenously.

Decompression schedules incorporating a substantial margin of safety for average diving do not provide a guarantee against decompression sickness in all divers which they should do if physical factors only are involved.

After what has been considered complete saturation of the body with inert gas, it is impossible for a subject to remain longer at that depth without a comparable extension of decompression time, which is contrary to the laws of physics upon which decompression tables are based.

A person's physical condition, as influenced by such factors as illness, age, and fatigue, often determines his susceptibility to decompression sickness.

An increase in the CO_2 content of the air breathed under pressure greatly increases the incidence of decompression sickness.

This last observation—the most difficult one to reconcile with the laws of physics—was made by Hunter (14) in 1887. Moir (15) in 1896 was able to reduce the incidence and severity of decompression sickness among workers in the Hudson tunnel by providing better ventilation. Snell (18) devoted much space in his book to the toxic effects of CO_2 and showed statistically that increased levels of CO_2 in poorly ventilated working areas correlate with a high incidence of decompression sickness. Momsen (2) put this knowledge to a practical test when two divers suffered from decompression sickness, apparently caused by an accumulation of CO_2 in their inadequately ventilated suits. He required them to perform the same dive on the following day, but with their suits adequately ventilated, and neither suffered from decompression sickness. Physiological factors rather than physical laws apparently determined their reaction.

Agglutination

First described by Swindle and demonstrated by End in rapidly decompressed animals, agglutination of erythrocytes appears to be a phenomenon in which the formed elements of the blood lose their common repulsion and tend to agglomerate and to adhere to vessel walls. Up to a certain point, this is a reversible phenomenon, after which true thrombosis and embolization occur. Swindle reported that CO_2 tends to increase agglutination and O_2 to reverse it—just as CO_2 tends to increase the incidence and severity of decompression sickness, and O_2 tends to reduce it (1). The body retains CO_2 while it is under pressure, and an increased CO_2 output persists for a long time after the diver surfaces (12, 13). These facts may explain the often protracted interval between a diver's surfacing and the onset of symptoms of decompression sickness, during which time agglutination may be taking place.

Inert gas bubbles, when present, probably appear first in areas in which circulation is impeded by agglutinated cells. Recompression not only reduces the size of the bubbles, but also tends to reverse the process of agglutination. Use of O_2 recompression at pressures of 2 to 3 atm abs has been very successful (4, 20) in the treatment of decompression sickness, probably

more so from the effect of O_2 on agglutination than from the removal of bubbles by the pressure.

Cockett and Kado (7) have shown that fatty–gaseous particles probably cause a pulmonary blockage in decompression sickness that low molecular weight (LMW) dextran is effective in treating. LMW dextran apparently acts as an excellent dispersant of agglutinated cells as well as a means of correcting hemoconcentration. As Bornmann says (4), "Dextran would also serve to ameliorate the microcirculatory stasis and sludging which accompany severe decompression sickness." He also mentions that "heparin in small doses acts to promote vasodilatation and plasma clearing, and use of it in the treatment of decompression sickness has been reported. . . ."

A theory of Davis (8) can be applied to agglutination. The stress of rapid decompression destabilizes the physiological emulsion of fat in the blood and probably produces the "fatty–gaseous" particles mentioned by Cockett (7). At the same time, the formed elements and vessel walls, which are normally in a state of equilibrium and mutual repulsion, are disturbed by stress-induced elevation of ionic fatty acids and the deposit of microgels on lipid-rich platelets and red blood cells.

The whole system then shifts from a stable hydrophilic state to one in which charged centers expose hydrophobic chains to similarly destabilized plasma and fibrinogen particles (H. L. Davis, personal communication). The end result is agglutination and, finally, thrombosis. Hemoconcentration and circulatory stasis contribute to the disorder. Recompression, LMW dextran, hyperbaric O_2, and the effect of anionic heparin in restoring a negative charge to formed elements deserve study in connection with the apparently primary role that agglutination plays in all but explosive decompression sickness.

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CIRCULATING LIPIDS AND INERT GAS EXCHANGE UNDER HYPERBARIC CONDITIONS

Paul W. Lange, Alf Martinsson, and Hans O. E. Röckert

A diver may develop symptoms of decompression sickness after deep diving (to depths greater than 50 m) during which he breathes air, even if decompression tables and other safety precautions have been followed carefully. Preliminary test results (5) have shown that lipemia occurring after a big meal considerably influences the gas solubility and saturation capacity of the blood that are observed under hyperbaric conditions. The present investigation was performed to study the *in vitro* relationship between the gas solubility and saturation capacity of blood, on the one hand, and its content of lipids, triglycerides, and cholesterol, on the other.

Material and Methods

Two identical experiments were run simultaneously in our pressure chamber (Fig. 1). Blood, contained in ordinary clinical infusion bottles with added ascorbic acid–citrate–dextrose (ACD) solutions, was obtained from a blood bank. In all experiments, 30 ml of fluids were used—in control experiments, 30 ml of blood; in the other experiments, 2, 4, or 8 ml of Intralipid 20% (Vitrum) were added to the necessary amount of blood to total 30 ml.* The blood, in glass jars, was put in a small plexiglass pressure chamber and exposed to compressed air for 4 hr at 6 atm abs. A pipet (length, 350 mm) with a stopcock at the top was placed in a vertical position through the wall of the chamber into the blood. During the pressure exposure the blood was slowly and constantly agitated with a magnetic stirrer to ensure maximum saturation. After saturation, the stopcock was carefully opened and the pipet was filled with blood. The stopcock was closed again, and the chamber was decompressed; the gases dissolved in the blood in the pipet then collected at the top of the pipet where their volume could be read. The pipet was placed at the same distance from the bottom of the jar in each experiment.

The serum samples as well as the different emulsions were analyzed for triglycerides and cholesterol. The lipids were extracted with isopropanol according to the method of Lofland

* Intralipid is an emulsion for parenteral nutrition and has the following composition: Oleum sojae fractionat. 20 gm; lecithin fractionat. e. vitello ovi 1.2 gm; glycerol 2.5 gm; aqua steril ad 100 ml f.emuls.steril.

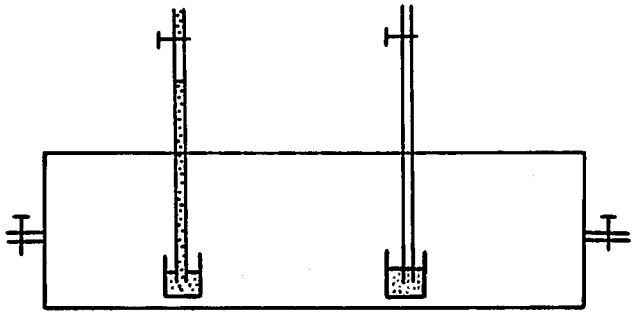

FIG. 1. Experimental apparatus with pressure chamber and pipets demonstrating method of measurement of released gas volume (left) and position of blood during saturation period (right).

(12); but instead of Doucil (Zeolite only), a mixture of Zeolite Lloyd reagent, $CuSO_4 \cdot 10H_2O$ and $Ca(OH)_2$, recommended by Kessler and Lederer (9), was used. Samples 47 and 57 (Table I) were diluted with physiological saline before extraction to decrease the methodological error in this cholesterol concentration range. The triglyceride content was determined through use of the method of Handel and Zilversmit (15), as modified for use with an Auto-Analyzer by Lofland (12). However, the concentrations of periodic acid and sodium arsenite were lowered to 0.025 and 0.05 *M*, respectively, to obtain better reproducibility. Triolein was used as the standard for this test. Cholesterol was determined by the method of Levine and Zak (11), modified for the Auto-Analyzer technique.

TABLE I

GAS EVOLVED FROM VARIOUS BLOOD SAMPLES AFTER DECOMPRESSION COMPARED WITH THE CHOLESTEROL AND TRIGLYCERIDE CONTENT

Control—no Intralipid added[a]				2 ml Intralipid added[a]				4 ml Intralipid added[a]				8 ml Intralipid added[a]			
No.	mm	chol	trigl	No.	mm	chol	trigl	No.	mm	chol	trigl	No.	mm	chol	trigl
44	10	173	115	32	8	237	365	13	11	315	430	8	13	265	810
53	10	173	115	45	12	240	305	15	11	350	430	10	16	215	810
58	5	173	115	46	8	240	305	17	18	275	430	14	19	350	810
60	6	170	170	49	10	240	305	19	13	340	430	16	19	355	810
64	6	170	170	53	9	240	305	21	17	280	430	18	28	345	810
66	6	170	170	56	7	240	305	26	10	280	380	20	16	315	810
67	6	170	170	59	8	240	305	29	15	304	555	22	21	300	836
48	4	173	115	61	11	237	360	31	10	304	555	27	15	315	836
68	6	170	170	63	8	237	360	33	6	304	555	47	15	441	875
69	7	170	170	65	10	237	360	34	9	304	555	57	11	441	875
$\overline{M}$	7	171	148		9	239	328		12	306	475		17	334	828
±SD	1	0.5	9		1	0.5	9		1	8	22		1	22	8

[a] mm = length of column of gas evolved after decompression.
chol = serum cholesterol in mg/100 ml.
trigl = serum triglyceride in mg/100 ml.

Professional deep-sea divers are physically well-trained persons, typically possessing a good appetite. To ensure that the *in vitro* experiments had been performed within the extreme physiological limits of lipemia, we gave five male students a big meal consisting of bacon, eggs, sausages, fried potatoes, milk, and cheese sandwiches, plus a cigarette—the meal representing an intake of some 4600 calories. Their blood was analyzed for cholesterol and triglycerides immediately before and again 3 hr after the meal.

The following sources of error must be taken into account in our investigation: (*1*) the readings; (*2*) the pipetting; (*3*) the analyses of cholesterol and triglycerides; and (*4*) the amount of dissolved gas (because the length of blood columns varied during the experiments).

The total effect of the random errors (*1*), (*2*), and (*3*), in addition to others, is shown as the errors of the mean in Table I. However, (*4*) could give rise to a systematic error in all the measurements.

During decompression, the gas collected in the upper part of the pipet reduced the length of the blood column from which it was released. To determine an eventual systematic error caused by the small relative variation (up to about 4%) of this length, the following hypothesis was analyzed. Instead of collecting in the upper part of the pipet, let us assume that the gas was passed into a second pipet above the first one (and of the same diameter) at a pressure of 1 atm minus the pressure exerted by the length of the blood column. Thus the length, L, of the blood column would be constant during the release of the gas. At equilibrium after decompression the length of the gas column in the second pipet would be l, which is also a measure of the gas volume released. It is reasonable to assume that at any time, t, following the decompression, the rate at which the dissolved gas is released from the blood in the pipet will be directly proportional to (*1*) the volume of blood, and (*2*) the difference between the amount of gas dissolved in the blood column before decompression and the amount of gas remaining in the blood at time t. The rate of gas release can be represented by the following formula:

$$\frac{dx}{dt} = k \cdot L\,(l - x)$$

in which x is the length of the gas column in the second pipet at time t and k is a constant. After integration ($x = 0$ for $t = 0$) we obtain

$$x = l\,(l - e^{-kLt})$$

At equilibrium ($t = \infty$) $x = l$, as expected. Now in our actual experiment, L is changed toward $L - x$ and $l - x$ toward

$$[(L - x)/L]\,l - x$$

This latter expression for the remaining gas above that at equilibrium in the blood column is only approximately correct, since the amount of gas released at the time t (x) has been released from a blood column varying in length from L (at time $t = 0$) to $L - x$ (at time t). However, the error introduced in this way is small, since in our experiments x is small in comparison with L ($x < 0.05L$). We thus obtain

$$\frac{dx}{dt} = k\,(L - x)\left[\left(\frac{L - x}{L}\right)l - x\right]$$

which gives

$$x = \frac{l - Le^{-kLt}}{[(L + l)/L] - e^{-kLt}}$$

At equilibrium ($t = \infty$) we obtain

$$x = [L/(L + l)]\, l$$

This value of x is lower than l. In our experiments l is always $< 0.05L$, which means that the systematic error introduced by our experimental conditions never exceeds 5%.

Results

The values obtained in the *in vitro* experiments are shown in Table I and are also plotted in Fig. 2.

As shown in Fig. 2, a linear relationship exists between the released gas volume, expressed in millimeters on the pipet, and the amount of added Intralipid. The volume of released gas is proportional both to the amount of triglycerides and the amount of cholesterol. A linear relationship was obtained between the amount of released gas and the amounts of both triglycerides and cholesterol (in mg/100 ml).

The results of the analyses of cholesterol and triglycerides in the students' blood before

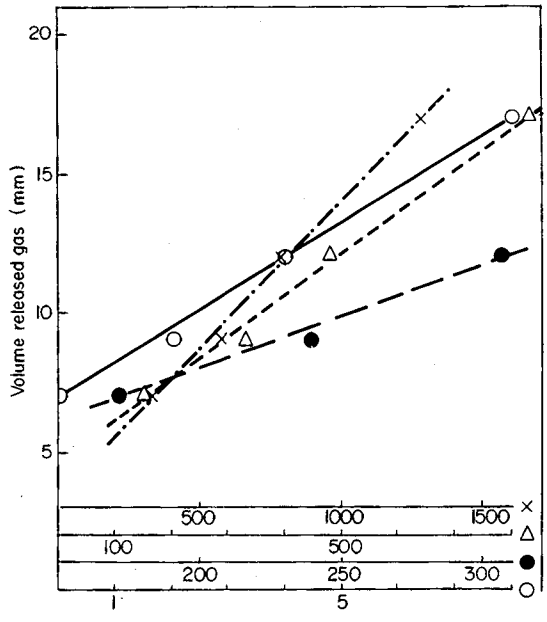

FIG. 2. Relationships between released gas volume and amount of added Intralipid, amount of cholesterol, and amount of triglycerides. The curves are obtained from the values in Table I. (×—·) Cholesterol + triglycerides; (△ – –) triglycerides, mg/100 ml; (● —) cholesterol, mg/100 ml; (○ ——) ml added Intralipid.

TABLE II

CHOLESTEROL AND TRIGLYCERIDE CONTENT IN SUBJECTS' BLOOD BEFORE AND AFTER A BIG MEAL

Subject	Cholesterol (mg/100 ml)			Triglycerides (mg/100 ml)		
	Before	After	Difference	Before	After	Difference
1	190	195	5	85	220	135
2	215	220	5	150	460	310
3	180	190	10	100	320	220
4	215	210	−5	120	450	330
5	205	210	5	85	180	95
			Mean: 4 ± 3			Mean: 218 ± 46

and after a big meal are shown in Table II. As can be seen in the table, there were no significant differences in cholesterol levels. The mean values for triglycerides, however, increased by a mean factor of 3. This demonstrates that the *in vitro* experiments were performed with similar amounts of lipids that might exist *in vivo* (4).

Discussion

The authors are anxious to stress that the aim of this investigation was to study the relationship stated in the first paragraph and to develop a simple method for that purpose. No doubt our method could be refined further, but to obtain the information we desired from the present investigation, it was not necessary.

In view of our results, it seems likely that the probability of developing decompression sickness increases if the diver has eaten a fatty meal shortly before diving. The clinical importance of this will be tested in animals and the results will be presented at a future date.

It is known that the Greek sponge divers from Kalymnos have for generations not eaten before diving. Their knowledge of diving procedures is purely empirical, of course, but their attitude regarding food intake before diving seems to support our laboratory findings. Further experiments will tell us if lipemia induced at the end of decompression, or immediately after diving, will decrease the risk of decompression sickness.

Few studies have been made of the 24-hr variations in serum lipids after fat consumption. Havel (3) found an increase in serum neutral fat, the maximum peak being reached about 3 hr after fat intake. The serum triglyceride level then returned to the fasting value 24 hr afterward. These results agree in general with those of Hollister and Wright (4). These investigators, as well as Kuo and Carson (10), showed that the diurnal variations of cholesterol and phospholipids are minimal after fat intake.

Analyses have also been made of the relationship between ethanol intake and alimentary intake, and it is now recognized that alcohol causes the lipids in the blood to increase (2, 6, 13, 14). In one investigation (14), increased triglyceride levels in the blood were registered more than 4 hr after intake of only alcohol. But the serum triglyceride level was more than three times its initial value 6 hr after intake of both alcohol and a fatty meal. Conscientious

divers know that they should not dive immediately after alcohol intake; but diving the day after alcohol intake is certainly not uncommon. Consideration, therefore, must also be given to the effect of alcohol on deep divers who drink the day before diving.

The free fatty acid fraction of the lipids also rises immediately after smoking, but the increase appears to dissipate within an hour (7, 8). Patients with abnormal serum fat levels have been studied by many investigators through fat tolerance tests (1). Different techniques have been used in these several studies, making the results difficult to compare; but they showed that maximum serum lipid concentration was reached between 3 and 7 hr after the lipid ingestion. The reasons for these variations in time are not clear, but individual differences in gastric emptying, lipase action, and fat resorption must be considered.

Using fat tolerance tests, Angervall (1) found that in patients with hypercholesterolemia and hypertriglyceridemia, the serum triglyceride level remained elevated over a longer period than it did in control subjects. While persons with a high serum lipid level show an abnormal type of response to alimentary fat intake, at present it is impossible to state whether a constantly high serum lipid level is of practical importance in deep diving.

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COEXISTENCE OF LIPID AND GAS EMBOLI IN EXPERIMENTAL DECOMPRESSION SICKNESS

A. T. K. Cockett, S. M. Pauley, J. C. Saunders, and F. M. Hirose

A traditional concept of nitrogenous bubble embolization has arisen as the etiological factor in decompression sickness. Quite properly, recompression has been used to treat this syndrome, and the results of treatment usually have been dramatic and effective. However, many factors are influential in the etiology and treatment of decompression sickness.

In 1961 we first pointed out the importance of hemoconcentration in bends occurring at altitude, and through standard clinical tests demonstrated the degree to which plasma deficit is involved (8). Subsequent studies by our group, using mongrel dogs who had been splenectomized, further documented the extent of this plasma loss (4). Low molecular weight (LMW) dextran replacement was effective in treating shock and maintaining an effective circulating blood volume (3, 6). Recompression was deliberately withheld.

These early findings suggested to us that the causative factors in the syndrome of decompression sickness are more complex than simply the formation of inert gas bubbles. LMW dextran reverses the blood viscosity caused by fibrinogen by forming a dextran–fibrinogen complex. This role is additional to its colloidal, plasma expansive functions. Dextran also affects hemostasis; it apparently alters the red blood cell surface by increasing its negativity, thus encouraging repulsion among the red blood cells.

A chance finding, later reported by our group (5), helped to clarify our understanding of this syndrome. We discovered bone marrow emboli in pulmonary arterioles following experimental decompression sickness, and suggested that a number of emboli in the lungs, kidneys, and liver were lipid in content. These findings formed a new, wide-based approach to therapy that was amply supported by our results in treating humans suffering from decompression sickness. Dextran infusion begun immediately after diagnosis improved the patients' shock-like state and permitted a shortening of the recompression schedule.

Recently we restudied the lung areas of 14 dogs known to have developed emboli during experimental decompression (2). These pulmonary tissues were obtained through biopsy or at autopsy. Pulmonary localization of air emboli has become possible with the development and refinement of radioisotopic pulmonary scanning techniques.

The purpose of the present discussion is to report the findings in microscopic examination of lung tissues of animals that were compressed in the manner described below.

Materials and Methods

Thirty-four mongrel dogs were used in this investigation. Five were killed and served as controls for baseline data on the pulmonary tissues studied for fat emboli by oil-red-O staining techniques. The other 29 animals were compressed to the pressure equivalent of 165 FSW (73.5 psig) in an air atmosphere. The dogs were maintained at that pressure for a period of 60 min before they were decompressed to surface pressure at the rate of 7 psi-min.

Of the 29 dogs, five were used as experimental controls and were not treated for decompression sickness. Of the others, 14 received therapy in the form of LMW dextran administered intravenously at various times after decompression. The remaining 10 animals received therapy in the form of heparin administered intravenously 3 hr after decompression.

The bodies of the five experimental controls and of the five unexposed controls were submitted to postmortem analysis. Their lung tissues were removed and studied for air embolism and stained for the presence of fat emboli.

The treated animals were given a baseline radioisotopic lung scan prior to pressurization. The dextran-treated group was then submitted to a second lung scan 48 hr after the pressure exposure in order to localize areas of embolization. Biopsies were taken of these areas, and the tissues were then oil-red O-stained and microscopically studied. The heparin-treated animals were also given a second radioisotopic lung scan to localize the areas of embolization. Lung biopsies were made and the tissues stained for the presence of fat emboli.

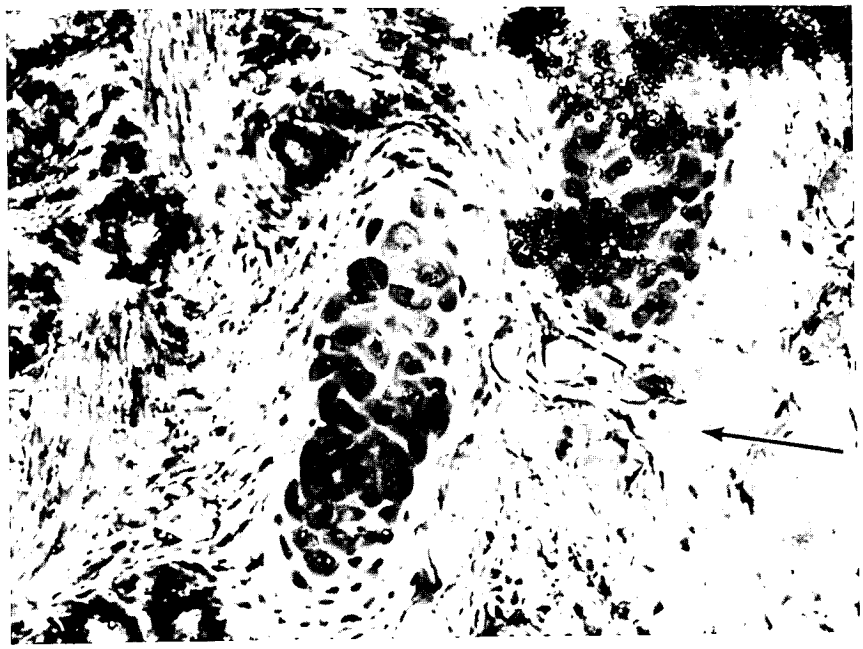

FIG. 1. Photomicrograph demonstrating fat emboli in small capillaries in the pulmonary region of a dog exposed to 165 FSW for 60 min but untreated after decompression (× 100).

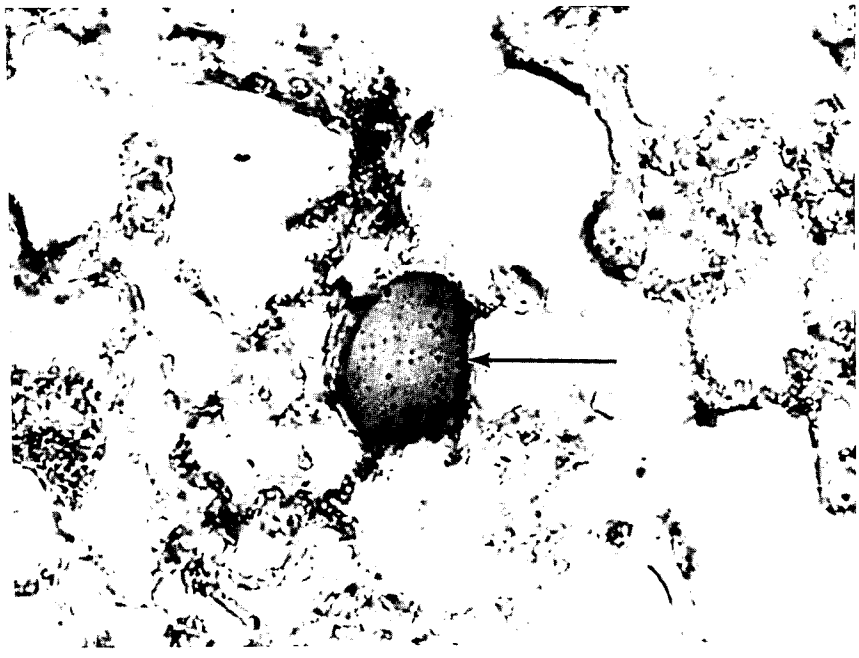

FIG. 2. Fat embolus (oil-red-O) in capillary in the lung of a dog exposed to 165 FSW for 60 min but untreated after decompression (× 125).

Results

UNEXPOSED CONTROL ANIMALS (5 DOGS)

Occasional fat globules were seen in the lungs of these animals, who had not been fed during the 24 hr prior to death. The fat globules were difficult to locate, and were observed in areas containing the smaller blood vessels near the bronchial cartilages.

EXPOSED BUT UNTREATED ANIMALS (5 DOGS)

Numerous lipid emboli were easily identified in routine sections (Figs. 1 and 2). The emboli appeared most frequently in areas where hemorrhage and pulmonary edema were present.

TABLE I

THE EFFECTS OF HEPARIN TREATMENT ON ANIMAL SURVIVAL FOLLOWING DECOMPRESSION FROM 165 FSW

Sample	Number of dogs		
	Total	Survived	Expired
Control	5	0	5
Heparin	10	10	0

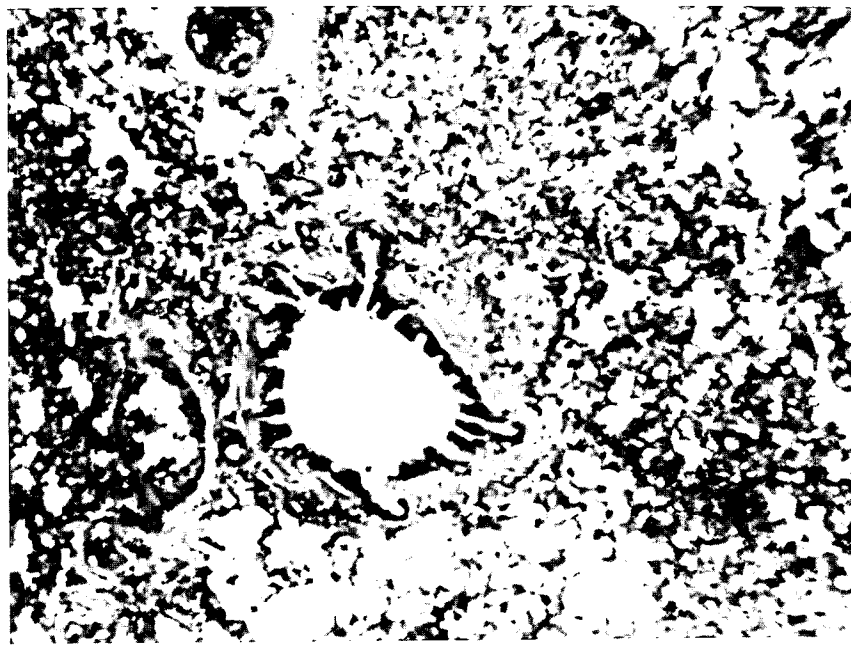

FIG. 3. Hemorrhage and pulmonary edema in the lung of a dog exposed to 165 FSW for 60 min and treated after decompression (× 60).

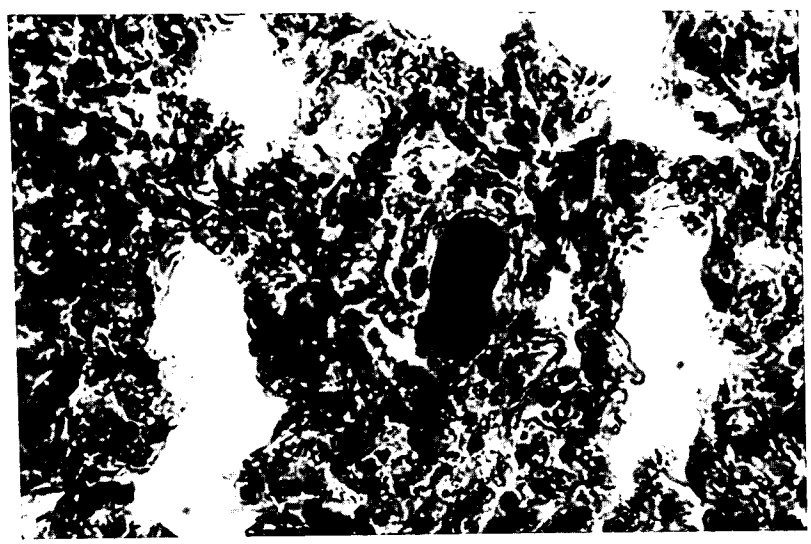

FIG. 4. Fat emboli (oil-red-O) in small vessels surrounding the bronchioles of a dog exposed to 165 FSW for 60 min and treated after decompression (× 100).

EXPOSED AND TREATED ANIMALS (24 DOGS)

Of these animals, 14 were treated by dextran alone (data not shown), and 10 with heparin alone (Table I). Evidence of hemorrhage and pulmonary edema existing in pulmonary areas, as diagnosed by radioisotopic lung scanning, can be seen in Fig. 3. Fat emboli were also observed in biopsied areas 48 hr after decompression (Fig. 4).

Discussion

Philp *et al.* (9) and Barthélèmy (1) have reported the benefits of heparin as a lipemic clearing agent in decompression sickness. Survival rates among their subjects were significantly higher when heparin was used in treatment. We ourselves recently confirmed the benefits of heparin in experimental decompression sickness (7). Lipid embolization seemed to us to be significant since recompression was not employed, and heparin therefore appeared to provide the necessary protection.

Although gaseous embolization is undoubtedly a major factor in the genesis of decompression sickness, our experience with dextran suggests the more important role of lipid emboli. Lipid content of blood is likewise reduced by dextran treatment. There are striking similarities in the clinical syndromes of traumatic fatty embolization and decompression sickness:

1. A latent period occurs in each before the onset of symptoms.
2. Symptomatology is identical, and includes pulmonary edema, hypoxia, shock, and CNS signs.
3. Petechiae in patients with decompression sickness follow the skin discoloration occurring in fat embolism.
4. Successful therapeutic regimens are similar.

Summary and Conclusions

1. Lipid embolization appears to play a major role in the genesis of decompression sickness.
2. There is evidence of the coexistence of lipid emboli and gaseous emboli.
3. Heparin or dextran, both effective lipemic clearing agents, are beneficial in treating experimental decompression sickness.
4. A combined therapeutic approach, recompression and dextran, should be employed in treating human decompression sickness. Heparin can be used alone as a substitute in selected instances.

ACKNOWLEDGMENTS

This investigation was supported in part by Contract NRO 0014 66 CO295 (NR 102–669) between the Office of Naval Research, Department of the Navy, and Harbor General Hospital, Torrance, Calif.; and in part by a grant from the National Aeronautics and Space Administration (NsG 237–62).

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ASEPTIC BONE NECROSIS IN ROYAL NAVY DIVERS

D. H. Elliott and J. A. B. Harrison

The feasibility of a prolonged dive to a depth of 1000 ft is well established. Although many scientific problems remain to be solved, it would appear that, from the practical point of view, no obvious hazards to operational deep diving exist. Unfortunately, not all hazards are obvious.

While overt manifestations of decompression sickness can be easily recognized and then treated successfully, it is already accepted that other pathological effects of dceompression may exist that do not cause an acute form of the illness. Even at relatively shallow depths, for instance, a diver—however asymptomatic he remains—continues to run the risk of damage from "silent" bubble formation in the body, a risk that becomes even greater with the prolonged decompression that must follow deep diving. Of special significance are two sites that may possibly suffer damage without early symptoms—the CNS and the skeletal system.

With respect to the CNS, Rózsahegyi (14) has recently described residual neurological findings in compressed air workers. He also found psychiatric changes, EEG changes, and other long-term pathological effects in as many as 60% of the 800–900 workers whom he examined. He stated, furthermore, that many of these changes might not have been discovered through a routine neurological examination.

With respect to the skeletal system, aseptic necrosis of the bones of compressed air workers has long been recognized as producing symptoms that may not appear until months or even years after the pressure exposures. In a recent survey (10) radiological examination revealed bone damage in nearly 20% of a group of caisson workers, many of whom had no relevant symptoms. It can be predicted that, in some of these workers, the "silent" damage to such sites as the hip and shoulder joints will be followed at a later date by the collapse of a juxtaarticular bone and the onset of joint pain. (Lesions around the knees are not usually juxtaarticular.) Thus, potential disabling lesions can be assumed to exist in a proportion, perhaps a large one, of compressed air workers who are at the present time symptom-free.

In the first cases of aseptic necrosis of the bone reported among compressed air workers, pain was the initial symptom. It was not until some years later that radiological examinations of sample populations of compressed air workers were made to detect the presymptomatic lesions. Similarly, among divers the first reports of aseptic bone necrosis involved patients who had sought treatment for joint pain. Until more is understood about the differences in

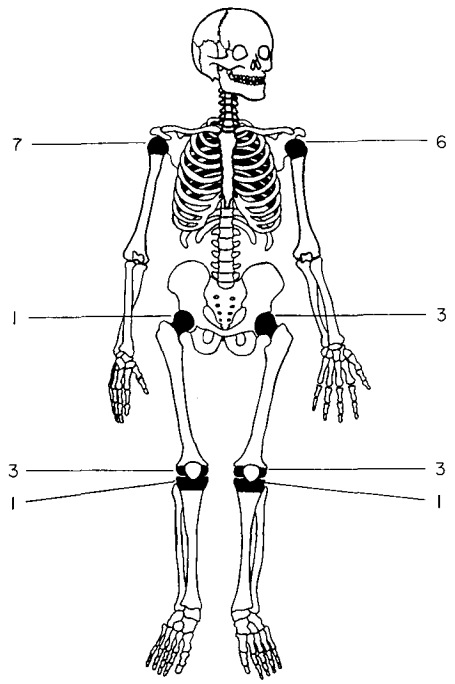

FIG. 1. Sites of bone lesions, taken from individual case reports on divers (3, 4, 6, 11, 13, 15, 18, and R. Barnes, personal communciation). The black areas indicate both shaft and juxtaarticular lesions.

the physiological mechanism of decompression and decompression sickness thresholds between men who work in compressed air and those who work immersed in water, an evaluation of these risks to divers must be a separate investigation.

A search of the literature indicates that the first case of aseptic bone necrosis that could possibly be connected with diving was reported by Seifert in 1936 (16); but since his patient worked on the seabed within the protection of a diving bell, and consequently cannot be considered a true diver, the case has not been included in our survey. It therefore appears that the first report of a suited diver with bone necrosis was made by Grutzmacher in 1941 (6). The characteristics of this case, as well as those contained in later reports on small numbers of individuals—most of whom sought medical help because of symptoms (3, 4, 11, 13, 15, 18, and R. Barnes, personal communication)—are summarized in Table I. The approximate sites of lesions in these patients are indicated in Fig. 1.

There are, of course, cases of bone necrosis in divers other than those tabulated in Table I. But even if the data had been published by the divers' physicians, they would have only limited value. The difficulty of drawing any general conclusions from any such data lies partly in the nature of the original diagnosis—in particular, the differences in radiological interpretations made by different radiologists of the nature and extent of the lesions—and partly in the case reports themselves. In some reports, for instance, the authors list the symptom-free lesions as well as the original lesion that produced symptoms; but none gives the negative findings. It is therefore possible to conclude from many reports that some anatomical site is symptom-free when, in fact, it was never X-rayed.

TABLE I

SITES OF LESIONS FROM INDIVIDUAL CASE REPORTS OF DIVERS

Survey	No. of cases	Humerus (shoulder)	Femur (hip)	Femur (knee)	Tibia (knee)
Grutzmacher (6)	1	R, L	—	—	—
Sartor (15)	1	R	L	—	—
Dale (4)	2	R, L	—	—	—
		—	R	—	—
Ronald (13)	1	R, L	—	R, L	R, L
Angei and Cossu (3)	1	—	—	R	—
Pirastu and Perra (11)	3	—	—	L	—
		—	—	R	—
		L	—	—	—
Barnes (personal communication)	1	R	—	—	—
Uhl (18)	4	—	L	—	—
		R, L	L	L	—
		R	—	—	—
		L	—	—	—

Since Grutzmacher's 1941 report (6), several other surveys have been made of selected samples of the diving population. Of 47 divers examined radiologically by Herget (7, 8), 13 had aseptic bone necrosis. Seven of these had bilateral lesions of the shoulders; one, bilateral lesions of shoulders and hips; and five, lesions of one shoulder. These 13 men had been exposed to a maximum pressure of 5 atm, and nine of them had experienced episodes of acute decompression sickness.

In a later paper, Herget (9) stated that in 29 out of 90 divers examined, there was radiological evidence of necrosis of the epiphyseal region, notably of the head of the humerus. Herget described the particulars of six of these cases, but did not give a detailed analysis or summary of the lesions in the other 23 divers.

Slørdahl (17) examined 13 divers and found three to have aseptic necrosis of bone. One had bilateral lesions of shoulders and hips, with associated symptoms in the left hip and impaired movement of both hips and of one shoulder. Another had bilateral lesions of the shoulders; and the third, a lesion of one shoulder, which also caused symptoms.

An extensive survey was conducted at Kiel, Germany, by Alnor (1), who found 72 cases of bone necrosis in 131 divers. Of the 131, 65 had been kept under observation for more than 10 years, and 22 of them remained free of radiologically identifiable lesions. Of the 43 with lesions, 17 had related symptoms; 10 of these had some physical incapacity; and 7 were totally unable to work. Shoulder lesions were present in 39, hip lesions in 12, and other sites were affected in an unspecified number. Alnor *et al.* (2) later tabulated, according to the anatomical site affected (Fig. 2), the total number of lesions in this group of 72 divers.

Although their description of aseptic necrosis was quite generalized, Fournier *et al.* (5) found bone lesions in 132 men, 24 of whom were divers. These researchers suggested that the incidence of the malady in divers was 25%, and that the lesions were more common in the humerus than in the femur.

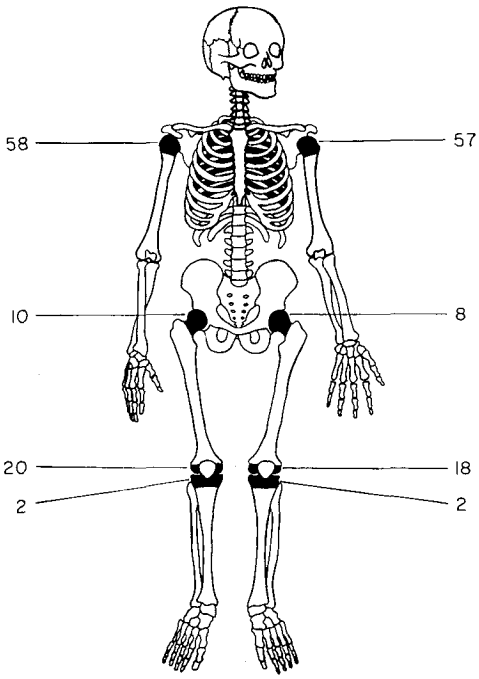

FIG. 2. Sites of bone lesions in 72 divers, found by Alnor *et al.* (2). The black areas indicate both shaft and juxtaarticular lesions.

Ohta (personal communication) found 152 cases of bone necrosis among 301 professional Japanese divers (Fig. 3). He further noted that the incidence was 15% among divers aged 16 to 19 years, and 76% among those over 40 years.

In the cases contained in these surveys (Table II)—none of which, incidentally, appear to have included O_2–He divers—the mean incidence of lesions was approximately 50%. Of the divers assessed by Herget (7, 8), Slørdahl (17), Alnor (1, 2), and Ohta (personal communication), 147 men had lesions in the head of the humerus, and 72, lesions in the head of the femur.

Although earlier investigations revealed no case of bone necrosis in the Royal Navy, it was deemed worthwhile in 1966 to initiate a radiological survey of R.N. divers so that a larger proportion of professional divers would be included. This survey is still under way; and although the complete results will not be available for some years, the preliminary findings are of interest.

If future comparisons of the pathology found in divers from different parts of the world are to be valid, some standardization of investigative procedure is essential. It is therefore considered to be of particular practical importance at this time to describe the techniques of the present survey, including those of radiological interpretation, and also to define the classification of the lesions found.

The objective of our pilot study was to X-ray as many as possible of the professional divers serving in the Royal Navy in order to discover the incidence, if any, of bone lesions. These men were all trained to use compressed air and gas mixtures to depths of 250 ft; many had been

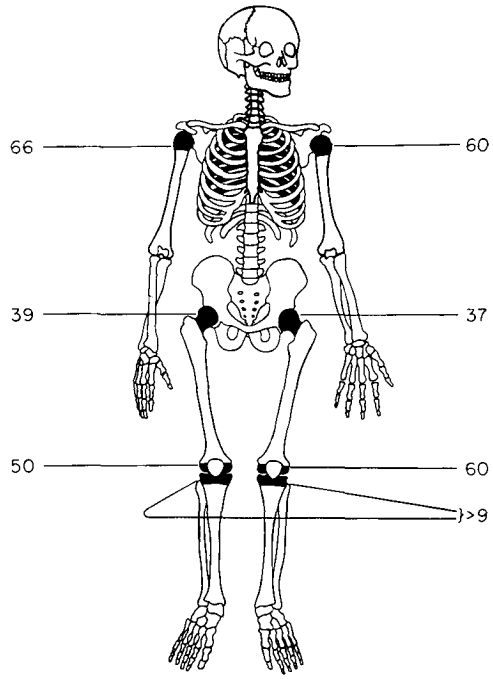

FIG. 3. Sites of bone lesions in 152 divers, found by Y. Ohta (personal communication). The black areas indicate both shaft and juxtaarticular lesions.

TABLE II

DISTRIBUTION OF BONE LESIONS IN PREVIOUS SURVEYS OF DIVERS

Surveys	Divers examined	Divers with lesions	Incidence (%)	No. of divers with lesions (*No. of lesions in parens.*) Head of humerus	Head of femur	Elsewhere (e.g., tibia)
Herget (7, 8)	47	13	36	13 (21)	1 (2)	—
Herget (9)	90	29	32	—	—	—
Slørdahl (17)	13	3	23	3 (5)	1 (2)	—
Alnor (1)	131, total	72	55	— (115)	— (18)	— (42)
	65 of the 131, examined for 10 years	43	—	39 —	12 —	>1 —
Ohta (personal communication)	301	152	50	92 (126)	58 (76)	81 (110)

subjects in O_2–He experimental deep diving, and many more, breathing air, in shallower diving trials. It was originally thought that an annual X-ray for 3 consecutive years might be sufficient to define the extent of the problem. Although it has to date been possible to X-ray only a portion of the total number of divers, the number of lesions thus far revealed is sufficiently large to justify making this radiological evaluation a regular part of the annual medical examination of all our professional divers.

Radiological Technique

High quality radiographs with good trabecular bone detail are essential for the detection of early lesions of bone necrosis. Even so, standardization of techniques is difficult to achieve, although we attempt to adhere to the following techniques and procedures.

A screen film using a Bucky grid, when suitable, is employed.

Anterior–posterior (A–P) radiographs are taken of each head and proximal shaft of the humerus. The trunk is rotated to bring the shoulder in contact with the table, with the arm on that side pulled down into a neutral position. The field is coned so that only the head and as much of the shaft of the humerus as possible are shown in the X-ray. A 10 in. × 12 in. film is used.

A–P radiographs are taken of each head and proximal shaft of the femur. The field is centered over the head of the femur and is coned to give a 4 in. × 4 in. result. The feet must be at a 90° angle to the table top.

A–P and lateral radiographs of each knee—including the distal femur from its midpoint, and the proximal tibia and fibula—are taken on 12 in. × 15 in. film.

A gonad shield is essential. It has been stated that the dosage of ionizing radiation is low enough to permit the examination to be repeated without hazard at a minimum of 4-month intervals.

Radiological Interpretation

The importance to be placed on minor variations in the trabecular structure of the bones X-rayed must remain part of the evaluation of the radiologist, on whose skill and experience much depends. The films of the Royal Navy survey are read initially by the service radiologist of the department in which the diver is X-rayed. The films and the radiologist's report are forwarded to the Royal Naval Hospital, Haslar, at Gosport, Hants, England, where the films are compared with any previous X-rays made of the individual. All the films sent to Haslar are read by a single radiologist (J.A.B.H.), and selected ones are reviewed by the Medical Research Council (M.R.C.)Decompression Sickness Panel in Great Britain. At the end of the first 3 years of this program, it is proposed that the films be reread and that any in which the interpretation has changed be critically reexamined. The current findings must therefore be considered as provisional.

The bone lesions revealed in the films are categorized, according to the M.R.C. Panel classification (10), as being *juxtaarticular* or *shaft* (Table III). Each individual is then classified according to these criteria: *negative*: having no doubtful or definite lesions; *doubtful*: having one or more doubtful lesions; or *positive*: having one or more definite lesions.

TABLE III

RADIOLOGICALLY IDENTIFIED BONE LESIONS, BASED ON CLASSIFICATION OF THE M.R.C. DECOMPRESSION SICKNESS PANEL (10)

Juxtaarticular
J1. Dense areas with intact articular cortex
J2. Spherical segmental opacities
J3. Linear opacity
J4. Structural failures
a. Translucent subcortical band
b. Collapse of articular cortex
c. Sequestration of cortex
J5. Osteoarthritis
Head, Neck, and Shaft
S1. Dense areas (*not* bone islands)
S2. Irregular calcified areas
S3. Translucent areas and cysts
S4. Cortical thickening

A case with doubtful and positive lesions is classified as positive.

In addition to being so classified, all films are also reported on in detail. Minor variations in bone structure are recorded, even though they are not currently accepted as evidence of aseptic necrosis (or of any other disease). At a later stage of the survey, statistical analysis may reveal some significance in these minor variations. A review of the early films of those subjects who subsequently develop necrosis may reveal additional characteristics of early lesions that can aid in diagnosis.

The Differential Diagnosis

It is well known that aseptic bone necrosis has many causes other than decompression from elevated pressure. But unless some other obvious cause can be attributed to it, a necrotic bone lesion discovered in a diver must be assumed to have been caused by diving. Among the alternatives that need to be excluded in each individual are sickle-cell anemia, alcaptonuria, polyarteritis nodosa, syphilis, long-term corticosteroid therapy, and chronic alcoholism.

In its investigations of compressed air workers, the M.R.C. Panel found no necrotic lesions in a control sample of 50 new workers who had not been previously exposed to elevated pressure (10). A radiological investigation of 100 nondiving Royal Navy officers and ratings is also planned so that the negligible incidence of bone necrosis lesions in a nondiving population can be confirmed.

The Management of Divers with Lesions

A radiological diagnosis of doubtful or positive bone lesions requires an immediate course of action. In each case the affected Royal Navy diver is interviewed and examined. He is told of the diagnosis and that he must restrict his diving to what is described as being within the

"limiting line" by the Royal Navy compressed air table. This restriction permits, for instance, a maximum of 20 min at a depth of 180 ft of sea water and of 55 min at 100 ft. The hazardous decompressions following experimental and O_2–He diving are obviously no longer permitted.

If a diver has a positive bone lesion that is found to be juxtaarticular—whether or not there are accompanying symptoms—it becomes necessary to reconsider his whole future diving career. He should also avoid such physical stress to the affected joint as weightbearing. Until the clinical condition has been assessed in detail by appropriate specialists, individuals with juxtaarticular lesions are not, except under one condition, permitted to dive. The single exception is diving to 25 ft with closed-circuit O_2 apparatus, since such a dive is considered to be without decompression sickness hazards.

Results

Of the 250 divers X-rayed by March 1969, 13 were found to have bone necrosis (Fig. 4). Of these, eight had at least one positive lesion (Figs. 5 and 6), five had only doubtfully positive lesions, and two had associated symptoms. The incidence of radiologically identified lesions in serving Royal Navy divers is, therefore, provisionally 6%.

Beyond the scope of this survey we have found positive lesions in a civilian O_2–He diver, in two persons exposed on many occasions to the Royal Navy High Altitude Selection Test, and in one Submarine Escape Training Tank instructor.

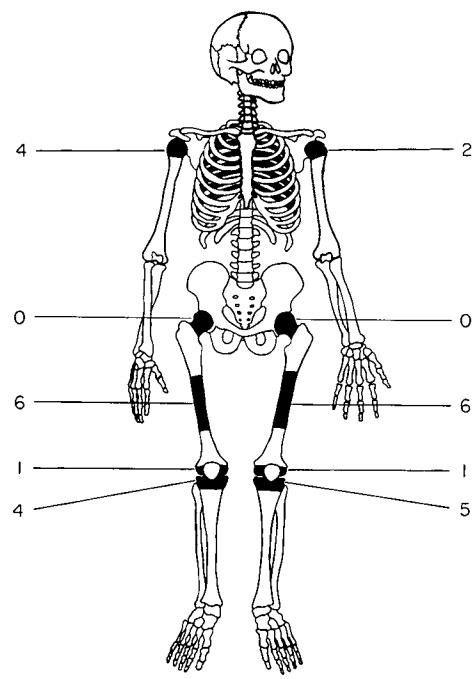

FIG. 4. Sites of bone lesions in 13 divers of the British Royal Navy. The black areas indicate both shaft and juxtaarticular lesions.

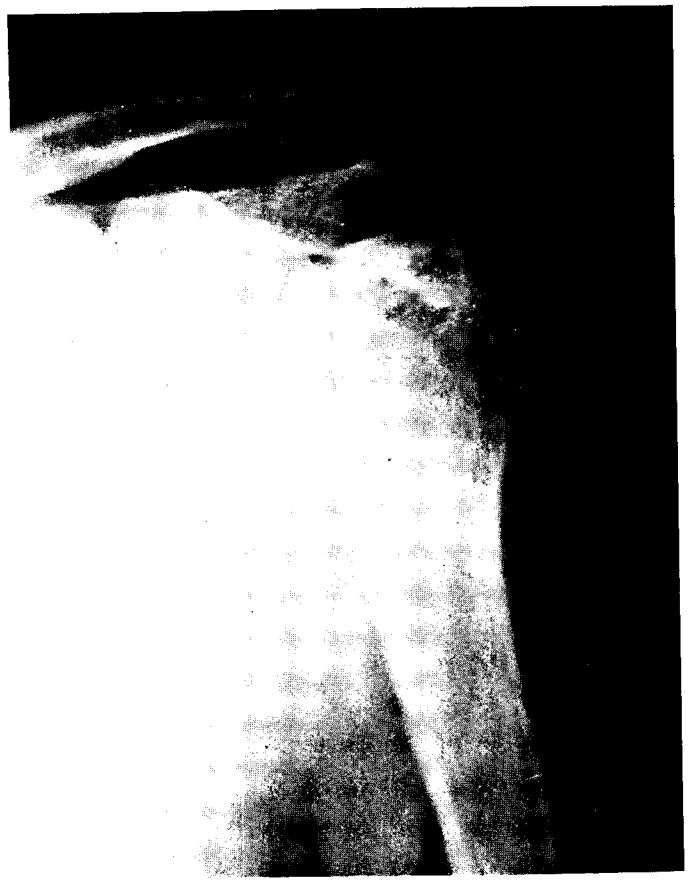

FIG. 5. Radiograph illustrating a positive bone lesion at an early stage.

TABLE IV

DISTRIBUTION OF BONE LESIONS FOUND IN SURVEY OF 250 NAVAL DIVERS COMPARED WITH THAT OF OTHER SURVEYS

Surveys	Divers examined	Divers with lesions	Incidence (%)	No. of divers with lesions (No. of lesions in parens.)		
				Head of humerus	Head of femur	Elsewhere (e.g., tibia)
Alnor (1)	131	72	55	— (115)	— (18)	— (42)
Ohta (personal communication)	301	152	50	92 (126)	58 (76)	81 (110)
Royal Navy	250	13	<6	6 (6)	0 (0)	11 (23)

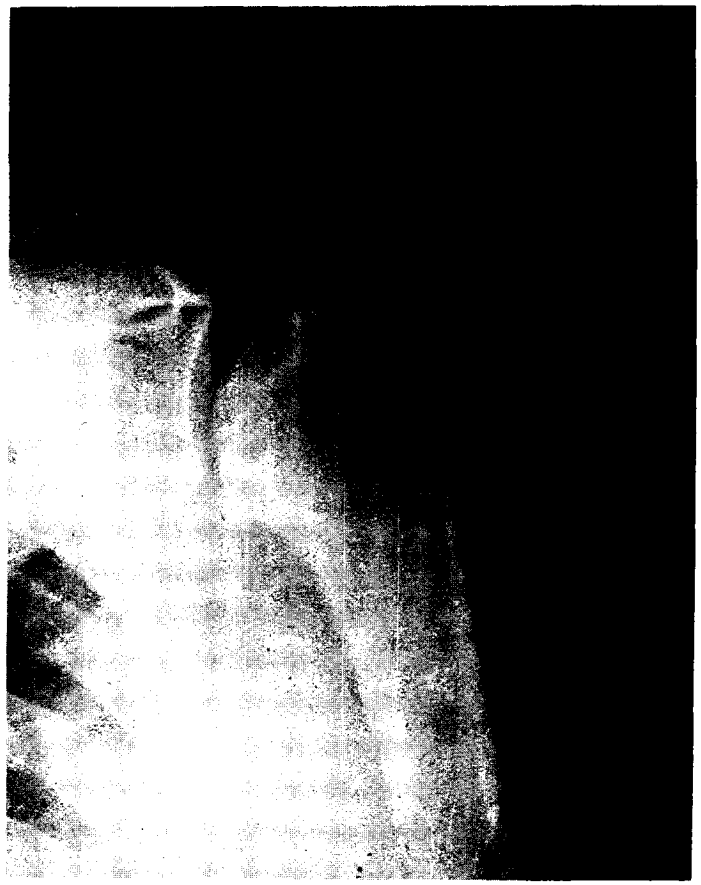

FIG. 6. The same lesion shown in Fig. 5 after an interval of 12 months.

A comparison of the sites of lesions found in Royal Navy divers with those in divers of other surveys (Table IV) reveals in the former a proportionately greater number in other than the clinically significant sites—the heads of the humerus and femur. This divergence, however, is probably only a reflection of the more extensive radiological search in the present survey, and could be taken to suggest that the incidence of necrosis in the civilian divers of other studies in comparison to that of naval personnel is even greater than is indicated here.

Summary and Conclusions

Aseptic necrosis of bone is indeed an occupational hazard of naval divers; and although it is reassuring to find that the incidence appears to be very much less than it is in their civilian counterparts, it would seem that an annual bone X-ray of all divers is the minimum safeguard that can be recommended.

After the present survey has been enlarged to include a greater number of Royal Navy

TABLE V

LESIONS OF SELECTED ROYAL NAVY CASES TO ILLUSTRATE THE TABULAR CLASSIFICATION

Case no.	Status	Humerus (head and shaft)		Femur (upper)		Femur (lower)		Tibia (upper)	
		R	L	R	L	R	L	R	L
85	+	—	—	—	—	S2+	S2+	—	—
93	+	J1+	—	—	—	—	—	—	—
118	?	—	—	—	—	—	—	S2?	—
123	+	—	—	—	—	J4+[a] S2+	J4+ S2+	S2+	S2+
127	+	—	J4+[a]	—	—	—	—	—	—

[a] Patient reports associated symptoms.

personnel, the next step is a thorough study of their diving and decompression sickness history. Until the detailed pathology of the illness is known, it must be assumed that the etiology is a past decompression procedure that was inadequate for the particular individual. Correlations must therefore be sought between bone necrosis and the circumstances of the individual's diving history, no matter how tedious the investigation may prove to be.

It is important that the results of the several surveys of different groups of divers be collated so that valid conclusions regarding the etiology of bone necrosis can be reached. To satisfy this requirement, however, standardization of techniques and diagnosis—and tabulation of findings along the lines suggested in Table V—are essential.

ACKNOWLEDGMENT

We are grateful to the Medical Director General of the Royal Navy for permission to present this work.

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PART VI. FACTORS IN DECOMPRESSION. THE CIRCULATION AND THE CIRCULATING BLOOD*

DISCUSSION

R. D. Workman, Chairman

Chairman Workman: Dr. Madsen wishes to make some remarks about the work that has been going on in his laboratory in terms of assessing the changes in perfusion of muscle and fatty tissues.

Dr. Madsen: The purpose of the work is to study the effects of O_2 breathing on the blood flow of skeletal muscle and subcutaneous adipose tissue. Measurements are done with a ^{133}Xe technique, and our subjects have been normal male volunteers. When we submitted our abstract for this meeting, we had a series of experiments showing consistent reduction in muscle perfusion during O_2 breathing. However, two months ago we discovered that this reduction was an artifact, caused not by the O_2 but by expiratory back pressure in the breathing system we used. Therefore we are doing the experiments again, avoiding this source of error. In eight subjects we now have found the resting muscle blood flow unaffected by O_2 breathing at 1 atm. In four out of seven subjects subcutaneous adipose tissue perfusion was unaffected by O_2 breathing at 1 atm, while the other three subjects showed reductions of 25, 26, and 35% in blood flow rate. Two subjects breathing O_2 at 2 atm showed reductions in flow rates of 25 and 43%. We do not know how consistent these effects may be in a larger number of experiments. We feel that the effects of changes in O_2 tension on adipose tissue perfusion must be of considerable interest since it implies changes in half-time for one of the slow tissues under conditions which are often met in diving, particularly during O_2 decompression.

Dr. Schreiner: For those on the panel concerned with bone necrosis, I wonder whether any studies have been made to determine if continued diving accelerates the deterioration called bone necrosis. Does anyone know that continued diving would aggravate a symptom-free, radiological finding?

Dr. Griffiths: You could not have asked a more difficult question. During our surveys of men actually engaged in compressed air work, when we find what we call the juxtaarticular lesion in its early stages, the first thing we have to decide is what is going to happen to this lesion. If I tell a man who is earning £80 a week that he has to stop working in compressed air because he has a lesion at the head of his femur, he'll say, "Well I'm going to go down to £20 a week. What's going to happen if I don't stop working in air?" And it's a question we cannot answer.

Take lesions classed A-1, which are simply areas of density with the articular surface not yet involved. If you follow this man by X-ray every 6 months, the lesion may not deteriorate. However, within 18 months it may have caused a destruction of the joint surface and then he will have symptoms. There is no possible way of saying which is going to happen.

Lesions radiologically looking identical can in 18 months cause pain by involvement of the joint surface or in 6 years may look radiologically unchanged. But if a man has a lesion near an articular surface, my advice is to stop working in compressed air. One has to make a decision, and I think that is the only sensible one. But if he has a lesion, there is nothing you can do about it. You can only wait and see what is going to happen to it.

What people would like to know is: Is it going to get any worse if I go on working in compressed air? I don't know, but he may get one in the other shoulder and if you have two shoulders upset then, of course, earning a livelihood is much more difficult. It is worse in the hips, of course.

* *Panelists:* E. End, H. Röckert, A. T. K. Cockett, J. Madsen, D. H. Elliott, A. R. Behnke, P. D. Griffiths.

Chairman Workman: Dr. Behnke, you are at this time involved in working with tunnel workers in San Francisco. Would you care to add to this aspect of aseptic bone necrosis?

Dr. Behnke: What little I know I have learned from Dr. Griffiths. We have not seen lesions in the tunnel workers. One difficulty is that it requires perhaps a year or longer for the lesions to be seen radiographically. We have followed some of Dr. Sealy's tunnel workers in the state of Washington, and in California. A much longer decompression time has been followed by tunnel workers than ever prescribed heretofore for these workers.

We have seen one or two individuals with lesions in the shaft that are symptomless. However, the percentage is very, very small and we have some hope that if decompression is adequate, these lesions will not occur. This will not necessarily eliminate bends or decompression sickness. We have just as many cases of decompression sickness as have occurred in England where decompression time is one-half what it is in the United States, but we have good reason to believe there will not be these symptoms.

In our experience with divers we have not been aware of any crippling lesions at all. The problem here is the small number, 5% out of 250—a rather small number of individuals involved. In the cases where you cited authors who gave a percentage as high as 50% of divers, I wonder if it is a matter of inadequate decompression.

One more thing is important in this matter. Why is it that some individuals can work maybe years and be entirely free from any changes whatsoever?

It has been brought out by Dr. Cockett and others that we have to consider the liberation of fat in alcoholics. With fatty livers fat will get out of the liver easily, and particularly in compressed air workers it is likely that we are dealing with two things, bubbles and fat, which predispose to the lesions.

Dr. Buckles: Dr. Schreiner has presented the idea that when he handles the problem of fat versus lean tissue, he merely adds together an effective solubility coefficient based on the volume fraction of fat in that tissue. Dr. Röckert's presentation suggests that there is now some way to check how good an assumption that is. I wonder, where you compared the effective gas solubility to the fraction or to the concentration of fats, if you have analyzed that from the point of view of either volume fraction of fat or percent of fat by weight to see whether you could indeed correlate solubility with fat content.

Dr. Röckert: I have not done that. One has to keep in mind that one cannot calculate as if blood were a mixture of water and oil. It is the blood corpuscles and also the small fat droplets which make the surfaces within the blood very complicated. So I cannot give you any figures.

Dr. Philp: With respect to the formation of fat emboli or perhaps, more properly, fat thrombi, I think it might be worth pointing out that we should not be too rigid in believing that they must necessarily originate from a fatty tissue. There is evidence in the formation of atherosclerotic lesions and platelet thrombi, which Dr. End referred to, that plasma lipids themselves may become incorporated into a thrombus which may therefore be composed of both platelets and lipids. Lipid may not necessarily have to originate either from bone marrow or from liver or some other fatty tissue site.

I think Dr. End's comment on the fact that that was indeed a platelet thrombus is also significant because the interrelationship of platelets and lipids in the formation of thrombi in arterial thrombotic disease is well established and perhaps we should utilize this information as it applies to the situation with decompression sickness.

Dr. Uhl: Regarding the nature of aseptic bone necrosis, I think it has been well-documented that these lesions do progress from minor symptomatology to massive destruction of bone and crippling in the absence of continued diving. Therefore the effect of continued diving is really an unknown in this illness.

Second, to make the record of this meeting correct, at the aerospace meeting a year ago I reported a case of bone necrosis. This disease has been found in U.S. Navy divers. The etiological importance of this is not at all clear; but lest the proceedings of this meeting be inaccurate, I will say that U.S. Navy divers have been found to have this illness.

Dr. Austin: Dr. Elliott, what was the minimum diving exposure of divers with aseptic bone necrosis?

Dr. Elliott: We are accumulating this information. It has occurred in people whose only exposure to raised environmental pressure had been submarine escape on one occasion.

Dr. Lambertsen: It has also occurred in individuals who have never dived. Dr. Cockett, what forces are involved in producing the extrusion of marrow into the circulation? I understand that you identified cells which were marrow cells in some of the occlusions of blood vessels. How do you visualize a pressure force other

than perhaps the formation of gas bubbles in the marrow cavity causing extrusion of material from the bone marrow?

Dr. Cockett: I think it is a gaseous pressure within a marrow cavity with extrusion. There is no trauma here. In fact, when we first saw our bone marrow emboli in our dogs, the pathologist who was working nearby and who was traumatizing long bones in rabbits and was having difficulty producing bone marrow emboli was quite alarmed that ours came without trauma.

Dr. Lambertsen: In that event the extrusion would be a consequence of rather massive bends, would it not, rather than a primary precipitating cause of symptoms? This would be an additive or secondary factor in bends unless the emboli are caused to enter the circulation by some primary means other than bubble formation itself.

Dr. Cockett: Yes, but let me point out what Dr. Philp has stressed and Dr. Pauley, working with our group, has emphasized from reviewing the literature. That is, a very small portion of the total number of emboli come from the marrow and the extrusion or the emboli coming from the liver might be more important. Finally, the coalescence concept has to be kept in mind. So I would certainly want to minimize the role of bone marrow embolization at this time.

Dr. Beckman: I somehow have missed the information that has to do with the appearance of idiopathic osteoporosis or bone necrosis in the general population, and particularly the population which relates to the divers. I had an opportunity to discuss this not long ago with an orthopedic surgeon who had done experimental work in this field, and my general impression in talking with him was that while there have been cases in the U.S. Navy, certainly the numbers that occur are not significantly different from the numbers appearing in the normal population who are not exposed to other than normal stress. This is what Dr. Behnke was referring to.

Would Dr. Elliott comment about the frequency of occurrence of idiopathic osteoporosis in the general population?

Dr. Elliott: We just do not agree that there is no significant difference between divers and the normal population. You have seen the evidence. As far as the normal population is concerned, the controls that have been done—more than a hundred men not previously exposed to pressure, not showing those lesions—in fact show that the incidence, therefore, in the normal population is for practical purposes zero.

Dr. Griffiths: We have done a lot of controls. We started X-raying the major joints of 120 compressed air workers who had been passed to work in compressed air, but before they had been compressed. Those were our major controls. During the last five years we have had five radiologists in various areas in Great Britain looking for lesions that we maintain are early lesions of aseptic necrosis. I think the total number of men examined this way is between 500 and 600 now, and not one of them has reported any lesion anything like the lesions we show and consider to be early lesions of aseptic necrosis following working in compressed air.

This is something that is going to be debated later on in the meeting, I think. If aseptic necrosis lesions are found among the general public in the United States to this extent, I don't know the answer to this. It certainly is not so in England. Mind you, we do not get sickle cell anemia. I think steroid therapy is much less given in England than in the United States, though I am not sure about this. But I cannot imagine any of our young compressed air workers ever having had steroid therapy. I think these two things eliminate a large proportion of the commoner causes of aseptic necrosis.

We are convinced that the lesions we show are due to working in compressed air. I want to say due to compression or to decompression, but we really don't know. But I think they are all due to working in compressed air.

Dr. Beckman: I did not in any way intend to imply that there is no correlation between what you see in compressed air workers and the number of lesions that you see. This is not the point in question. I am asking what the percentage occurrence of this phenomenon is in the normal population, but not in a hundred cases. The reason that I ask is that one of my friends who did work in this field for about five years gave up because he could not understand it. He said that in his experience the spontaneous occurrence was not uncommon. He is in practice now in orthopedic surgery and has three cases now that he is treating that had no known etiology.

What I am really pointing out is that it is not uncommon in the general population, and particularly in the male population.

Dr. Griffiths: The question of differential diagnosis of bone islands, for instance, is a very difficult one. We think we have overcome this, but I know that in many cases bone islands have been diagnosed as aseptic necrosis and men have had compensation for bone islands. But I would say that in the general population in

Great Britain, men or women, with aseptic necrosis of bone, we always know the reason why they have it. For the type of people who work in compressed air, examined before they work in compressed air or have not worked in compressed air in England, I would say that the incidence is nil; we just cannot find anybody in the general population who has lesions that we think are early aseptic necrosis of bone.

Dr. Hoke: Dr. Cockett, I would like to know the reason for doing the splenectomies on the dogs before you put them into your series. What is the role of the spleen in decompression sickness or in your results?

Dr. Cockett: We started doing splenectomies in our blood volume studies, where in the dog you must get rid of this large blood trapping organ. Once we started it we had no good reason for not doing it. I suppose we could leave the spleen in now. I do not know exactly what the role of the spleen might or might not be.

Chairman Workman: I suspect that perhaps in the manipulation of surgical procedures the possibility of fat emboli being liberated cannot be ruled out. Have there been control studies of animals following splenectomy to determine whether fat emboli were in fact present before ever being exposed to pressure?

Dr. Cockett: These procedures are done 3–4 weeks before the chamber exposure, and we always allow that time in order to have the animal back to what we consider the normal condition. They are not done the same day or immediately thereafter.

Dr. Behnke: I think a new parameter has been added to our therapeutic armamentarium here. Some of us have used recompression but have overlooked what Dr. End called to our attention, namely agglutination and the condition of the blood. From Sweden we learn the dangers of diet, and from Dr. Cockett's work the use of dextran and of heparin. We must really pay attention to the blood.

Specifically, in much animal work there are fat emboli seen along with gas emboli. In the preservation of kidneys that are removed surgically for transplantation, blood may be oxygenated by bubbling O_2 through it. This process will liberate considerable quantities of fat from lipoproteins, particularly the low density lipoproteins, in which the lipid is rather loosely linked with the protein. So it may well be that even the presence of bubbles will cause a separation of lipid from lipoprotein. Certainly the concentration of the blood and replacement of blood is very real contribution to this symposium.

Chairman Workman: Certainly any therapeutic adjuncts to pressure and O_2 that may be beneficial to improve the status of the patient who still remains paralyzed should be considered. We certainly have a great deal of experience yet to be gained with these. Work with the animals has helped to give us hope for the usefulness of restoring circulating blood volume, the prevention of shock, and also the prevention of agglomeration of cells. If there is any possibility that pressure and O_2 therapy do more to restore bladder and bowel functions when sphincters have been lost, these things are tremendously to be wished for.

Dr. Hoke: Dr. Cockett, did you look for fat emboli in your control animals that were not compressed and decompressed? Shim recently compressed and decompressed some rabbits, decompressed others to altitude and his controls were neither compressed nor decompressed. And when he sacrificed his controls, he found fat emboli in the controls—not to the same extent as in his experimental animals, but he did find fat emboli in all three groups. In the animals that had symptomatic decompression sickness, bubbles were seen, but not in the controls.

Dr. Cockett: Yes, we did examine the controls and we found fat emboli with difficulty. But you will note that I said 24-hr fasting animals. This is a big difference. Also, I think in my presentation I mentioned that we found bubble and lipid emboli. The tendency is to overplay the gaseous aspects.

Dr. Flynn: Dr. Röckert, did you look at the time course of evolution of gas from the blood following decompression? How long did it take for this gas to separate quantitatively from blood postdecompression?

Dr. Röckert: Within the first 5 min about 99% of the gas was collected at the top of the pipet.

Dr. Flynn: Did this seem to follow any particular mathematical function, such as an exponential function?

Dr. Röckert: I think so, but I must admit I have not calculated that mathematically.

Dr. Winter: Dr. Cockett, you reported very marked changes in plasma volume in an LD_{100} preparation. Have you done such measurements on decompression profiles which gave you a safer return at the surface?

Dr. Cockett: No, but let me answer the question in a wider sense. We have examined humans with decompression sickness, and the same concept holds true. You can find a plasma deficit in abalone divers who have severe type of bends, and certainly in those with fullblown decompression sickness this very same plasma deficit can be found. Amazingly, dextran infusion, while the patient is on the way to the recompression chamber, is extremely helpful.

Dr. Winter: But you have no information about plasma changes in the absence of marked decompression sickness? I wonder whether there is any data on plasma volume during decompression when the decompression is successful rather than when gross bends occurs.

Dr. Cockett: I don't have such information. We did not use recompression, as you know, and did not study this aspect of changes during the protocol.

Dr. Bühlmann: We have determined the blood volume before and after a sufficient decompression and found nothing, but with three cases with bends we had loss of plasma and had to treat them with plasma. In one case we had the clinical picture of fat embolization in the lungs; in the two other cases nothing abnormal was indicated in the lungs. But we are not sure whether fat embolized to the lungs or not.

Part VII. SENSES AND COMMUNICATION

VISION AND VISIBILITY

Jo Ann S. Kinney and S. M. Luria

One does not need to be a competent diver to realize that the underwater world appears quite different from what it really is. Nor does one need a long diving history to have experienced the panic of suddenly discovering that one cannot see underwater when one needs to. The modified appearance to air dwellers of the underwater world has, of course, a physical basis—the fact that light energy is traveling through water rather than through air. In this paper we shall consider some of the changes in vision that occur underwater and how they affect divers.

First, water absorbs electromagnetic energy, vastly more than air does. The amount of absorption depends upon the distance that light travels through the water, and is approximately proportional to the square of the distance. Furthermore, water selectively absorbs different wavelengths of light (according to the same power function), and this selective absorption varies with the body of water—varies, that is, according to whether the water is clear or contains such absorptive material as plankton, silt, and pollutants. Thus the visibility of colors underwater varies dramatically according to the particular body of water involved.

Figure 1 shows characteristic light-transmission curves for several bodies of water in which we have worked (4).

The transmittance curve of water from Morrison Springs, Florida, a freshwater body famous for its clarity, is essentially the same as that of distilled water, and has a maximum transmittance of over 90% at 480 mμ, i.e., of blue-green light. The only difference between the samples from Morrison Springs and the Gulf of Mexico is a lower transmittance of the Gulf water in the violet and blue portions of the spectrum, presumably because of plankton (transmittance curves for Caribbean waters are similar).

The Long Island Sound water, which is typical of coastal water, shows more consistently reduced transmittance throughout the spectrum than Morrison Springs and Gulf waters do, with the greatest loss being in the blue and blue-green light. Finally, the Thames River in New London, Conn., a fine example of highly turbid, polluted river and harbor water, transmits very little light at all. The shape of its curve is the reverse of the other curves, showing the greatest transmittance in the long wavelengths. These curves represent an absorption distance of only 1 m of water, the transmittance differences between waters increasing exponentially as the distance that light travels through them increases.

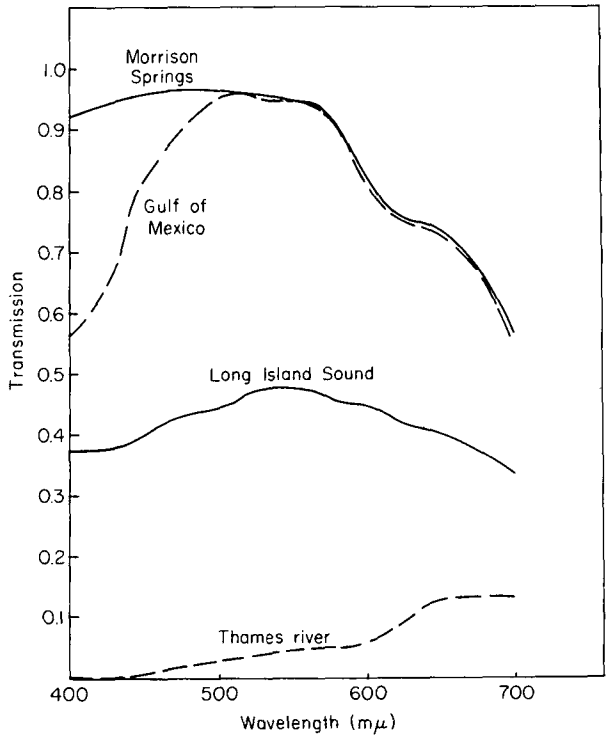

FIG. 1. Spectral transmittance through 1 m of water from various sources. (From Kinney *et al.* (4), with permission of the publisher.)

We have made several investigations into the effect of these differences on the visibility of colors. Figure 2 summarizes our findings under conditions of natural illumination. Relative visibility is plotted as a function of the dominant wavelength of the colors tested. Fluorescent paints prove to be consistently superior to the nonfluorescent ones of the same color; the colors most visible in clear water are not so in murky water; white is highly visible everywhere; gray and black are rarely visible. Additional data for artificial light sources underwater and data on color confusions (from which one can determine the best colors to use for color coding) have been previously reported (4, 6).

A second physical modification of light energy underwater occurs when particles in the water scatter light energy, causing a blurring of the natural contrast of objects against their backgrounds (2). If the contrast is reduced sufficiently, the object will become invisible, no matter what its size. In certain cases only a perfectly diffuse, homogeneous field—a sea of light—can be seen. The same effects in air are well known (1); they can cause "space myopia," visual illusions, loss of orientation, and other disturbances. Deaths of airplane pilots and arctic explorers have been attributed to the phenomena called "empty-field myopia" and "whiteout," which are quite similar to the visual impairment characteristic of the underwater world.

We have studied changes in the appearance of colors and in man's ability to judge the relative distances of objects as they are affected by this homogeneous visual stimulation. Objects

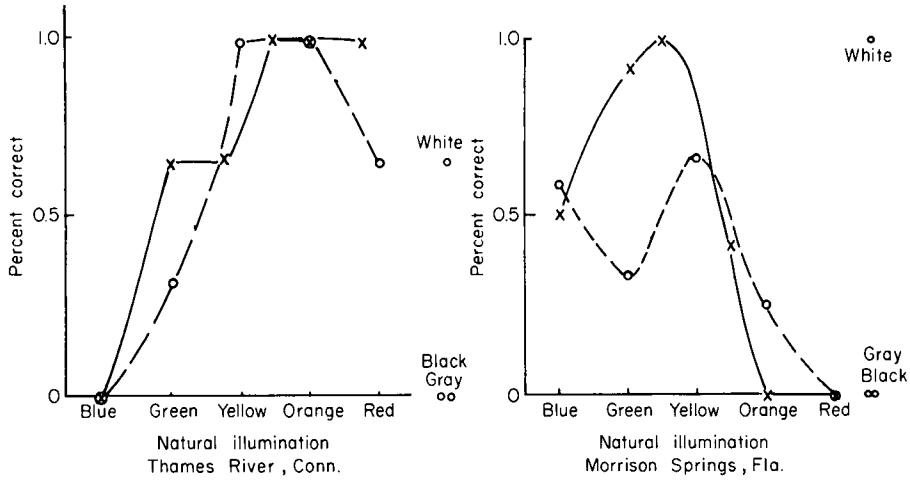

FIG. 2. Examples of relative visibility of naturally illuminated colors at a distance of 1.8 m in the Thames River, and at a distance of 26 m in Morrison Springs. (——) Fluorescent paint; (– – –) regular paint. (From Kinney *et al.* (4), with permission of the publisher.)

of neutral color appear reddish (3) and relative depth judgments based on color are severely hampered (8).

We wish now to discuss the implications of another major change in physical energy occurring underwater—the refraction or bending of light rays as they pass from one medium to another. This physical phenomenon has many important visual consequences.

Normally, most of the refraction of light that focuses the image on the retina occurs at the corneal–air interface. When the unprotected eye opens underwater, this interface is lost, and with it most of the refractive power of the eye. The interior of the eye has almost the same refractive index that the water does, and therefore no refraction or bending occurs. The optical image of an underwater object, therefore, is dreadfully out of focus; the diver becomes farsighted with a 50-diopter hyperopia.

We recently conducted an investigation of the underwater visual acuity of individuals not using a face mask to determine just how bad the situation is. Visual acuity proved to be exceedingly poor under all conditions. Under the most ideal circumstances, acuity was reduced to the level of night vision—that is, to be seen the underwater target had to be at least 10 times as large as a similar target viewed in air.

Fortunately, individuals do not usually find themselves under water without face masks. With the exception of a downed pilot or an escaping submariner (for example), men under water normally wear a face mask, which restores the corneal–air interface and allows more normal vision. The use of the mask, however, brings with it a second interface, between the water and the air in the diver's mask. This interface in turn refracts light rays and has important visual consequences.

The effect of the water–air refraction at the face mask is diagrammed in Fig. 3. The light rays from the underwater object are refracted or bent away from their normal axis at the water–air interface. This creates a virtual image at three-fourths of the actual distance between

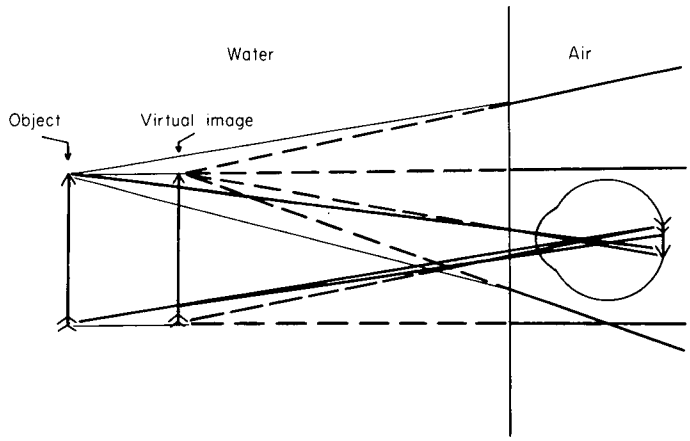

FIG. 3. Optical images produced by a real object (solid lines) and its virtual image underwater (dotted lines) due to refraction at the water–air interface.

the interface and the object. The retinal image created from this virtual image is thus larger by about 30% than the actual image from the same object if it were in air.

We have studied perception of both size and distance under water. For inexperienced subjects, sizes were perceived exactly as one would predict from the magnification of the optical image. We are currently measuring the size perception of experienced divers to determine whether they have adjusted to this distortion.

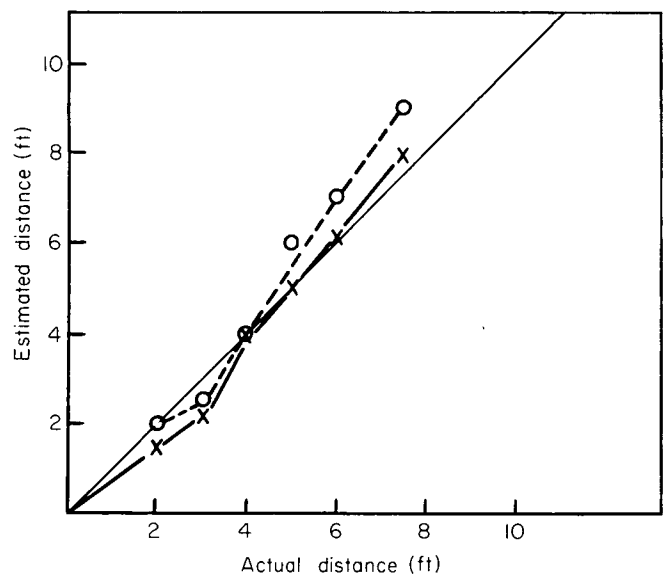

FIG. 4. Effect of water clarity on the perception of distance. Median values of subjects tested. (– – –) Turbid water; (—) clear water.

On the other hand, the subjects' perception of distance underwater was not what we would have predicted from the optical image or from what is taught in standard diving manuals. Some of our distance-perception data are shown in Fig. 4. Distance estimates are plotted as a function of actual distance for two conditions of water clarity (7, 9).

Most distances were overestimated, and the overestimation increased with greater turbidity. As the object distance decreased, there was a crossover point, after which underestimation occurred at very short distances. The data in Fig. 4 for subjects viewing through clear water at distances of 2 or 3 ft can be predicted from the size of the magnified optical image.

An additional problem confronts the diver who attempts to perform tasks underwater. Because of the distortions of size and distance, objects cannot be located in space where they appear to be. The diver, particularly the novice, is constantly plagued with reaching out for an object only to miss it; often many trials must be made before successful contact is made. Some experienced divers do successfully perform many tasks underwater; one must therefore assume that some adaptive visual process occurs. In fact, responses to distorted images in air have been extensively investigated. A major conclusion is that human beings are remarkably adaptive, eventually responding appropriately even to the most distorted images.

In our first study of adaptation to the underwater environment (5), we tested the hand–eye coordination of subjects with various degrees of underwater experience. Data from this experiment are shown in Fig. 5. We expected that if a subject were not visually adapted, he would respond to the test objects as if they were closer to him than they actually were. This

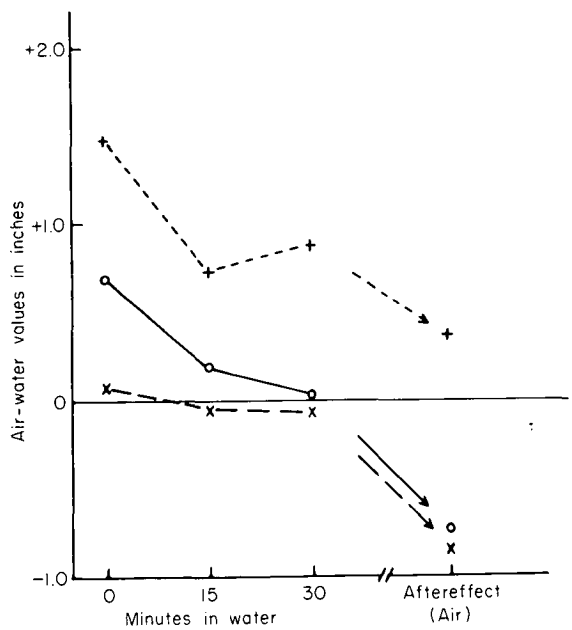

FIG. 5. Adaptation to changes in the apparent location of underwater objects as a function of time spent in visual–motor underwater activities. Subjects: (+) No experience; (○) novice divers; (×) experienced divers. (From Kinney *et al.* (5), with permission of the publisher.)

is, in fact, what happened initially in the tests involving subjects with little or no underwater experience. However, after 15 min of underwater activity (playing games, working crossword puzzles, building a tower of rocks), they made fewer errors. For those subjects having some underwater experience, another 15 min of underwater activity was sufficient to eliminate errors in hand–eye coordination; but the totally inexperienced subjects did not completely adapt during this 30-min period. The performance of the latter group as a whole was relatively poor, undoubtedly because they were preoccupied with the process of breathing. Breathing must become routine before an individual can even pay much heed to the visual world underwater, much less learn from it.

The results obtained from the experienced divers were completely different. They never underestimated, even when they first entered the water, and they showed excellent hand–eye coordination to distorted images throughout the test periods.

We recently attempted to plot the course of visual adaptation by following the progress of students attending scuba school for a 4-week period. Surprisingly, the men showed very little adaptation. Even after 4 weeks of intensive daily underwater training, their underwater vision was no better than that of our group of inexperienced subjects after 15–30 min of specially designed visual–motor activities. It appears that there is tremendous room for improvement in the training of beginner divers in visual–motor coordination, and we are currently working on means of raising underwater visual adaptation to its maximum level.

In summary, the image of an underwater object is altered in size and distance, its color is changed, its brightness is dimmed, and its outline is fuzzy and indistinct. The object is not where it appears to be; often it is invisible. We are studying all of these problems with the aim of improving all aspects of divers' underwater vision that lend themselves to improvement.

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HEARING LOSS IN DECOMPRESSION

J. Donald Harris

The structures of the ear are so delicately formed and balanced that man is sensitive to acoustic stimuli over the very broad range of 10 octaves in frequency and to pressures near 0.0002 dynes/cm^2, at which value particle movements of the air are as minute as 10^{-10} cm. Yet over and above this extreme sensitivity, the ear can support acoustic pressures a billion times greater, or 140 db. But even this does not exhaust the elasticity of the ear, for the middle ear can undergo static displacements, as in the Valsalva maneuver, at least an order of magnitude greater than is found in response to intense sound.

It is only because of these large safety factors and pressure release mechanisms that we are able to withstand the innumerable severe insults to our auditory mechanism which are the everyday lot of the diver. The fact is that a corps of men can be formed by sharp selection and training which can continue to perform well for years while undergoing the constant auditory rigors and dangers of a diving career. Shilling and Everley (69) show that the mean audiogram of divers who have had no special exposure to intense noise was not appreciably worse than controls. Coles (14) examined 57 very experienced divers and found their hearing perfectly normal except for those who had experienced "much" gunfire or small arms noise. Evidently, in a cadre of men who do not experience barotrauma and who are protected by proper procedures from bubble formation, hearing may remain perfectly normal. There is thus no suggestion that repeated compression–decompression cycles per se have any effect on auditory acuity.

There are, however, many individuals for whom pressure equalization across the drum is not semiautomatic, or even possible, and there are occasions when even experienced divers find it difficult, for one reason or another, to "clear" their ears, or to do so with sufficient rapidity. Furthermore, there are those who have, during a dive, experienced sudden hearing losses of which the etiology is still obscure, though it is thought to be associated in some way with their diving experience. We shall deal with these two types of cases in this paper.

Aerotitis Media

Armstrong and Heim (2) thoroughly explored the condition they termed aerotitis media, caused by a lack of pressure equalization of the middle ear. The drastic changes which can

sometimes occur in the eardrum and lining of the tympanic cavity, with blood coming from the Eustachian tube, can easily be seen. The classification of increasing damage by Teed (75) into categories 1–5 is well known.

From this laboratory Shilling and Everley reported a series of 2751 submariners, only 9 of which finally failed to pass the 50-lb pressure test (69). In this series, 90% of men taking the test for the first time required no change in pressurization rate to completion, while 91.5% of those who had passed the test at least once required no change.

The condition is worse with air than with $He–O_2$ mixtures because of the increased speed with which $He–O_2$ can pass through the Eustachian tube. Thus in acute conditions it has been found that caisson workers could obtain relief, reenter a pressure chamber, and suffer less infection and suppuration if the pressure change was performed in $He–O_2$ (16, 30, 47, 62).

Haines and Harris (29) performed otoscopy, nasopharyngoscopy, and pre- and postpressure audiometry on 6149 men. Almost no effect on hearing was found unless the middle ear contained serosanguineous fluid. Alfandre (1) in another series of 432 men from this laboratory generally confirms these conclusions.

Occasionally, even an experienced diver will experience an ear "squeeze," a more or less severe case of aerotitis media, such as when there is a temporary catarrhal obstruction of the Eustachian tube or if the rate of descent is too fast, as in a free fall. Most eardrums will then rupture, at about one-half atmosphere difference across the drum. But when such damage has occurred and healed, and the eardrum is scarred, it is never quite as strong again, and may rupture at much less than half an atmosphere. Coles (14) mentions one case of a very thin scar which ruptured at a pressure differential the equivalent of 6 ft. Such a person would be quickly screened out of a diving cadre.

Etiology of Sudden Deafness of Obscure Origin

An early case of obscure origin was mentioned in 1922 by Kobrak (43), and since then hundreds of cases have been fully described (4, 5, 7–13, 15, 17–23, 25, 26, 28, 31, 32, 34–37, 40, 42, 44–46, 48–51, 53, 55–58, 61, 63, 64, 67, 68, 71–74, 77, 78, 80, 82–84).

The observations of Bosatra and DeStefani (10) may be taken to be typical of these surveys: 1 patient experienced bilateral loss after emotion strong enough to redden the face; 7 patients after cooling, usually driving with an open window; 5 patients probably in connection with Asian flu; 34 patients with no clue whatever. The loss was bilateral in 5 of 47 patients.

Five types of possible etiology have been suggested by most authors for unexplained sudden deafness.

1. Acute neuritis of the 8th nerve. Some patients show an audiological picture of acoustic neurinoma rather than hair cell involvement, usually with vestibular disorders, and in these an allergic or serous neuritis might have occurred. In one clinical case from Bosatra and DeStefani (10), histology showed an extreme edema interrupting 8th nerve fibers at the base of the modiolus. The edema and disruption may also occur more distally, within the cochlea itself.

2. Virus infections. Van Dishoeck and Bierman (79) proved by immunological research the strong connection between sudden deafness and virosis. Schuknecht *et al.* (65) noted that about 25% of patients with sudden deafness complain of a "cold." In four patients diagnosed as viral labyrinthitis, histology showed in fact that the picture was *not* that seen in experi-

mental vascular occlusion in the animal, and they concluded that a mumps-like virus may be important in sudden deafness. Beal *et al.* (6) confirmed this, and stated that the port of entry of the virus particles into the cochlea was via the stria vascularis during viremia. Jaffe (34) reported 40 of 143 patients had viral symptoms; Van Dishoeck (78) reported this in 50 of 153 patients, about one-third overall. Jaffe pointed out that virus particles could influence intracochlear blood flow by: (*a*) causing a hypercoagulable state, (*b*) causing edema of the endothelial cells of the capillaries, narrowing the lumen, and (*c*) attaching to the erythrocytes and causing hemagglutination *in vivo*, and sludging the blood as seen in the fingernail bed and in the retina. His cases included five pregnant or postpartum patients and eight patients postsurgery. In both of these situations there is a rise in platelet number and adhesiveness, either of which, and especially both, may lead to microthrombi in the inner ear. Thus, it is likely that the pathogenesis of viral infections leading to sudden deafness is in fact a vascular obstruction to the intracochlear structures. In this connection one recalls that the capillaries feeding the organ of Corti are actually on the basilar membrane in intimate association, rather than further off in the stria vascularis as formerly thought.

3. Vascular accident. Many writers have suggested thrombosis, embolism, hemorrhage, or vasospasm due to generalized or localized cardiovascular disease—labyrinthine ischemia. These concepts are clarified by a review of the cochlear circulation (59). For example, a thrombosis of the labyrinthine artery or of the internal acoustic artery immediately distal to its origin can result in complete loss of function in both cochlea and vestibule. A thrombosis of the common cochlear artery will suppress the cochlea, while the vestibular artery will feed the posterior labyrinth, and a thrombosis of the cochlear artery proper will suppress the low tones but not the high, since the basal spiral also receives from the cochleovestibular artery [see several cases in Maurer (52)].

The histological picture is one of the hair cells of the cochlea being most susceptible, followed by the nerves and finally by the vestibule. Thus, more or less transient spasms of the labyrinthine vessels affect the hair cells first, as determined by, for example, the presence of recruitment. Jerger *et al.* (36) studied a series of 12 patients who knew almost the minute their loss occurred, and whose hearing did not subsequently change. Four gave flat audiometric losses at 50 db with an audiological test pattern typical of peripheral organ disorder, while eight gave sloping losses of 60–90 dB with test pattern typical of 8th nerve lesion. Tetu (76) offers a series of 33 patients, 14 with confirmed vascular accident and retinal vascular disturbances, and 19 in which it was concluded that hemorrhage, paralysis of the vessels, or obstruction by vascular spasm must have caused the hypacusis. They felt their vascular hypothesis was correct since treatment for that condition improved most of the cases. These patients must have been highly selected.

It is not difficult to picture severe and sudden vasomotor lesions in the branches of the internal acoustic artery. The vascular bed of the guinea pig has been examined, for example, by Weille (81), Irwin *et al.* (33), and Perlman and Kimura (59). Vascular spasms, stasis, and blood sludge may follow such stressors as CO_2 breathing, cooling, intake of histamine, etc., bringing about alterations in the endolymph and perilymph with probable deleterious effects on the neuroreceptors.

4. Vasomotor neurosis. Bosatra and DeStefani (10) advance the interesting hypothesis that a "vasomotor neurosis" may underlie much unexplained sudden deafness, just as it may underlie chronic glaucoma and Ménière's disease, by way of a primary alteration of the capil-

lary and precapillary circulation. Such alteration would lead directly to deterioration of structures maintained metabolically, such as hair cells, dendrites, etc. Arnold and Ohsaki (3) point out that blood vessel sludging can stem from disorders in neurovascular regulation. Jakobi and Skurczynski (35) feel indeed that many cases of sudden deafness should be looked on as acute extreme hydrops labyrinthi, neurovegetative in nature. They immediately trephine the footplate, reporting nine complete cures in 41 patients, 10 more improved, and 5 able to hear loud speech. This is over 50%, better than often is reported by other treatments. Borasi and Sperati (8) had three skindivers with unilateral hypacusia, from diving under apnea at 5–10 m. The precipitating causes were pressure imbalance, cold temperatures, hypercapnea, and hypoxemia; but the authors felt the etiopathogenesis was a neurovascular angioneurotic mechanism, which yielded within a month in all three cases to antiedematous drugs and vasodilators. Kleinfeldt (42), in the largest sample yet published (N:64q), concluded that the pathogenesis of the condition is based on an abnormality of the nervous regulation of the smallest blood vessels in the internal ear. It is important here to recall that the cochlear and cochleovestibular branches of the internal acoustic artery are "end branches" and there is no collateral circulation.

5. Acoustic trauma. Reports persist that some episodes of sudden deafness occur in or shortly after exposure to loud noises, but of an intensity not usually thought to be noxious. Kawata and Suga (38) followed 20 patients over 7 years, almost all with flat or U-shaped audios, about half with recruitment, but only two with vestibular symptoms. Faltýnek and Veselý (22) presented five cases. Kecht (39) had four patients presenting bilateral and partly severe asymmetric inner ear losses, and one total unilateral, to noises of pneumatic drills, circular saws and steam engines. This is now termed "akustische unfalles."

Additionally, Arnold and Ohsaki (3) report a patient with diagnosis of collagen disease, successfully treated with a 2-week regime of prednisone. Mörl (54) reports a case from violent physical effort, with spontaneous recovery in 6 weeks, apparently a transient ischemia. Kleinfeldt (42) noted that in 16% of his large sample there were alterations of the cervical spinal column. This reminds one of Fields and Weibel's demonstration (24) of effects on vestibular function of ischemia due to compression of the vertebral arteries by hyperextension of the neck and extreme rotation of the head.

In summary, one can say that in many cases the precipitating causes mentioned, such as viremia, exposure to low temperature, loud noises, emotion, sudden physical work, etc., must be overlaid on a previous state of sensitization and abnormal reactivity of the local or general vegetative nervous system.

Decompression Sickness and Hearing Loss

Even after discounting some cases of sudden deafness in a cadre of divers as arising from such causes as discussed heretofore, there remain a number of cases of sudden hearing loss in such a population which seem inescapably to be more directly connected to decompression. Ten cases of hearing loss are described in the Appendix: R. C. from our own laboratory, and nine from the Experimental Diving Unit (EDU), courtesy of Dr. Summitt and the EDU staff.

In the case of R. C., an open-water diver, two episodes a year apart gave a picture of intracochlear hemorrhage, probably of the internal acoustic artery, producing in the first episode a severe and in the second episode a mild flat loss, but recovering to normal, except for a per-

manent 15-dB dip at 1 kc/sec. The nine cases from EDU all occurred during decompression from dives of 300 ft or more on $He–O_2$. The fact that two of these men, H. J. and C. K., recovered their hearing during recompression almost as suddenly as they had lost it would seem to indicate that bubble formation was the sole cause of their defect, with resulting hypoxia in the hair cells. In a similar case, R. J. suffered a sudden profound loss of hearing, which responded only partly to recompression. Most bubble formation had led to anoxia of sufficient duration to damage the receptor cells permanently.

Of those with residual permanent defects, S.'s audiogram is U-shaped down a maximum of 45 db at 1–2 kc/sec and R. J.'s is U-shaped down a maximum of 30 dB at 500 kc/sec, while generally flat audiograms are found in R. W., down 40 dB; W. D., down 70 dB; T., down 50 dB; and B., down 70–80 dB. Evidently, if bubble formation is the etiopathology, it is rather general throughout the cochlea.

Of those whose hearing returned substantially during subsequent days or weeks, R. C. recovered after 3 weeks, S. recovered 25 dB after a few weeks, and M. after 4 weeks. From the time course and the extent of recovery, one may conclude that these men are likely to have suffered hemorrhage rather than bubble formation. On the other hand, those men who did not respond to recompression and whose losses did not improve back to normal (R. W., W. D., R. J., T., and B.) are likely to have suffered bubble formation.

In summary, one can say that among those who are exposed repeatedly to compression–decompression cycles, such as aviators, the breath-holding Ama shellfish divers of Japan, military and industrial divers, and caisson workers, etc., a relatively high incidence of otological symptoms including hearing loss is often reported. Most of this can readily be attributed to the residual effects of repeated aerotitis media. There exist, however, cases of hearing loss, usually sudden, directly connected to decompression. A review of the topic of sudden deafness reveals that every large hospital where such records have been published sees about one to two patients every month whose sudden deafness is not easily explained. Possible causes have been suggested: acute neuritis of the 8th nerve, virus infection, vascular accident, vasomotor neurosis, acoustic trauma at levels of noise not usually noxious, collagen disease, transient ischemia from violent exercise or strong emotion, or from alterations of the cervical spinal column.

None of these causes, however, would seem to underlie sudden hearing loss of a moderate or often profound level during decompression, and which responds favorably to immediate recompression. Five patients are presented with hearing loss under decompression, two of whom recovered their hearing loss completely during recompression, and three with only slight recovery. It seems likely that these patients had sustained bubble formation in one or more branches of the internal acoustic artery. In the others, not responding to recompression, treated with decongestants or vasodilators, the possibility also exists of microhemorrhage in the cochlea.

In cases of sudden hearing loss during decompression, recompression is always performed, though if the etiopathology is a vascular accident such as a spasm or hemorrhage, it would not be expected to help. Several authors (4, 28, 34) advocate immediate hospitalization for every case of sudden deafness, with virological, vascular, humoral, and audiological examination. All authors agree that treatment is hopeless if deferred. In a large series Sheehy had 62% recover if treated within 4 days, only one if treatment was deferred beyond 6 weeks (67). Segal

(66), Piesel (60), and Lumio and Aho (48) confirm this. Cocks (12), Maurer (52), and Kessler (41) have had good success with stellate ganglion block if given very early. Kessler performed 610 stellate ganglion blocks on 94 patients over 7 years, for Ménière's disease and for sudden loss of hearing, although with three fatal accidents; he cautions against inexperienced use. Gaillard (27) notes that in the case of a hemorrhage, an anticoagulant may obviously do more harm than good. Coyas (15) gives a regime of testing for clotting time to govern dosage of heparin and acenocoumarol. In cases of supposed ischemia of the labryinth, Boriani (9), Faltiner (21) and Faltýnek and Veselý (22) report very good success with ATP. The latter present 13 patients with supposed ischemia of the organ of Corti, and 5 with acute acoustic trauma, but from generally nonnoxious levels; only 1 patient did not show entire (14 patients) or at least substantial (3 patients) improvement. Jaffe (34) gives a summary of a suggested regimen with the most recent drugs. Stange (70) cautions that treatment over weeks is not only justified but necessary, as with some patients he found improvements through the 70th day. He gave, on alternate days, (*1*) Complamin*, Ozothin*, Neurobion*, and Rovigon*, and (*2*) infusions of 7% $NaHCO_3$ and stellate ganglion block.

Appendix

Audiological Notes on Sudden Deafness among Divers—Ten Cases

1. R. C.

Episode (1). Audiogram normal in 1963. Tank instructor. On Jan 18, 1964, while not actually in the water, suffered severe loss of hearing in right ear, with some slight nausea. Two days later, audiometry showed a rather flat loss at 70–80 dB. A dozen audiological tests (speech, recruitment, temporal integration, differential intensity and frequency discrimination, tone decay, Bekesy audiometry) indicated intracochlear peripheral organ involvement. Within 3 weeks hearing was normal except for a residual 15-dB loss at 1 kc/sec.

Episode (2). Had been in water 45 min on Oct 14, 1965, up and down to 118 ft. Sudden disorientation of 90°, but no nausea. Within a few minutes he seemed normal and made another 118-ft dive. Six days later he appeared for an audiogram, showing a U-shaped curve down to 30 dB, within 6 more days back to previous acuity. No further episodes, though he subsequently broke the world free-dive record, but the 15-dB dip at 1 kc/sec remains today.

Impression: two episodes of hemorrhage throughout the labyrinth, cause unknown.

2. S.

In early 1968, on a dive to 350 ft for 30 min on $He–O_2$, patient had fluid in middle ear and hearing loss. Placed on decongestants; 2–3 days later audiometry showed U-shape at 70 db through 3 kc/sec, up to 40 dB at 8 kc/sec. Four days later recovered to 60 db at 1–4 kc/sec, and 1 year later still U-shaped, down 45 dB at 1–2 kc/sec.

3. R. W.

450-ft dive on 28–31 Jan 1969. Pain in knees during decompression; 2 hr after compression, decompression, and surfacing, noted mild hearing loss in right ear with tinnitus. Vasodilators

* German proprietary name.

and recompression were given. Irregularly flat audiogram loss, 50 db at 1 kc/sec of which 5–10 db only recovered later.

4. W. D.

Same dive as R. W. At 56 ft, patient noted hearing loss in right ear. Some fluid in middle ear; decongestants were given. At 36 ft, tinnitus occurred, and lowered hearing. Recompression was given, but flat loss of 70 db continued, with little subsequent recovery.

5. R. J.

Sudden profound loss of hearing at 110-ft decompression stop. Partly recovered during recompression at 165 ft after 5 min of breathing 36% O_2. Diagnosis by Capt. Taylor, ENT Clinic, Bethesda Naval Hospital, as "bends involving left internal acoustic artery." Residual U-shaped audiometric defect, maximum of 30 db at 500 cps.

6. H. J.

At 40 ft during decompression, noted sudden but moderate loss in right ear. Recompression to 60 ft, and later slow decompression, patient felt hearing improve back to normal.

7. C. K.

After 11 min at 95-ft stop during decompression, patient noted sudden loss in right ear, confirmed by low and high frequency tuning forks. Recompression to 165 ft brought acuity to normal after 15 min.

8. T.

Late 1966, in air compression chamber, he reached down to pick up something, and felt something happen to his left ear. One hour later, audiogram showed flat 50-db loss bilaterally. Only slight improvement after 4 months, and loss persists to this date.

9. B.

Much like R. J.; episode at first of low-tone loss during decompression, treated by recompression but with deficit of 70–80 db remaining.

10. M.

Episode (1). Jan. 1968 had vertigo only during 350-ft dive.

Episode (2). June 1968 at 30–40 ft on He–O_2, suffered unilateral profound acuity loss. On vasodilators, back to normal over 27 days, but tinnitus remains.

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VESTIBULAR DERANGEMENT IN DECOMPRESSION

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Loss of auditory or vestibular function during or after decompression is an old and continuing concern in the diving community. Present-day interest in the problem stems from an apparent increase in incidence of such derangement, which seems to parallel the burgeoning number of deep dives made in recent years.

The U.S. Navy Experimental Diving Unit has reports on about 1050 military and civilian diving accidents of all types from 1960 to the end of the first quarter of 1969. We collected the reports of vertigo and/or hearing loss, and eliminated the cases of those subjects who: (*1*) had difficulty clearing their ears during compression; (*2*) had inner ear symptoms while at maximum depth; (*3*) underwent uncontrolled or rapid emergency ascents, with the possible consequence of traumatic air embolus; (*4*) had extensive decompression sickness involving the CNS in which vertigo was quite secondary; or (*5*) whose histories obviously contained inadequate information.

After this elimination there remained 16 cases—seven with vertigo alone, three with vertigo and hearing loss, and six with hearing loss alone. As indicated in Table I, there has been a sharp increase in the occurrence of such cases over the period from 1960 to the first quarter of 1969. The incidence rate of this problem is not available since there is no tally of the total number of dives performed at the various depths.

TABLE I

CASES OF VERTIGO AND/OR HEARING LOSS IN DIVING

1960–1962	0
1963	1
1964	0
1965	2
1966	2
1967	2
1968	7
1969 (First quarter)	2

TABLE II

DESCRIPTIONS OF TEN DIVES IN WHICH VERTIGO OCCURRED

Case no.	1	2	3	4	5	6	7	8	9	10
Type of dive	Wet chamber	Wet chamber	Open sea	Open sea	Open sea	Wet chamber	Open sea	Wet chamber	Wet chamber	Wet chamber
Depth (ft)	250	450	149	45	149	300	70	400	160	350
Bottom time (min)	120	60	30	2–3	30	15	114	60	35	90
Work level	Moderate	Moderate	Moderate	Light	Moderate	None	Moderate	Moderate	Heavy	Moderate
Breathing mixture	$He–O_2$ air	$He–O_2$ air	Air	Air	Air	$He–O_2$ air	Air	$He–O_2$	$He–O_2$	$He–O_2$ air

Table II describes the 10 dives in which vertigo occurred. Since the large majority of U.S. Navy dives have been to depths of less than 100 FSW, the four cases of vertigo occurring in dives to 300 FSW or greater constitute a relatively high incidence rate. In case 4, incidentally, the loss occurred after 30 successive breath-hold dives to 45 FSW. Paulev (8) has reported on the possibility that bends will occur after repeated breath-hold dives, and we felt that this mechanism might have been involved in this case.

The accompanying symptoms in our 10 cases are listed in Table III. The findings are those

TABLE III

SYMPTOMS ACCOMPANYING TEN CASES IN WHICH VERTIGO OCCURRED

Case no.	Symptoms	Case no.	Symptoms
1	Nausea	6	Fatigue
	Vomiting		Nystagmus
	Visual disturbances		Nausea
			Visual disturbances
2	General weakness	7	Nausea
	Nystagmus		Vomiting
	Tinnitus		Nystagmus
	Nausea		Diplopia
3	Nausea	8	Hearing loss
	Vomiting		Tinnitus
			Nausea
4	Nausea		
	Vomiting	9	Hearing loss
	Headache		Dyspnea
	Nystagmus		
5	Localized pain	10	Hearing loss
	Rash		Tinnitus
			Nausea

TABLE IV

RELIEF OF SYMPTOMS

Case no.	1	2	3	4	5	6	7	8	9	10
Onset of symptoms	90 FSW during ascent	98 FSW during ascent	Surface +13 min	Surface +1 min	10 FSW during ascent	Surface +20 min	Surface +32 min	120 FSW during ascent	Surface +25 min	110 FSW during ascent
Delay in treatment (min)	Not treated	Not treated	96	68	42	2	151	5	19	17
Maximum treatment depth (FSW)	—	—	165	165	165	60	165	210	165	165
Depth of relief (FSW)	—	—	No relief	165	165	60	165 (80% relief)	210	165	165
Time of relief	3–4 weeks	5 days	Not reported	+20 min	+2 min	+22 min	+68 min (80% relief)	+103 min	+38 min	+6 min

usually associated with vestibular dysfunction. The visual disturbances, other than diplopia and nystagmus, experienced in cases 1 and 6 were bright flashes of light; the subjects reported neither blind spots nor constriction of visual fields.

Recompression, when used, was generally successful in relieving the symptoms (Table IV). In the two cases in which recompression was not carried out, the subjects gradually became asymptomatic (over 5 days in case 2, and over 3–4 weeks in case 1).

Discussion

Most reports of vestibular problems in diving involve middle-ear squeeze during compression. However, earlier literature on the subject, on caisson workers, contains reports of vestibular symptoms, at least some of which were related to decompression problems. For example, Heller *et al.* (2) around the turn of the century reported 24 cases of a Ménière-like syndrome. Four in 10 of their cases suffering only from vertigo had normal-appearing tympanic membranes, and 6 in 11 having hearing loss as well as vertigo displayed normal-appearing tympanic membranes. These pressure exposures were rather shallow, but the bottom times were relatively long. The controlling half-time tissues and the pressure–time relationships in the caisson exposures are similar to those in our own deep dives, suggesting that these factors might conceivably account for the appearance of decompression-related vestibular and hearing symptoms in both instances.

The sudden onset of vestibular symptoms during or after decompression naturally leads one to assume that the etiology is decompression-related, and the odds favor such an assumption. However, we must at least consider other possible causes of these symptoms and we should rule them out insofar as it is practical. Changes in the tympanic membrane and the middle ear due to barotrauma may develop slowly, and the onset of symptoms may be delayed until some minimal threshold of stimulation is reached. The case history and an examination of the ear should be sufficient to make a correct diagnosis. Infections of the middle ear, external auditory canal, or surrounding structures—infections known to cause vertigo—can develop during the long decompression from a saturation dive or from a deep nonsaturation dive. Dizziness or vertigo can be a manifestation of hypotension and decreased perfusion of blood from any cause, including bradycardia or tachyarrhythmias, as examples. The emphasis again must be placed on an immediate, careful history and physical examination. Hypertensive crisis can manifest itself as vertigo, but one hopes that a person with this potential does not dive.

Of the primary demyelinating diseases of the CNS, multiple sclerosis is practically the only one that can begin with the sudden development of true and unaccompanied vertigo, or of nystagmus and ataxia without subjective vertigo. Reports indicate that vertigo is the first symptom in from 5 to 12% of multiple sclerosis patients (9). There is nothing clearly distinctive about the vertigo in multiple sclerosis, and diagnosis of the disease based on vertigo can only be made in retrospect.

Paroxysmal positional nystagmus, which is a poorly understood disease entity, may be central or peripheral in origin. The vertigo occurs only when the head is in certain positions or during change to a certain position (6).

Localized cerebrovascular insufficiency—whether from a recompressible intravascular bubble, from a noncompressible thrombus or embolus, or from reversible hemoconcentration effects—can cause dizziness or vertigo as the first or sole symptom. The internal auditory artery, which supplies the 8th nerve and the vestibular and cochlear structures, arises either from the anterior inferior cerebellar artery or directly from the basilar artery. Occlusion of any of these three arteries can compromise vestibular blood flow (1).

Histories of a group of stroke patients at the Massachusetts General Hospital, in whom specific vascular lesions could be identified, revealed dizziness or vertigo in a large percentage of those with occlusions of the basilar artery, anterior inferior cerebellar artery, internal auditory artery, or vertebral artery. For many this was the initial symptom, and for some, the only one. The vestibular symptoms in the vertebral–artery occlusions correlated with the finding of a wedge-shaped infarction of the lateral medulla, including the inferior portion of the vestibular nucleus (1).

It is desirable to differentiate between CNS and labyrinthine vertigo, and a clinical evaluation is helpful in doing so. True rotatory vertigo usually is labyrinthine in origin. Labyrinthine vertigo is further characterized by a history of discrete attacks and by other symptoms of proportionate severity, such as nystagmus, nausea, vomiting, ataxic gait, pallor, and sweating. Central-vestibular vertigo often involves a more or less constant sense of imbalance; its other symptoms are disproportionate to its severity, or are absent altogether (10).

Existing information about Ménière's disease, also known as *endolymphatic hydrops*, suggests another possible cause of vestibular dysfunction: intralabyrinthine pressure changes. Ménière's original case report, as reported by Walker (11), involved hemorrhage into the

labyrinth, but subsequent reports have consistently stressed the autopsy finding of dilatation of the endolymphatic system. In addition to the triad of unilateral low-tone sensorineural hearing loss, tinnitus, and attacks of vertigo, many patients with typical (and atypical) Ménière's syndrome report a sense of pressure or fullness in the affected ear (10). Lest we jump too rapidly to a conclusion regarding pathogenesis, however, we should note that endolymphatic hydrops may be present in individuals who never have had symptoms of Ménière's disease (11). Several questions, therefore, need to be answered. What is the effect of pressure, as such, in different parts of the labyrinth? Does increased pressure really cause the observed dilatation? Is the pressure effect a continuum, or does it occur only after disruption of labyrinthine structures? Do changes in the ionic composition of the endolymph and perilymph occur before membrane disruption occurs? How is the pressure increased? Some of the answers are available, or at least on the horizon.

Henriksson *et al.* (3, 4) reported, in 1966, a series of experiments on the effects of pressure variation in the membranous labyrinth of the frog. They developed and tested a simple, reproducible means of applying and of quantitating pressure and volume changes in the endolymphatic labyrinth. They demonstrated its elasticity by showing a linear relationship between changes in volume and pressure, and a consistent rupture of the frog saccule at applied pressure exceeding 5–8 cm of water. In some of their preparations, these investigators further showed an effective block between the utricle and the saccule, and made use of this block to show that, at pressures below the rupture point, the intact utricular membranes do not permit passage of fluid. When the utriculosaccular connection was intact, the rise in pressure, for a given increase in volume, was followed by an exponential decline that was due to loss of the added volume from the system. One cannot tell from this report, however, whether this loss in volume was due to increased removal or to decreased formation of endolymph. By the use of fluid-staining techniques, applying pressures below the rupture point showed fluid uptake into the capillaries surrounding the endolymphatic walls. A small amount drained into the cranial cavity via the endolymphatic duct, but no leakage occurred through the membrane into the perilymph. Subsequently—again with pressures below the rupture point—Henriksson *et al.* (3, 4) showed a transient rise in ampullary nerve activity in response to increased pressure, and suggested that more persistent nerve activity would result from pressure applied to the point of membrane disruption.

We suggest several ways in which such pressure changes might occur either minimally or with enough force to create membrane disruption during decompression: bubble formation in the endolymph; decreased circulation of the blood, perhaps caused by hemoconcentration or autonomic responses involving the supplying vessels (10), which could result in decreased resorption of endolymph; rapid buildup and release of middle-ear pressure, as suggested by Lundgren (7) in his discussion of alternobaric vertigo; or pressure differences within the membranous labyrinth because of blocks in the endolymphatic circulation. Additionally, osmotic gradients from dissolved gases, as suggested by Kylstra *et al.* (5), could raise endolymphatic pressure by causing fluid shifts.

The goals of this presentation have been: (*1*) to call attention to the problem of vestibular dysfunction caused by decompression; (*2*) to review what is known regarding possible causes of such malfunctions; and (*3*) to stimulate interest in possible avenues of research. Definitive knowledge about the pathophysiology of vestibular derangement will, one hopes, help to avoid it and to treat it more effectively when it does occur.

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SPEECH DISTORTION AT HIGH PRESSURES

G. M. Fant, J. Lindqvist, B. Sonesson, and H. Hollien

There are several factors that affect verbal communication at hyperbaric pressures. Speech distortion is the primary disorder, whereas middle ear hearing loss (1, 4) is less disturbing. Psychological and physiological factors account for disturbances in human operators' articulatory and discriminatory abilities. However, the basic cause of the speech distortion can be attributed to physical disturbances in the sound generation process. This disorder, dependent as it is on the pressure and gas mixture of the breathing atmosphere, cannot be compensated for to any considerable extent by speech training.

The changes in speech occurring when a speaker breathes a $He–O_2$ or other mixture are well known. The effects of the particular ambient pressure, on the other hand, are less well known and constitute the main theme of the present paper, the purpose of which is to describe some Swedish work done on the subject (2, 3) including an analysis of speech recordings of SeaLab aquanauts while they breathed a $He–O_2$ mixture at pressure. These recordings were put at our disposal by the Communication Sciences Laboratory of the University of Florida.

The Effect of Pressure on Speech in Air

Our interest in these matters dates from 1962, when we heard a tape demonstrating the typical nasal quality of a subject's speech in a tank pressurized to the equivalent of approximately 300 FSW, or 11 atm abs, compared with his speech at normal pressure in air. We first looked for a physiological explanation. However, an X-ray investigation in the pressure tank revealed no insufficiency in the velopharyngeal closure (see Fig. 1). The nasality, therefore, was not induced by velar muscle dysfunction. So we turned our attention to the physics of speech generation in the search for possible factors to explain the distortion. This study was quite successful.

In normal speech, the distribution of maximum energy in the speech spectrum is principally determined by the shape of the vocal cavities from the larynx to the lips and by the velocity of sound in the exhaled gas mixture. Figure 1 illustrates the cross-sectional area function of the vocal tract and the frequency spectrum of the vowel *i* spoken in air at 1 atm. Since in-

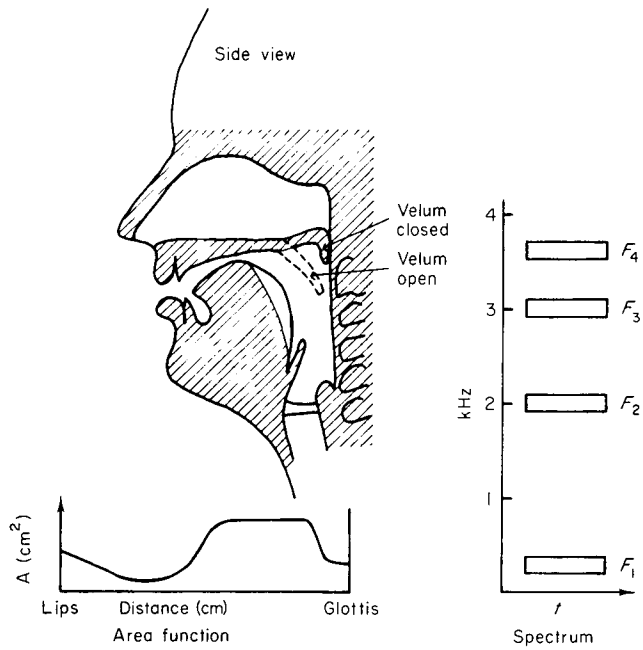
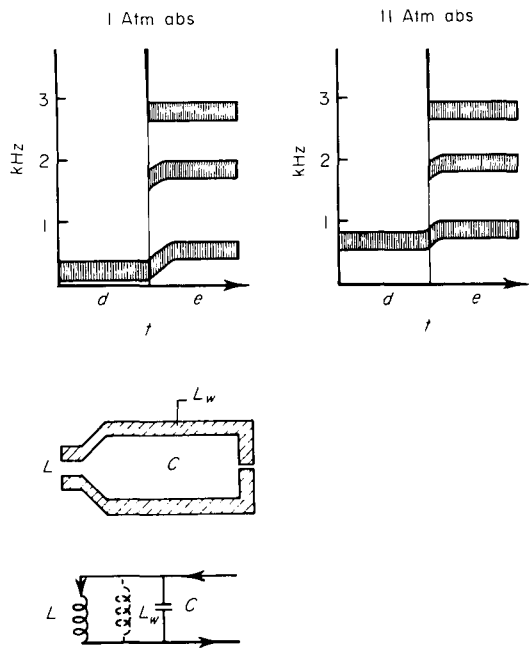


FIG. 1. Vocal tract outline, velopharyngeal valve, area function and stylized spectrogram of a nonnasalized vowel *i*.

creased air pressure does not affect the velocity of sound to a significant degree, the effects of pressure distortion are not the same as the "Donald Duck" transposition effects that are characteristic of He speech. The main spectral distortion observed under hyperbaric conditions can be described as a transposition of formants in some inverse relation to their frequency. The lowest limit of the formant range is radically raised and the dynamic frequency range of formant 1 is accordingly compressed, as illustrated in Fig. 2.

When one speaks in an air atmosphere at increased pressure, the increased density of the air causes an increase in the impedance of the air, and accordingly reduces the mismatch between the impedance of the cavity walls and air. The cavity walls therefore participate more effectively in sound propagation and in tuning of vocal resonances than they do at normal pressures. In an electrical circuit, as shown in Fig. 2, that is equivalent to the vocal resonator mechanism, the cavity wall impedance enters as an inductance (L_w) that evidently causes a relatively heavy loading of the resonator at a low frequency, but is of no importance at a very high frequency.

The lower limit of F_1, the first formant frequency, is the resonance frequency of the closed vocal tract, F_{wo}, which in speech under normal atmospheric conditions is about 150–200 Hz, but which can be shown to increase with the square root of the pressure. At 164 FSW—i.e., 6 atm abs—this limiting frequency is raised to approximately 350–500 Hz, and at 11 atm abs, 500–650 Hz. Figure 3 provides a comparison of predicted and measured formant frequency shifts at 6 atm abs. The data refer to the average formant frequency shifts of four



$$\begin{cases} F = (1/2\pi)\ (1/L_e C)^{1/2} \\ L_e = (L\ L_w)/(L + L_w) \end{cases}$$

$$F_{wo} = (1/2\pi)\ (1/L_w C)^{1/2}$$

$$F^2 = \left(F_i \frac{c'}{c_0}\right)^2 + F_{wo}^2 \frac{\gamma}{\gamma_0} \frac{P}{P_0}$$

$$F^2 = \left(F_i \frac{c'}{c_0}\right)^2 + F_{wo}^2 \left(\frac{c'}{c_0}\right)^2 \frac{\rho_0'}{\rho_0} \frac{P}{P_0}$$

F_i at conditions $c_0\ \rho_0$ and no wall shunt
F_{wo} closed tract resonance at $c_0\ \rho_0$
$c_0\ \rho_0$ air at sea level, P_0 = 1 atm abs
$c_0'\ \rho_0'$ other gas at sea level, P_0 = 1 atm abs
$c'\ \rho'$ other gas at pressure P

FIG. 2. The effect of vocal tract wall vibrations on the closed tract resonance and the formant pattern in air at 1 atm abs and 11 atm abs pressure.

male subjects. The measured data agree very well with the theoretical predictions. As can easily be demonstrated analytically for the lowest resonance, acoustic theory predicts relation:

$$F_n^2 = F_{ni}^2 + F_w^2 \tag{1}$$

in which F_n is the actual resonance frequency, F_{ni} is the resonance frequency without consider-

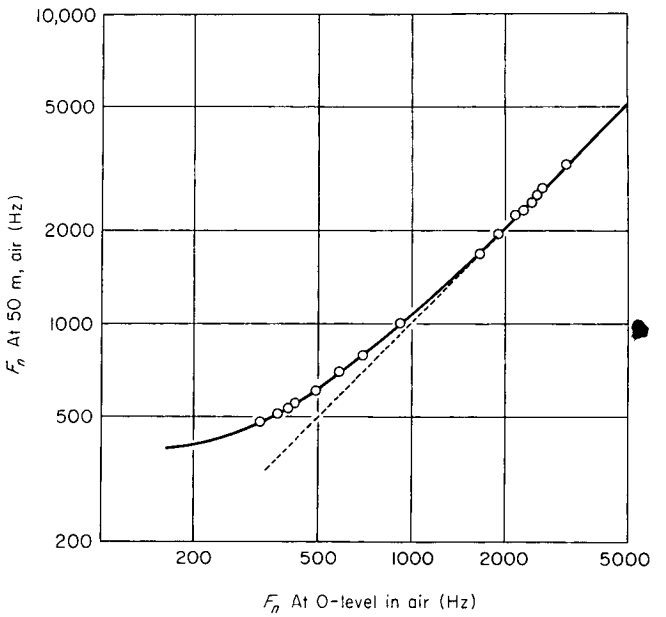


FIG. 3. Predicted and measured formant frequency transpositions in air at 50 m equivalent depth (164 FSW). Average of four male subjects. (—) Predicted; (○) measured; (– – –) $k = 1$.

ation of vocal wall vibration, and F_w is the resonance frequency of the closed vocal tract. The last frequency, which sets the lower limit of the sound spectrum, is proportional to the square root of the density of air, i.e.,

$$F_w^2 = PF_{wo}^2 \tag{2}$$

in which F_{wo} is the closed tract resonance frequency at a pressure P of 1 atm abs. Alternatively, the cavity wall vibration can be introduced as a frequency-dependent change in the effective velocity of sound propagated in the vocal tract (c_e):

$$c_e = c_0(1 - F_w^2/f^2)^{-1/2} \tag{3}$$

Other Gas Mixtures

In the study of the combined effects of a pressure P, a density ρ', and a velocity of sound c', which are different from P_0, ρ_0, and c_0 of air at 1 atm abs, the following formula may be used instead of Eq. (1):

$$F_n^2 = F_{ni}^2\left(\frac{c'}{c_0}\right)^2 + F_{wo}^2\left(\frac{c'}{c_0}\right)^2\frac{\rho_0'P}{\rho_0P_0} \tag{4}$$

The derivation is given in reference 2. The density of the gas at $P_0 = 1$ atm abs is ρ_0'. Thus

$\rho' = P \cdot \rho_0'$ under the specific condition

$$\rho_0' P = \rho_0 P_0 \qquad \text{or} \qquad \rho_0' = P^{-1}\rho_0 \tag{5}$$

Thus Eq. (4) takes the form

$$F_n = (c'/c_0)(F_{ni}^2 + F_{wo}^2)^{1/2} \tag{6}$$

Thus, assuming that a compressed gas has the same density as air at sea level, the specific low-frequency distortion is canceled out. Accordingly, the optimal diving depth (d) in meters is

$$d = 10[(\rho_0/\rho_0') - 1] \text{ atm abs} \tag{7}$$

Since for an ideal gas

$$c = [(\gamma P)/\rho]^{1/2} \tag{8}$$

the optimal diving depth for avoiding the pressure effect is

$$d = 10[k^2(\gamma_0/\gamma) - 1] \tag{9}$$

in which $k = c'/c_0$ is the overall frequency transposition factor, $\gamma = C_p/C_v$ for the gas, and γ_0 pertaining to air. Thus when $k = 2$, the optimum depth is about 35 m (115 FSW); and when $k = 4$, optimum depth is about 180 m (590 FSW). The closed tract resonance F_w is approximately independent of the particular gas mixture, and varies as a function of pressure

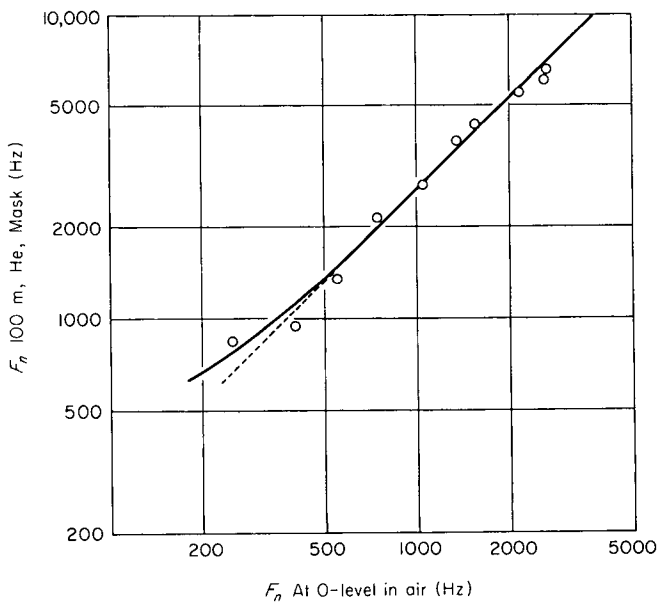


FIG. 4. Predicted and measured formant frequency transpositions of Subject B while he breathed a 2.5% O_2–97.5% He mixture through a light diving mask at 100 m equivalent depth (328 FSW). (—) Predicted; (○) measured; (– – –) $k = 2.63$.

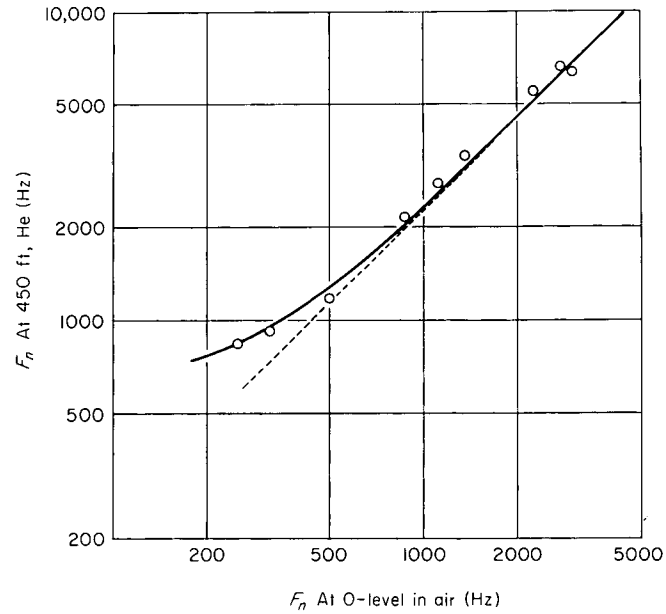


FIG. 5. Predicted and measured formant frequency transpositions of Subject C while he breathed a 90% He–7.8% N_2–2.06% O_2 mixture at 450-FSW pressure. (—) Predicted; (○) measured; (– – –) k = 2.26.

of the breathing mixture, i.e.,

$$F_w = F_{wo}[(\gamma/\gamma_0)P]^{1/2} \tag{10}$$

Here F_{wo} pertains to air at P_0 = 1 atm abs. The ratio γ/γ_0 is a constant of the order of 1.

A study of the formant transposition in a He–O_2 atmosphere was carried out at the research unit of the Swedish Navy in Stockholm. The subject used a closed circuit breathing system providing 97.5% He and 2.5% O_2. The velocity of sound in the gas mixture was experimentally measured and checked against calculated values (5). A transposition factor of k = 2.63 was measured. A prediction according to the complete formula given in Eq. (4) produced quite satisfactory results, as shown in Fig. 4.

Fant and Lindqvist (2) further evaluated the above theory by using formant data taken from the SeaLab III speech material. Figure 5 pertains to subject C at the equivalent of 450 FSW. He was speaking without a mask in a chamber containing a 90% He–7.8% N_2–2.06% O_2 breathing mixture. Even under these conditions, the results were rather well predicted by our theory.

Among other effects that can be predicted from acoustic theory is that voiced speech sounds increase in intensity by 3 db through doubling the atmospheric pressure, whereas the level of fricatives and other unvoiced speech sounds is not affected appreciably by pressure. A certain loss in high-frequency energy of unvoiced fricatives and stops is generally observed. Such effects probably reflect changes in turbulent source spectra, but could also be caused by insufficient frequency response of the microphone used for recording.

Technical methods for restoring the normal spectrum of speech articulated at high pressures are thus facilitated if the density of the gas is made low enough to suit the particular diving depth (Eq. 7 or 9). However, depth is not a very critical consideration as long as the density of the gas at a particular depth is not radically different from that of air under normal speaking conditions. This is also a requirement for comfortable breathing.

At high pressures, some speakers raise their fundamental voice frequency, and most speakers slow down their speaking rate. A correlation between speaking tempo and the P_{O_2} was found in the SeaLab III speech material (2). Intelligibility scores of the unscrambled He speech studied by one of us (Hollien) were of the order of 90% at zero level, 47% at 200 FSW, and 12% at 450 FSW. Developmental work on new systems for speech restoration is being carried out at the Royal Institute of Technology in Stockholm, Sweden.

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PART VII. SENSES AND COMMUNICATION*

DISCUSSION

J. K. Summitt, Chairman

Dr. Kylstra: Dr. Kinney, in your studies of hand–eye adaptation of trained diver subjects in contrast with the untrained subjects, there was a recovery period in which the trained subjects showed remarkable deterioration in hand–eye coordination, and this apparently persisted for quite a while. Would you comment on that? I wonder how anything that has to do with coming to the surface could affect this performance.

Dr. Kinney: There is an aftereffect that persists, but only for a very short time. It is measured after all optical distortion or, in this case, underwater distortion has been removed. The duration is usually 1–2 min but sometimes it lasts as long as 5 min.

Dr. Beckman: In the visual studies that Dr. Kinney did, it is my impression that one must really differentiate between the optical effect of wearing a piece of plate glass in front of the visual apparatus because of the magnification and the nearing effect on vision. In one of your slides you showed the effect of distance, and I want to know whether these studies were done with a flat plate in front of them so that visual distortion exists.

Second, in the proprioceptive studies done on grasping, was this purely a visual phenomenom or was there any differentiation between the visual effect and the proprioceptive effect of being in water?

Dr. Kinney: The subjects all wore normal diving masks, so that there is distortion due to the normal face mask. The overestimation apparently occurs at distances beyond 4–5 ft and is apart from the usual magnification, which accounts for the data at lesser distances.

In answer to your second question, concerning hand–eye coordination, the visual appearance of the object is quite different from where the physical location of the object is. This is what we are attempting to measure. I do not believe a kinesthetic component entered into our particular tasks. The tasks could be arranged so this would be a complicating factor. In our cases the subject simply was reaching to where he thought the object was located, not being able to see his own hand or to self-reach at the same time.

Dr. Godfrey: Dr. Kinney, could you expand on the kinds of tasks which your subjects performed while adapting?

Dr. Kinney: Without any great theoretical basis we chose for this particular experiment a group of tasks which involved manual manipulation of objects while the subject was looking at them. One of the tasks was a game like checkers, where you pick up pieces and move them on a board. Another of the tasks was working a crossword puzzle so that you had to fill in little spaces. Another was writing your name out of objects on the bottom of the underwater area.

In our new work we are going to choose some tasks on a theoretical basis, which should optimize things. But in every instance you need to have some feedback from where you think your hand is and where you actually see it come.

Dr. Godfrey: Did you have people who did not do anything, who did not physically interact with the environment but just sat on the bottom and looked at things?

Dr. Kinney: Not in the experiment that I showed you. We have done this and our data are not complete. But we have looked into this sort of thing. However, that would be the experience of the class of scuba divers that I mentioned. Presumably most of their 4 weeks of underwater experience was simply swimming and getting used to the equipment.

* *Panelists:* J. A. Kinney, J. D. Harris, C. J. Rubenstein, G. Fant.

Dr. Lambertsen: Concerning the occurrence of deafness and vertigo, I understand that there is a condition, an idiopathic acute deafness which is a fairly well-known phenomenon in the nondiving population. I also understand that the end organ is one in which the arteries which supply the tissue are end arteries. Therefore the discussion concerned with sludging of blood or the agglutination phenomena has for many years been considered important in clinical otolaryngology in this condition, and on that basis heparin has been used as the major method for managing this nondiving casualty.

Can you elaborate upon the possibility that heparin may be important in the management of sudden deafness that occurs in diving and that heparin treatment should be routinely used in this circumstance?

Chairman Summitt: Dr. Farmer is the best one to answer that, but I would like to make one comment. A hemorrhage into the cochlea is also a very real possibility, and in the reported cases of caisson workers in the early 20th century, autopsy cases demonstrated some hemorrhage into the end organ itself. In the cases we have treated we have not used heparin. We have been very hesitant even to use dextrans, which decrease the coagulation of the blood.

Dr. Farmer: I think Dr. Lambertsen is quite right. In otolaryngology sudden deafness in the nondiving population is not infrequently seen. However, our understanding about why this occurs still remains quite remote. There is a possibility, as Dr. Summitt has pointed out, that hemorrhage within the organ itself could be a possible explanation. Indeed there is also the possibility that vascular insufficiency plays a very real role, whether it is on the basis of intravascular sludging or intravascular bubble formation or localized spasm of the end artery.

Insofar as treatment is concerned, in otolaryngology there is very little agreement as to what treatment should be used in cases of sudden deafness in the nondiving population. Intravenous fluid therapy has been used; vasodilators have been tried. Intermittent respiration with 5% CO_2 has been used; diuretics, intravenous histamine, steroid therapy—in fact, there are several very well-known otolaryngologists who treat cases of Ménière's disease with every one of these modalities at the same time.

We might have evidence of experience in subjective feelings, but there is no experimental data which justify any one of these therapeutic measures at the present.

Chairman Summitt: I might point out, too, Dr. Lambertsen, that during our treatment schedules with recompression we frequently put our divers on 100% O_2 at 60 ft and we do not know the local vascular response to the vasodilators or many of the other drugs used in this kind of treatment.

Dr. Lambertsen: To extend that question, could you tell us, then, the usual pattern in the nondiving public when these sudden episodes of deafness and vertigo occur? It is my understanding that unless treatment is quite drastic and prompt the deafness is likely to be permanent. Is this the case? It would help the audience, I think, to know the usual end result.

Dr. Farmer: The pattern of cases of sudden hearing loss is very variable. When we better understand this phenomenon, prompt treatment is certainly going to be the rule. In our present understanding and knowledge of this problem, the pattern is so variable that I think it would be improper to say any one specific form of therapy at the present time is apropos.

Perhaps the best way for the audience to approach this problem is with the feeling that prompt therapy will probably do no harm and certainly has a better chance of helping than delayed therapy.

Probably what you are referring to is cases of sudden deafness in which it has been postulated that there are sudden membrane breaks. Again, this is not well understood. In a particular series of cases reported by a group in California, half of the patients had some hearing loss which was considered to be a sudden membrane break on the basis of being associated with some sudden stress or trauma. Half of these people had no return of hearing, and half had a return of hearing within 6–8 weeks. Again, a variable and very poorly understood pattern exists at the present time.

Dr. Rubenstein: I may complete part of the answer to Dr. Lambertsen's question: One of the things asked was whether use of heparin would be considered to be important in treatment. We want to make sure that there are no false ideas created about the role of recompression in treatment of these sudden losses of hearing in vestibular function. Of our cases of vestibular function loss, recompression was applied in some of these; and where recompression was applied immediately, there was a nearly immediate response to treatment. I think this is an awfully important thing to keep in mind. We do need to think about all the possible etiologies. Nevertheless, the odds are with us when we are in a decompression situation in recompressing, but we do want to take the time to look first to see what else may be going on.

Dr. Rehman: My question concerns the audiovisual aspects of decompression, the vestibular aspects

particularly. First, the possibility of pressure on the endolymphatic system was mentioned. To refresh your memory, the endolymphatic system is based in a position in the region of the middle ear, well protected by the cerebrospinal fluid externally and lying within an enclosed compartment internally, but not readily subjected to external pressure.

Second, in considering the vascular supply to the vestibular apparatus, mention was made that the branch might be derived from the anterior inferior cerebellar artery or the basilar artery or the internal auditory artery. These three vessels, in their order, are derived—the basilar particularly—from the vertebral and the vertebral from the subclavian. Might not a pressure effect be derived directly from an increase in the ambient pressure or the hydrostatic pressure on the root of the neck rather than to assume that the pressure is transmitted into the interior of the cranium to produce the effect?

In that case, of course, the question also arises: Might there not also be an effect upon the carotid sinus affecting not directly the lining and the medial layer, but the general area around it?

The third aspect of this deals with the possibility of the Eustachian tube being clogged, with resultant inability to dive. Is there a possibility that in such a situation there is a pressure effect on the vascular bed in the region of the promontory which in turn produces an effect upon the round window? There could result interference with the action of the cells and in the organ of Corti, or possibly the action upon the stapes leading to hyperemia in the area and interfering with the action of the stapes on the foot plate. If so, the temporary deafness and then the restoration of hearing on recompression might be due to a mechanical effect.

Dr. Rubenstein: To begin with the degree to which the membranous labyrinth is protected, it actually is a protected organ within the bony structure and separated from the bony structure by the perilymph. There is no good information now concerning how the pressure effect is created. We can only postulate ways that have at least been suggested by other experiments in the literature, and what we are postulating is the creation of a pressure effect within the endolymph itself.

If you look at the structure, the connection between the utricle and the saccule, for instance, there is a little slitlike orifice with a flap of the utricular wall directly over it. It is conceivable, for example, that this could act as a flap valve and produce transient problems of block within the endolymphatic channels themselves.

We are also suggesting the possibility of the formation of additional fluid or the occurrence of additional fluid within the endolymph, either because of decreased elimination or overproduction or osmotic shifts. I think the concept of osmotic shifts is intriguing, but we can only raise the question and not answer it.

Pressure on the vasculature itself in the region of the neck should be distributed in a more generalized sense to the vasculature and might have a greater tendency to cause venous obstruction and retrograde pressure. This might produce an additional likelihood of membranous rupture or of vascular rupture within the structures.

If one thinks in terms of vasodilatation, then the question should be raised whether, if you had vasodilatation on that basis, this would be likely to cause an increased extravasation of fluid into the endolymph.

Dr. Farmer: Perhaps so under certain circumstances. I think we might clear up this point of confusion. In order to think about possible etiologies of this problem on the basis of pressure changes in the endolymphatic system, one has to presuppose or to find that there is a pressure differential between certain parts of the endolymphatic system and the middle ear, or the subarachnoid space. At the present time we have no knowledge that would indicate that there is such a pressure differential.

The inner ear, as a matter of fact, even though it is encased in a very bony structure, is a distensible organ. There are three portholes of distensibility—the round window, the oval window, and the endolymphatic sac. Pressure in any one of these places, if there is such a pressure differential, will lead to distribution of this pressure through either of the other two openings if these openings are patent.

In regard to your questions about possible middle ear dysfunction, this brings us to what I feel is a very important point. In cases of middle ear dysfunction one is most likely going to have a conductive hearing loss rather than a neurosensory hearing loss, and if I may make a plea to people who are concerned with deep diving and who are having ear problems: When you do your audiometric testing, please differentiate. The first step in analyzing a hearing loss is to determine what type of hearing loss it is. If you are having middle ear problems with no inner ear problems, you are going to have a conductive hearing loss. With tuning forks and sophisticated use of tuning forks, this type of hearing loss can be ascertained even in a diving tank under high pressure conditions.

In addition, we should obtain reliable means of measuring hearing even under pressure, as well as before dives and after dives. Then we will have at our disposal a better means to diagnose exactly what kind of problem you are dealing with and can institute treatment more quickly.

Dr. Horvath: Dr. Kinney, in view of your pictures of the remarkable patterns of water effects on vision, were your experiments conducted under the most optimum conditions for water, namely, in distilled water?

Dr. Kinney: The graph which showed the various transmissions for different bodies of water applied only to the visibility of color data. The rest of the data were obtained in a clear pool where the turbidity could be controlled. In most cases the experiments were performed in clear water. Under certain circumstances—for example, some of the depth data—clarity was controlled and transmissions got down as low as 30%/m.

We have done other depth experiments in lakes where turbidity was extremely high. But most of the hand–eye data was clear water data.

Dr. Hughes: The ability to equalize the middle ear seems to be a problem with any diver and indeed sometimes eliminates some otherwise physically qualified people. Is there an antihistamine and/or decongestant regimen that is recommended for this problem, for the man with hay fever sometimes cannot dive at certain times of the year or cannot equalize well? And is the "reverse block" really a problem when using a decongestant and/or antihistamine?

Dr. Farmer: You will get a different opinion among various otolaryngologists. Some consider that decongestants, whether systemic or topical, are poisons in the sense that they destroy the function of the nasal epithelium temporarily. In some cases they are very useful.

It would be my opinion that if a person needs to use a decongestant to adequately clear his ears, he probably should not be diving. This does not help you in your problem of having to exclude some very talented and physically able people, but this is the best answer I can give you.

Dr. Hughes: There are those I know who, because of hay fever, have a hard time equalizing the middle ear. They use decongestant nose drops and some have said that there is a rebound phenomenon while they are diving, which can be a problem in that they rupture the ear drum on ascent.

Dr. Farmer: The rebound phenomenon is a definite problem with decongestants. Certainly in situations that you just described there could be a rare instance where air can get from the nose to the middle ear but cannot get from the middle ear to the nose.

Dr. Rubenstein: What about a ball valve phenomenon during decompression?

Dr. Farmer: Most experienced otolaryngologists do not believe that in the case of an uninfected normal ear you can have a situation where you have a higher pressure in the middle ear than you do on the outside and therefore have air trapped in the middle ear.

We do occasionally see a valve action in ears that have tumors, in which the tumor does indeed act as a ball valve and obscures the proxymal orifice of the Eustachian tube as it leads from the anterior part of the tympanic cavity.

Part VIII. RESPIRATORY LIMITATIONS OF HIGH AMBIENT PRESSURES

MECHANICAL LIMITATIONS OF EXERCISE VENTILATION AT INCREASED AMBIENT PRESSURE

L. D. H. Wood and A. C. Bryan

Man is mechanically inefficient in water. His inefficiency is compounded at depth by a reduction in his aerobic power owing to the progressive restriction of pulmonary ventilation. The use of additional He rather than N_2 in the breathing mixture helps alleviate this problem, but it arises again at greater depths where, despite the use of He, exercise may be limited by the inherently restrictive properties of man's respiratory pump. The increased work of breathing and the limitation of alveolar ventilation together play a major part in the diver's retention of CO_2. This retention is dangerous in itself and potentiates other diving stresses. Some sort of respiratory assistance is obviously desirable; it is less obvious, however, what form the assistance should take. We have therefore attempted to explain the mechanism of flow limitation and to assess its importance in the working diver.

Limitations of Expiratory Flow

Many investigators have demonstrated a nonlinear fall in maximum expiratory flow rates as air density increases (8, 10, 12, 19). We have used maximum expiratory flow–volume curves to define the effect of air density on maximum expiratory flow at six different lung volumes (16). At lung volumes greater than 25% of vital capacity (VC), these flow rates varied approximately inversely as the square root of air density (Fig. 1).

We have explained these results by using the equal pressure point concept of Mead *et al.* (11), who demonstrated the unique relationship between static recoil pressure of the lung and maximum expiratory flow. Alveolar pressure (Palv) is the driving pressure in expiration. It exceeds pleural pressure (Ppl) by an amount equal to the static recoil pressure of the lung [Pst(l)]. During a maximum expiration there is a pressure drop along the intrathoracic airway that at some point equals the static recoil pressure of the lung, so that intraluminal and extraluminal pressures are equal to Ppl.

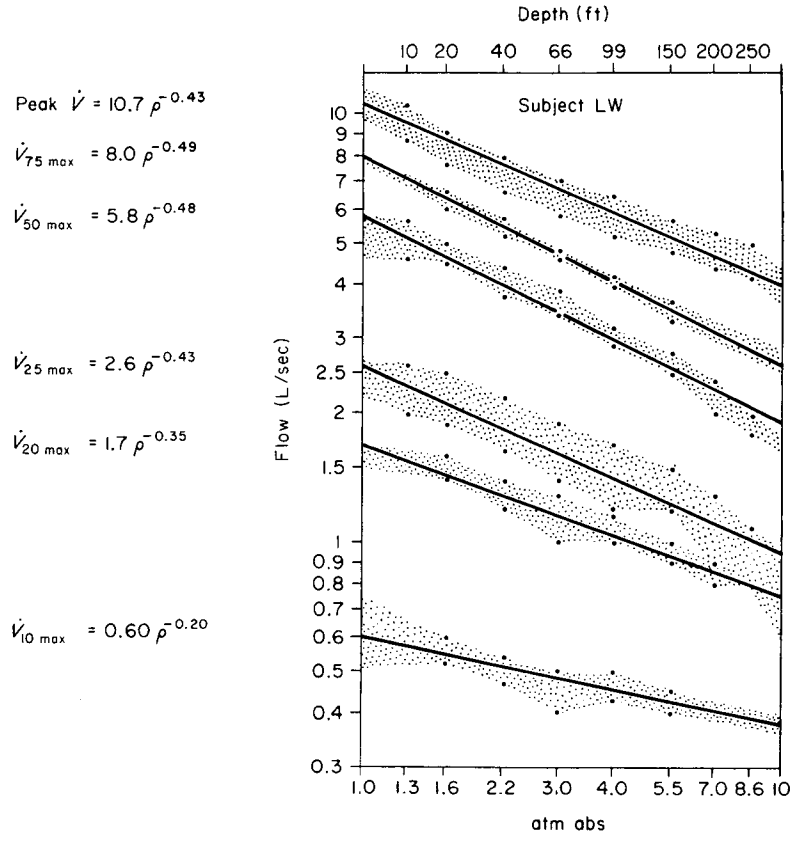

FIG. 1. Logarithmic plot in one subject of maximum expiratory flow rate at six lung volumes against air density (numerically equivalent to atmospheres). Shaded area is the range of measured values. Equations at left describe adjacent solid lines of visual best fit; i.e., $\dot{V}_{75\,max}$ is the maximum flow at 75% VC. Exponent is the slope of the line, and so describes the relationship of maximum expiratory flow to density at that lung volume. The decreasing slope with decreasing lung volume is shown (16). (By permission of publishers.)

These equal pressure points (EPP) divide the airway into two segments in series. The downstream segment has negative transmural pressures and is compressed as pleural pressure increases, so that flow cannot increase further—i.e., maximum expiratory flow is effort-independent (3, 11, 15). The upstream segment is characterized by a fixed driving pressure, Pst(l), and by a fixed geometric configuration of the airway at each lung volume.

The pressure drop along the upstream segment equals the static recoil pressure Pst(l) and has two components: (*1*) the frictional pressure losses (P_{fr}), composed of turbulent (P_{tu}) and laminar (P_{la}) pressure drops, and (*2*) the pressure losses caused by convective acceleration (P_{ca}). This last term—called the bernoulli effect—refers to the energy required to accelerate gas particles between two points in a tube with converging boundaries. Macklem and Mead (9) have shown that P_{ca} accounts for the largest part of the upstream pressure loss at lung volumes greater than 60% VC.

The components of the upstream pressure drop are described by the following equations:

$$P_{ca} = \frac{\dot{\rho}\,\dot{V}^2}{2g\,D_{EPP}{}^4}\,\dot{V}\alpha\rho^{-0.5}$$

$$P_{tu} = \frac{\rho^{0.75}\mu^{0.25}\dot{V}^{1.75}}{D^{4.75}}\,\dot{V}\alpha\rho^{-0.43}$$

$$P_{la} = \frac{L}{D^4}\,\mu\dot{V}$$

in which ρ = gas density, $\dot{V}$ = volume flow, g = gravity, D = diameter, and μ = gas viscosity. They show that if all the flow in the upstream segment were nonlaminar, maximum expiratory flow would vary between density $^{-.43}$ and density $^{-.50}$ (16). The observed relationship between maximum expiratory flow and density (Fig. 1) at lung volumes greater than 25% VC conforms with the predicted relationship. At lower lung volumes the slope decreases, so that laminar flow becomes an increasingly large component of the upstream flow regime.

These data show that maximum expiratory flow and, consequently, maximum voluntary ventilation (MVV) decrease approximately inversely as the square root of air density, because the pressure drop in the upstream segment is predominantly P_{ca} and P_{fr}.

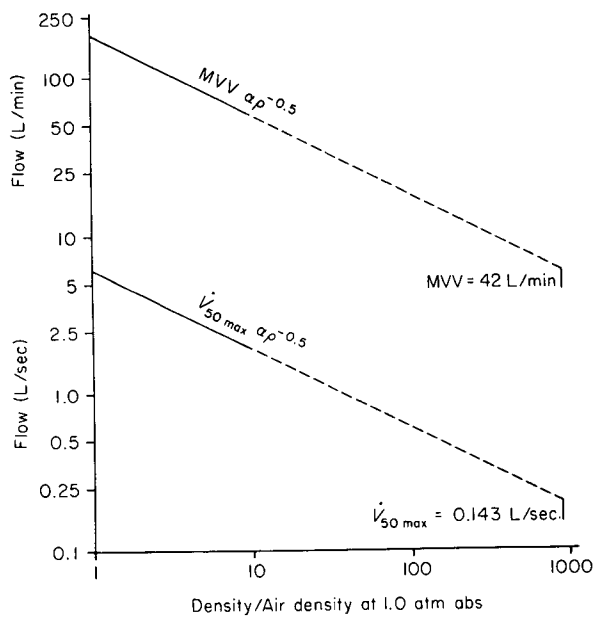

FIG. 2. Logarithmic plot of flow against density, extrapolated to the density of saline, predicting a 30 × reduction in MVV and maximum expiratory flow because of density-dependent increase in upstream resistance; and a further 1.4 × reduction because of decrease in static recoil pressure in the liquid-filled lung.

Limitations of Liquid Flow

Earlier workers [e.g., Kylstra (5)] have assumed laminar flow in predicting the mechanical limits of liquid breathing, so that breathing saline would be expected to cause a 40-fold reduction in MVV (saline viscosity being approximately equal to 40 times air viscosity). Since our data indicate that MVV is density-dependent, the data predict, rather, a 30-fold reduction in MVV (saline density is equivalent to 900 times air density) (Fig. 2). Since the pressure drop in the upstream segment varies as $\rho \dot{V}^2$, a twofold reduction in static recoil pressure caused by the lung's becoming filled with saline (7) will further reduce the MVV by the square root of 2. Our approach also predicts a 40- to 50-fold reduction in MVV, and suggests that liquids of lower density may increase liquid ventilatory capacity.

Limitations of Inspiratory Flow

Unlike maximum expiratory flow, inspiratory flow maxima are effort-dependent (3). However, as Fig. 3 demonstrates, at moderate flow rates at depth, very large pleural pressure increments produce little increase in flow. We have frequently observed a transient decrease in flow and an increase in esophageal pressure during maximum inspiration at depth. The tentative explanation is that extrathoracic airway pressure becomes so negative that there is tracheal or glottal narrowing, i.e., an inspiratory flow-limiting segment.

Maximum inspiratory flow also varies inversely as the square root of air density (Fig. 4). This density dependence is primarily due to turbulent flow in the upper airway. Moreover,

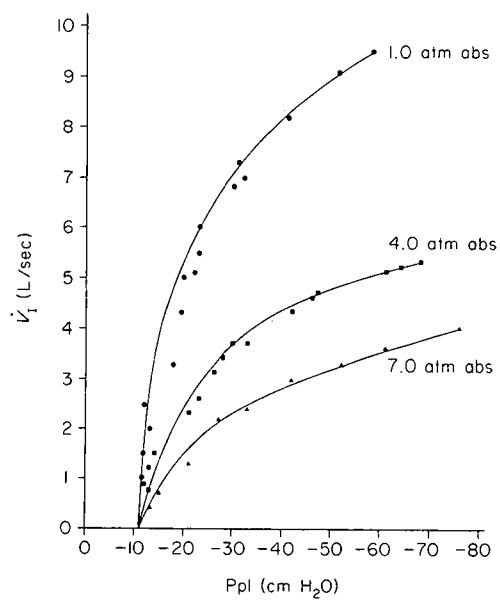

FIG. 3. Inspiratory IVPF curves at 50% VC. Note the large increase in Ppl required to produce moderate flow increases at 2–3 L/sec at 4.0 and 7.0 atm abs.

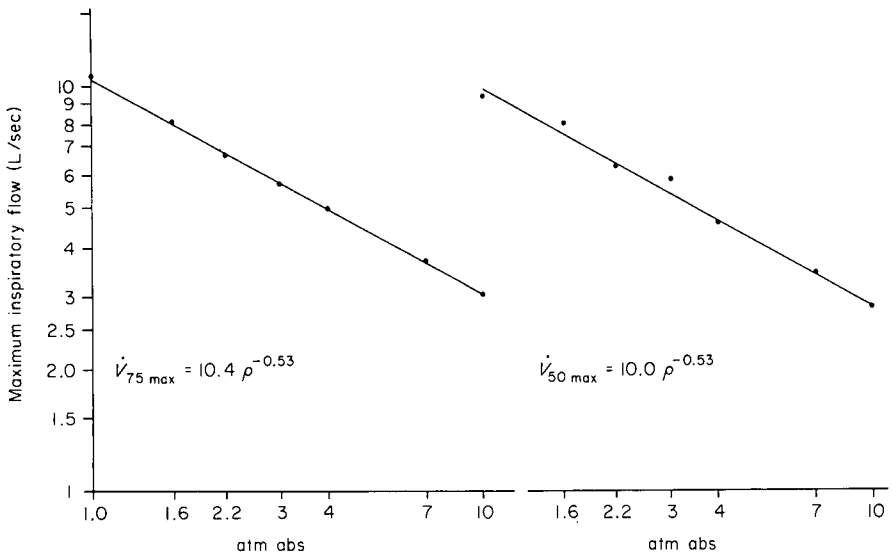

FIG. 4. Logarithmic plot of the maximum inspiratory flow rate against air density in one subject at 75 and 50% VC.

nonlaminar flow probably persists in the peripheral airway because of shear forces and the orifice effects of the branching airway (14), and because of the small length–diameter ratio of the airway segment between branching points (16). The significance of these observations is that while inspiratory flow maxima do not limit ventilation, inspiratory work increases greatly at depth.

Limitations of Exercise Ventilation

Both inspiration and expiration decrease as the same power of air density. However, expiratory flow maxima are substantially lower than inspiratory flow maxima are (cf. Figs. 1 and 4, containing data from the same subject), so that expiration limits total ventilation. Dynamic compression of the airway, which limits expiratory flow, occurs at sea level pressure only during coughing or voluntary forced expiration. It does not occur even during maximum exercise in healthy men, although it commonly occurs during exercise in patients who have obstructive lung disease (4).

To determine the effect of airway compression on exercise ventilation at depth, we have constructed expiratory isovolume pressure–flow (IVPF) curves for five lung volumes at five ambient pressures (17). Flow increased with pleural pressure until a maximum flow plateau was reached. The lowest pressure at which maximum flow was reached signaled expiratory flow limitation; and any further increase in pressure was wasted effort.

Figure 5 shows typical curves from which maximum transpleural pressure (P_{tp} max) values were obtained. The P_{tp} max values decreased with lung volume and also decreased at the

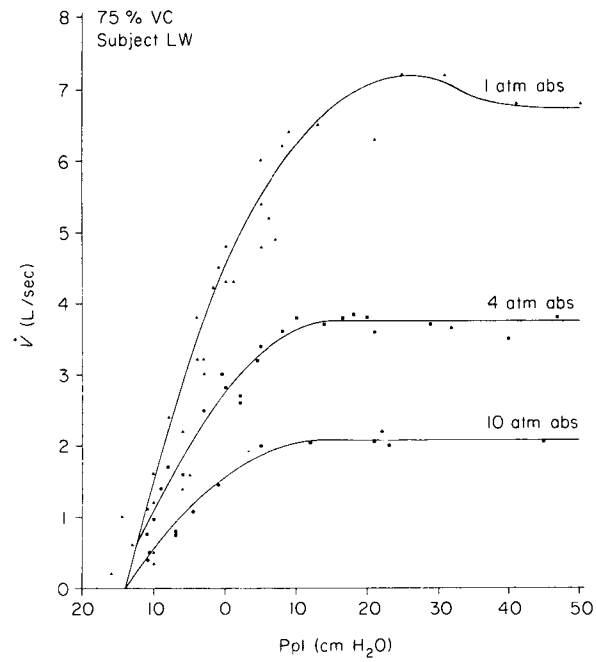

FIG. 5. Expiratory IVPF curves at 75% VC in one subject at 1.0, 4.0, and 10.0 atm abs.

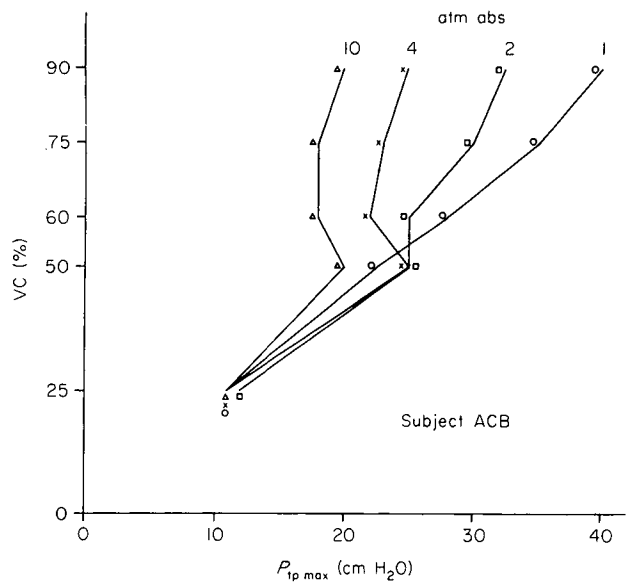

FIG. 6. Relationship of P_{tp} max and lung volume at four ambient pressures.

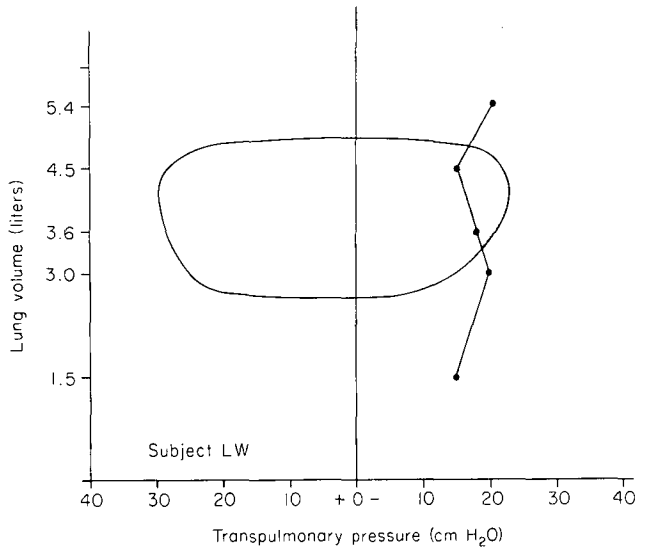

FIG. 7. Pressure–volume loop during heavy exercise at 4.0 atm abs showing expiratory pressures exceeding the superimposed P_{tp} max–volume line.

same lung volume as air density increased (Fig. 6). This latter observation may be explained by the upstream movement of EPP's as air density increases. The pressure–volume loop obtained during maximum exercise at 4.0 atm abs exceeded the P_{tp} max–volume line (Fig. 7). This occurred at lower work loads and lower ventilations as air density increased. At 300 FSW this dynamic airway compression limited exercise at an O_2 consumption ($\dot{V}_{O_2}$) of 1.3 L/min in a subject with a ground-level $\dot{V}_{O_2}$ max of 3.0 L/min. These data indicate that maximum exercise at depth is limited by dynamic airway compression.

The onset of airway compression during exercise produces an intense choking dyspnea that persists long after exercise has stopped. This symptom can be very dangerous to the working diver. Nadel (personal communication) has suggested that this dyspnea may be due to the stimulation of receptors in the compressed airway, for local anesthesia of this area in some clinical situations has relieved the symptom.

Figure 8 shows the marked rise in alveolar carbon dioxide (PA_{CO_2}) accompanying airway compression during exercise at depth. Minute ventilation decreased and PA_{CO_2} increased at each exercise level as depth increased. The terminal points at depth were obtained during maximum exercise, which was limited by airway compression during expiration. Milic-Emili and Tyler (13) have demonstrated a linear correlation between inspiratory work and end-tidal CO_2. It is possible that the increased inspiratory work at depth is responsible for this early rise in PA_{CO_2} and fall in ventilation.

It has been suggested (6) that altered sensitivity of the respiratory center plays a role in CO_2 retention, either because of high O_2 or N_2 tensions or because divers adapt in some undefined manner. Although these factors may play a part, our data suggest that the respiratory drive is there, but the respiratory pump cannot handle it.

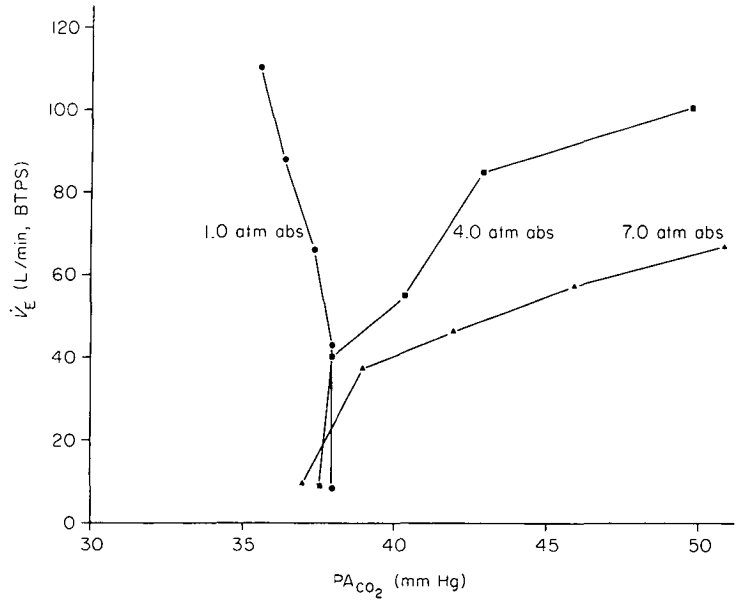

FIG. 8. Relationship of minute ventilation and PA_{CO_2} during rest and four exercise loads at 1.0, 4.0, and 7.0 atm abs.

Limitations of Regional Flow

Increased air density may disturb intrapulmonary gas exchange because either parallel or series inhomogeneity of inspired gases develops. We have used ^{133}Xe to measure the parallel distribution of inspired gas while subjects breathed mixtures of sulfur hexafluoride (SF_6) and O_2 at 1.0, 1.5, and 2.0 atm abs (18). At low flow rates (Fig. 9) the distribution of ventilation was normal for all gas densities; that is, alveoli in the inferior portion of the lungs were better ventilated than those near the apex. As flow rate increased, this distribution became reversed at higher gas densities. In view of this great variability in distribution of ventilation with depth and flow rate, many different patterns of ventilation–perfusion ratios may exist. It is possible that some of these patterns may impair gas exchange during exercise at depth. Furthermore, increased gas density will magnify any abnormalities in ventilation distribution that existed at surface pressure.

Cumming *et al.* (1) has presented convincing evidence for series inhomogeneity, or stratification, of gas partial pressures at sea level. Because intergas diffusion is density-dependent, stratified inhomogeneity might be expected to increase at depth. On the other hand, Farhi (2) has suggested that increased gas density may promote convective mixing in the lung. Recently, we have shown that alveolar–arterial oxygen tension differences decrease in subjects breathing 20% O_2 in SF_6. This suggests that, to a density 4.0 times greater than air, the improved bulk mixing more than compensates for any impaired diffusion.

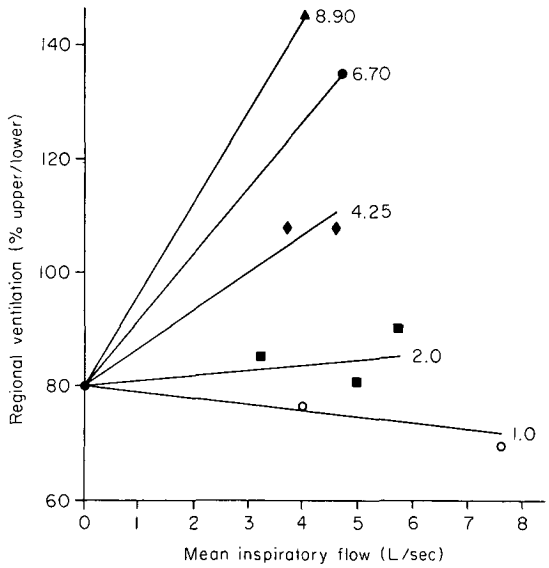

FIG. 9. Effect of flow rate of upper and lower lung regions in one subject breathing gases of different densities. The relative gas densities are indicated adjacent to each solid line.

Implications for Mechanical Assistance

The working diver breathing air at modest depths or O_2–He mixtures at greater depths has difficulty eliminating CO_2 because of the mechanical limitations of his respiratory pump. The similarity of this difficulty to that of patients with chronic obstructive lung disease suggests that divers may need assisted ventilation. Expiratory flow rates are limited by intrathoracic airway compression, so that neither pressure applied to the thorax nor suction at the mouth can increase ventilation. Assisted inspiration, however, would considerably diminish respiratory impairment at depth by decreasing inspiratory work.

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VENTILATORY LIMITATIONS ON EXERTION AT DEPTH

J. N. Miller, O. D. Wangensteen, and E. H. Lanphier

At normal atmospheric pressure the amount of work a man can do seems to be limited by his cardiovascular system (3). At depth, however, alveolar ventilation may be the limiting factor. Increased air density may prohibitively increase flow resistance through a breathing apparatus, or it may limit flow through a man's own airways.

Even if a diver uses superior breathing apparatus, his maximum voluntary ventilation (MVV) decreases markedly with increasing depth, as shown in Fig. 1. This figure is taken from Lanphier (4), who correlated data from the work of Miles (7), Wood (12), Maio and Farhi (5), and Seusing and Drube (10). The greatest difference in the values obtained by Maio and Farhi and by Wood appeared at 1 atm abs, whereas agreement was almost perfect (both about 135 L/min) at 2 atm abs. The average MVV when air is breathed is nearly 200 L/min at the surface, and falls to about half that figure at 4 atm abs.

We contend that the principal factor limiting MVV is the conductance of the airways themselves, rather than a man's ability to expend energy in moving dense gas, as had previously been supposed. The concept of dynamic compression of airways put forward by Fry and Hyatt (1), Mead *et al.* (6), and Pride and his associates (9) explains why airways rather than expiratory effort limit flow.

Figure 2 shows expiratory flow at 50% vital capacity (VC) plotted for pressures of 1, 4, and 7.8 atm abs when breathing air. Each curve is composed of many points obtained from measurements of lung volume, flow, and transpulmonary pressure during expiratory maneuvers ranging from gentle air leaks to maximal forced expirations. Pressure–flow curves were then constructed for isovolumes between 80 and 20% VC. Only the curves for 50% VC isovolume are shown. The crucial factor in expiration is that expiratory flow increases only to a certain point while expiratory effort continues to increase. Large intrathoracic airways then begin to collapse. Beyond that point additional expiratory effort produces no further increase in flow, only an increase in the degree of airway collapse. Maximum flow has become *effort-independent.*

Studies made under conditions of high pressure by Wood *et al.* (11) indicate that effort-independent maximum expiratory flow ($\dot{V}_{max}$) becomes progressively lower as gas density increases. Our own findings confirm theirs in most respects (8).

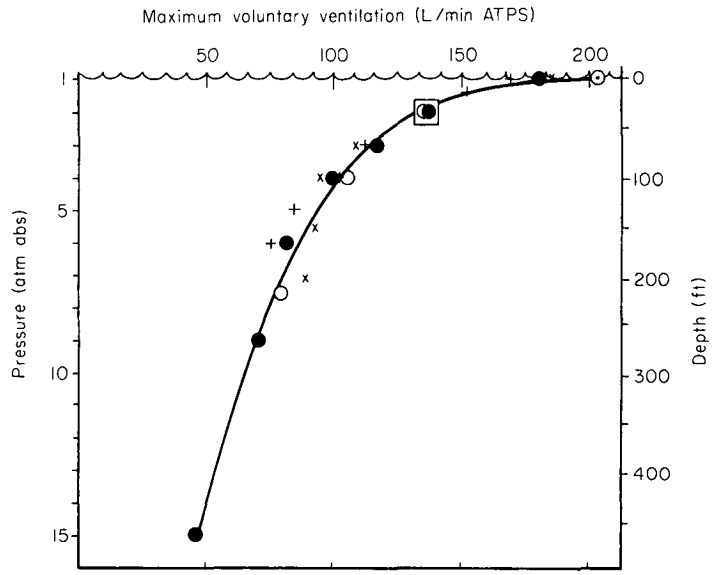

FIG. 1. Maximum voluntary ventilation with air at various depths, from the work of Lanphier (4); values derived from four different studies. (○) Maio and Farhi (5); (●) Wood (12); (×) Miles (7); (+) Seusing and Drube (10). (By permission of publishers.)

It seems certain that limitation of expiratory flow is the main reason for the reduction in MVV shown in Fig. 1. The work of breathing is indeed increased at depth. However, expiratory flow becomes effort-independent before an intolerable effort is required. It is essential at this point to differentiate between the mechanics of the human respiratory system and those of an external breathing apparatus. Airway collapse does not occur without relatively high expiratory flow. The addition of high external resistance in a conventional breathing apparatus

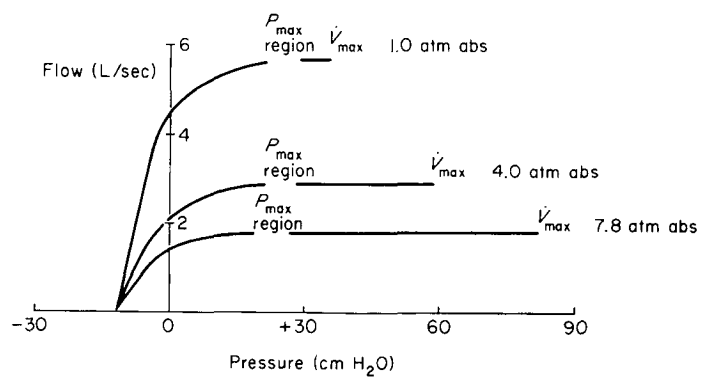

FIG. 2. Pressure–flow curves for 50% vital capacity isovolume at 1, 4, and 7.8 atm abs. Zero flow corresponds to static lung recoil pressure. $\dot{V}_{max}$ is maximum effort-independent flow, and P_{max} is pressure at which flow first becomes effort-independent.

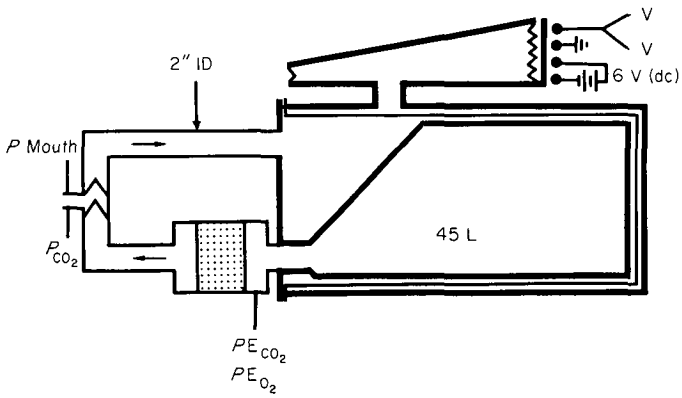

FIG. 3. Low-resistance rebreathing apparatus coupled to a Wedge spirometer. Low-resistance conical breathing valves were developed by Scott Aviation Division, Automatic Sprinkler Corporation of America, Erie Street, Lancaster, New York.

may restrict flow so much that effort limitation actually does occur before flow limitation in the airway occurs.

We have built a low-resistance rebreathing apparatus, which is based on the bag-in-box principle and is coupled to a Wedge spirometer (see Fig. 3). At the surface its resistance is

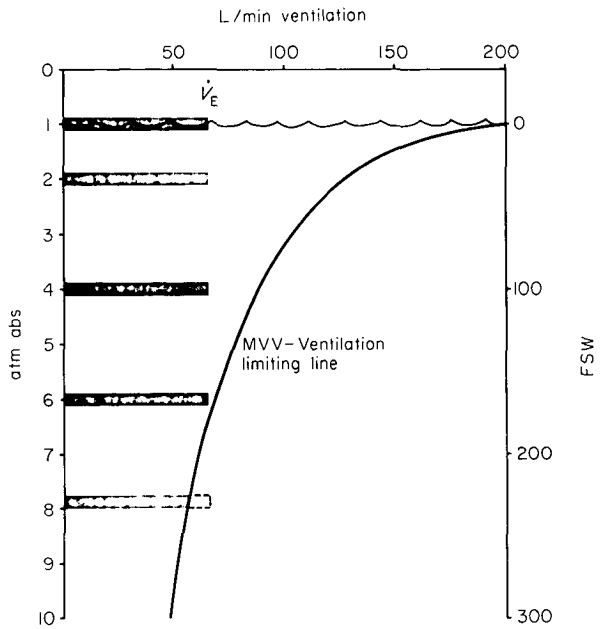

FIG. 4. Ventilation in air atmosphere at various depths. Exercise ventilations are shown as shaded bars. The dashed portion of bar at 7.8 atm abs shows what ventilation was required to prevent CO_2 retention; shaded area shows ventilation actually measured.

about 1/25th that of conventional scuba apparatus, and as depth increases this relationship becomes still more favorable. Dead space in the breathing valve is less than 100 cm^3. An automatic mechanism switches the bag-box connections to allow continuous rebreathing for long periods of time. Carbon dioxide is absorbed in soda lime, and O_2 is added volumetrically as required, based upon continuous gas analysis. We repeatedly measure ventilatory and respiratory variables, including mechanical factors and breath-by-breath CO_2 changes.

We are currently studying the effect of exercise on subjects compressed to the equivalent of 225 FSW (7.8 atm abs) while they breathe air in a pressure chamber. Density of air at 7.8 atm abs is the same as that of an appropriate O_2–He breathing mixture at about 2000 ft. The results reported in this paper pertain to the most complete of a series of experiments performed on four subjects. The data for the subject reported here correlate well with those for the other three subjects.

This subject exercised at a work setting of 200 W, using a bicycle ergometer at surface pressure and at 2, 4, 6, and 7.8 atm abs. This work load is heavy, but submaximal. Oxygen consumption ($\dot{V}_{O_2}$) is 2.8 to 3.0 L/min—heavier than that usually required in diving operations (4).

Minute ventilations ($\dot{V}_E$) during this heavy work load are shown in Fig. 4. The subject's MVV curve is shown, and the bars represent his minute ventilations. There was no marked change in $\dot{V}_E$ until the work was attempted at 7.8 atm abs, where MVV was less than the exercise ventilation required at shallower depths. His $\dot{V}_E$ diminished from 60 L/min at 6 atm abs to 54 L/min at 7.8 atm abs—a fall of 10%.

Figure 5 shows typical curves representing breath-by-breath CO_2 measurements made with an infrared CO_2 analyzer and recorded during the same exercise. There was a slight increase in end-tidal P_{CO_2} (from 36 to 41 mmHg between 1 and 6 atm abs), and a dramatic rise to 50 mmHg at 7.8 atm abs. The rise in P_{CO_2} coupled with the decreased ventilation at 7.8 atm abs indicates that the subject could not ventilate beyond his maximum voluntary level. He then began to retain CO_2 and after 4 min felt so tired and uncomfortable that he stopped work. At shallower depths he easily maintained 6–8 min of exercise, and at no time did he feel the need to stop.

We conclude that heavy work could be done in an air atmosphere at depths greater than 225 FSW (7.8 atm abs) if the diver could tolerate a high degree of CO_2 retention, as some divers can. However, the risk of CO_2 intoxication, particularly when there is a possibility of inert gas narcosis or a high P_{O_2}, could make very heavy work at great pressure extremely hazardous.

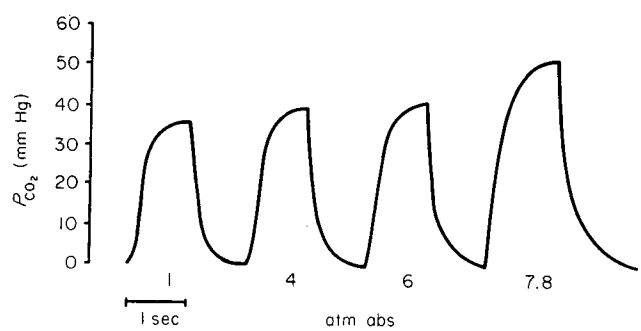

FIG. 5. End-tidal P_{CO_2} records during 200-W exercise at various depths.

Although ventilation during heavy work did not change much with depth down to the MVV, the expiratory effort progressively increased until expiratory-flow limitation developed. At the point of MVV, the subject did not significantly increase his expiratory work. He seemed to limit himself to the minimum effort required to generate maximum flow. Our data so far

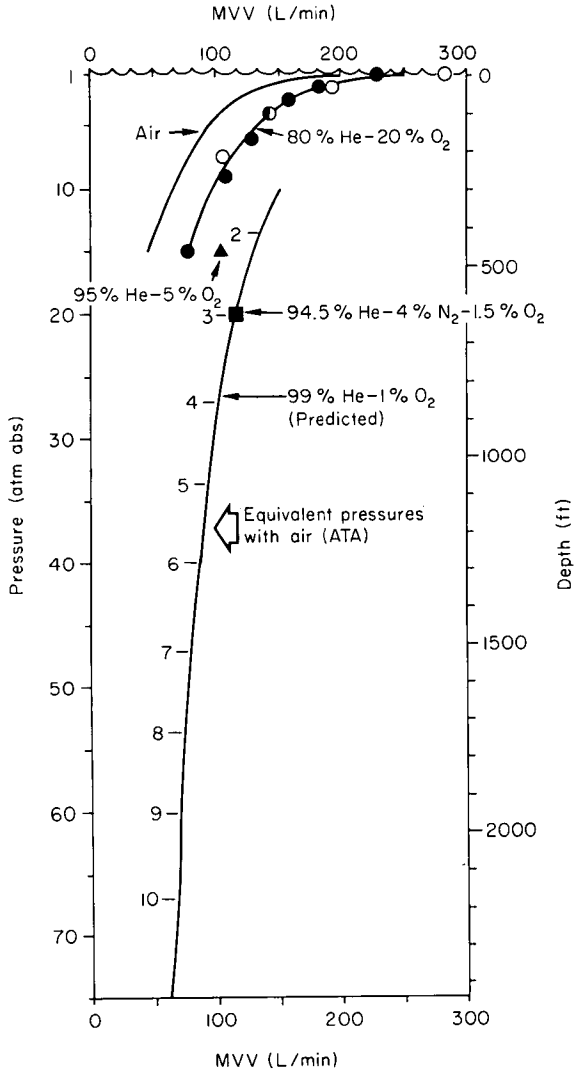

FIG. 6. Actual and predicted values of MVV with He–O_2 breathing mixtures, from the work of Lanphier (4) The curve labeled "99% He–1% O_2 (predicted)" is based on the air curve in Fig. 1. Air is 6.67 times as dense as the He–O_2 mixture. The assumption was therefore made that the MVV values obtained in an air atmosphere at various depths will be obtained at 6.67 times those depths if the breathing mixture is 99% He–1% O_2. (○) Maio and Farhi (5); (●) Wood (12); (▲) Wood (12); (■) Hamilton *et al.* (2). (By permission of publishers.)

suggest that subjects do not waste energy in attempting to maintain ventilation in the face of effort-independent flow.

This same subject also exercised at a lighter load, namely, 100 W. This load required an O_2 consumption of about 1.3 L/min, and is equivalent to such moderate exertion as swimming underwater at a rate of 20–30 yd/min or 0.8 knot (4). Minute ventilation was about the same at depth as it was at the surface. Expiratory flow never became effort-independent. End-tidal P_{CO_2} did not change. Although the subject's ventilation during heavy work in an air atmosphere became limited between 6 and 7 atm abs, it should be possible for a diver to maintain a moderate work load while breathing air at a pressure of 15 atm abs or more.

Predictions can be made regarding the maximum ventilation of subjects who breathe gas mixtures of various densities relative to air. Figure 6, also taken from Lanphier (4), shows actual and predicted values for MVV with He–O_2 mixtures.

From our data we conclude that our subject should be able to maintain heavy work to about 45 atm abs (approximately 1500 ft) while breathing a 99% He–1% O_2 mixture. Furthermore, he should be able to perform moderate work at much greater depths than those on the figure—i.e., at about 3500 ft or 100 atm abs. With optimal breathing apparatus, man perhaps may dive to such depths or even deeper.

ACKNOWLEDGMENTS

This work was supported by ONR Contracts NONR-969(03) and N00014-68-A-0216 (NR 102–722), between the Office of Naval Research, Department of the Navy, and the State University of New York at Buffalo. One of the authors, J. N. Miller, was the recipient of a Wellcome Research Travel Grant.

We are especially indebted to Dr. Hermann Rahn and Dr. Hugh Van Liew for their invaluable advice and criticism both during the studies and in the preparation of this paper. We wish to thank Messrs. C. H. Smith, Jr., and E. A. Gard for their assistance in the conduct of the studies; and Miss Augusta Dustan for her unfailing secretarial help.

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RESPIRATORY AND CARDIAC RESPONSES TO EXERCISE IN SUBJECTS BREATHING HELIUM–OXYGEN MIXTURES AT PRESSURES FROM SEA LEVEL TO 19.2 ATMOSPHERES

M. E. Bradley, N. R. Anthonisen, J. Vorosmarti, and P. G. Linaweaver

As yet there is little information about those physiological changes that occur during sustained heavy work at great diving depths. It is necessary to establish man's ability to perform hard useful work at extreme depths, and to seek evidence of changes that may represent physiological limitations. The purpose of this study was to evaluate the cardiopulmonary function of divers breathing He–O_2 mixtures from sea level to depths of 600 FSW during exercise.

Methods

Four experienced divers in excellent physical condition participated in this study. The conditions of physical activity studied were rest, moderate work (450 kg-M/min), and heavy work (900 kg-M/min). Each subject was studied predive and postdive while breathing a 30% O_2 in He mixture. The measurements obtained at the 150, 300, 450, and 600 ft depths were made while the subjects breathed the chamber atmosphere. Composition of this atmosphere was 0.3 atm of O_2 and 1.2 or less atm of N_2, the remainder being He. Ambient CO_2 levels were maintained at 2 mmHg or less.

The subjects performed exercise by pedaling a bicycle ergometer at a constant rate in time with a metronome. A low-resistance exercise valve and mouthpiece (Triple J) were used to administer the breathing mixture. Expired gas passed from the breathing valve through 2½-in. smooth bore tubing to a calibrated dry test gasometer and then to a 10-L mixing chamber. At sea level while air was breathed, mean inspiratory resistance of the equipment was 0.01 cm H_2O/L/sec, and during expiration, 0.02 cm H_2O/L/sec. Ventilatory volume and respiratory frequency were measured and recorded at 60-sec intervals.

Mixed expired gas was continuously sampled from the mixing chamber, through the chamber wall, and through infrared CO_2 and paramagnetic O_2 analyzers. The inspired chamber atmosphere was monitored by the same technique. ECG electrodes were attached to the subject's precordial region, and heart rate was continuously monitored.

Resting state measurements were obtained during the last 5 minutes of a 20-minute rest

period. After the resting measurements were made, the subject performed either the moderate or heavy work load. All exercise periods lasted 15 min. For the first 10 min of the work period, the subject was allowed to achieve a steady-state status, and measurements were made during the final 5 min of the work period.

Results

All of the subjects stated that the dyspnea and fatigue which occurred while they worked at 600 ft were no more severe than they were during exercise at sea level pressure. Pedaling the bicycle ergometer at the 450 and 600 ft depths aggravated the compression arthralgias of two subjects. Discomfort was minimal, however, and did not impair performance of the exercise.

The results of this study are presented in Tables I, II, and III. A three-way analysis of variance was performed with the data to determine whether the variables of exercise and depth exerted statistically significant effects upon the parameters studied. Exercise significantly ($P < 0.05$) affected all the cardiopulmonary and metabolic functions that were either measured or calculated in this study. Those functions that were significantly ($P < 0.05$) affected by the increasing depth will be discussed separately.

As depth increased, O_2 consumption (Fig. 1) and CO_2 production (Fig. 2) tended to rise

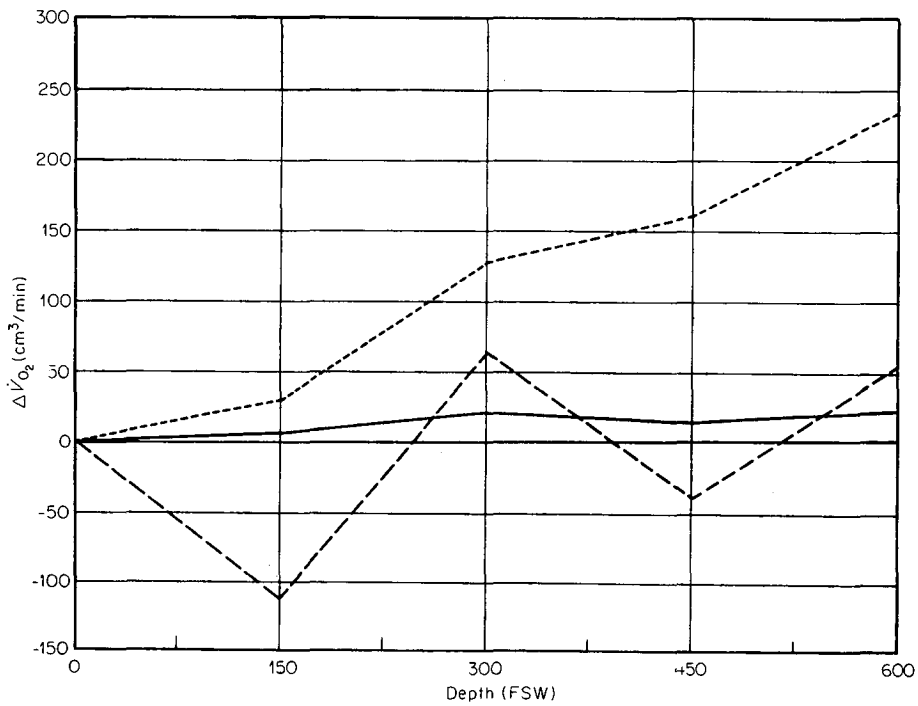

FIG. 1. Change with increasing depth in the O_2 consumption of four divers during rest and exercise. (——) Rest; (— —) 450 kg-M/min; (- - -) 900 kg-M/min.

TABLE I

OXYGEN CONSUMPTION, CARBON DIOXIDE PRODUCTION, AND RESPIRATORY EXCHANGE QUOTIENT OF FOUR SUBJECTS DURING REST AND EXERCISE AT WORK LOADS OF 450 AND 900 KG-M/MIN FROM SEA LEVEL TO 600 FSW

		O_2 consumption (L/min, STPD)					CO_2 production (L/min, STPD)					Respiratory quotient				
Subject	Work	Sea level	150′	300′	450′	600′	Sea level	150′	300′	450′	600′	Sea level	150′	300′	450′	600′
D. J.	Rest	0.339	0.314	0.349	0.313	0.239	0.262	0.240	0.293	0.233	0.241	0.77	0.76	0.84	0.74	1.0
W. L.		0.336	0.358	0.411	0.306	0.430	0.269	0.304	0.367	0.241	0.326	0.80	0.85	0.89	0.79	0.76
J. M.		0.264	0.270	0.310	0.340	0.280	0.226	0.223	0.253	0.271	0.288	0.86	0.83	0.82	0.80	1.03
P. W.		0.243	0.271	0.244	0.281	0.328	0.220	0.215	0.226	0.241	0.272	0.91	0.79	0.93	0.86	0.83
Mean		0.296	0.303	0.315	0.310	0.319	0.244	0.246	0.285	0.247	0.282	0.84	0.81	0.87	0.80	0.90
D. J.	450 kg-M/min	1.218	1.082	1.346	1.128	1.141	1.111	0.964	1.148	0.948	1.105	0.91	0.89	0.85	0.87	0.97
W. L.		1.261	1.150	1.356	1.288	1.557	1.086	1.171	1.211	1.211	1.184	0.86	1.02	0.89	0.88	0.76
J. M.		1.390	1.210	1.318	1.473	1.259	1.227	1.120	1.196	1.501	1.283	0.88	0.93	0.91	1.02	1.05
P. W.		1.218	1.193	1.315	1.211	1.339	1.188	1.165	1.385	1.263	1.290	0.98	0.98	1.05	1.04	0.96
Mean		1.272	1.159	1.334	1.230	1.324	1.153	1.105	1.235	1.210	1.216	0.91	0.96	0.93	0.95	0.94
D. J.	900 kg-M/min	1.983	1.954	2.138	2.255	2.122	1.828	1.826	1.916	1.880	1.803	0.92	0.93	0.90	0.83	0.83
W. L.		2.157	2.140	2.167	2.107	2.343	2.024	2.144	2.044	1.916	2.186	0.94	1.0	0.94	0.91	0.93
J. M.		2.293	2.486	2.313	2.291	2.170	2.155	2.034	2.287	2.179	2.223	0.94	0.82	0.99	0.95	1.02
P. W.		2.261	2.233	2.583	2.677	2.998	2.164	2.153	2.671	2.672	3.063	0.96	0.96	1.03	1.0	1.02
Mean		2.174	2.203	2.300	2.332	2.408	2.043	2.039	2.230	2.162	2.319	0.94	0.93	0.97	0.92	0.95

TABLE II

VENTILATORY VOLUME, RESPIRATORY FREQUENCY, AND TIDAL VOLUME OF FOUR SUBJECTS DURING REST AND EXERCISE AT WORK LOADS OF 450 AND 900 KG-M/MIN FROM SEA LEVEL TO 600 FSW

		$\dot{V}_E$ (L/min, BTPS)					f (breaths/min)[a]					$\dot{V}_T$ (L, BTPS)[a]				
Subject	Work	Sea level	150′	300′	450′	600′	Sea level	150′	300′	450′	600′	Sea level	150′	300′	450′	600′
D. J.	Rest	10.83	6.87	8.31	7.99	7.40	7.3	2.8	3.4	6.0	3.8	1.444	2.455	2.444	1.332	1.947
W. L.		7.63	6.62	8.47	6.00	7.10	4.7	2.3	3.3	3.0	2.7	1.630	2.878	2.606	2.000	2.630
J. M.		11.88	11.04	11.82	11.88	11.52	11.7	10.1	11.0	10.9	9.1	1.015	1.093	1.075	1.037	1.260
P. W.		9.72	10.00	9.65	8.86	10.16	15.8	12.6	13.0	11.0	10.6	0.615	0.794	0.650	0.805	0.958
Mean		10.02	8.63	9.56	8.68	9.05	9.9	7.0	7.7	7.7	6.6	1.176	1.805	1.694	1.294	1.699
D. J.	450 kg-M/min	28.60	20.06	24.18	22.76	26.50	9.6	5.4	6.9	7.4	8.4	2.980	3.694	3.525	3.076	3.155
W. L.		23.19	20.43	23.89	22.38	21.23	7.2	3.8	8.2	6.2	7.2	3.220	5.376	2.913	3.610	2.949
J. M.		39.50	43.70	42.75	42.75	43.36	25.0	24.2	24.4	18.8	21.6	1.580	1.806	1.752	2.274	2.007
P. W.		34.55	37.78	37.83	34.43	38.89	22.6	19.8	19.8	17.4	20.4	1.529	1.908	1.911	1.979	1.906
Mean		31.46	30.49	32.16	30.58	32.50	16.1	13.3	14.8	12.5	14.4	2.327	3.196	2.525	2.735	2.504
D. J.	900 kg-M/min	45.94	44.52	44.14	46.55	43.70	15.0	15.5	10.8	16.0	16.6	3.062	2.872	4.087	2.909	2.633
W. L.		43.00	36.90	37.92	33.52	40.62	13.2	11.8	11.0	10.6	12.3	3.260	3.127	3.420	3.162	3.294
J. M.		69.27	78.57	76.93	70.35	73.80	33.0	28.0	27.0	26.8	27.2	2.099	2.806	2.849	2.625	2.713
P. W.		64.31	67.56	71.22	71.76	71.02	29.2	25.0	25.8	27.2	28.4	2.202	2.702	2.760	2.638	2.501
Mean		55.63	56.89	57.48	55.55	57.29	22.6	20.1	18.7	20.2	21.1	2.656	2.877	3.266	2.834	2.785

[a] The changes in respiratory frequency and in tidal volume with increasing depth are statistically significant: $P < 0.05$.

TABLE III

ALVEOLAR VENTILATION, ALVEOLAR CARBON DIOXIDE TENSION, AND HEART RATE OF FOUR SUBJECTS DURING REST AND EXERCISE AT WORK LOADS OF 450 AND 900 KG-M/MIN FROM SEA LEVEL TO 600 FSW

		$\dot{V}A$ (L/min, BTPS)[a]					PA_{CO_2} (mmHg)					Heart rate (BPM)[a]				
Subject	Work	Sea level	150′	300′	450′	600′	Sea level	150′	300′	450′	600′	Sea level	150′	300′	450′	600′
D. J.	Rest	5.16	5.14	6.23	4.81	5.22	36.7	38.6	38.5	39.6	37.8	81	60	60	72	66
W. L.		5.06	5.16	6.46	4.25	5.41	45.9	48.8	46.5	46.3	49.3	66	60	72	56	60
J. M.		5.96	5.83	6.15	6.33	6.72	32.6	31.4	33.7	35.0	35.0	90	69	72	66	70
P. W.		4.59	5.03	4.73	5.12	5.92	40.1	35.2	39.0	38.4	37.7	54	58	54	54	54
Mean		5.19	5.29	5.89	5.13	5.82	38.8	38.5	39.4	39.8	40.0	73	62	65	62	63
D. J.	450 kg-M/min	22.33	16.26	19.48	17.91	20.96	42.9	49.0	48.3	44.9	43.2	120	78	90	96	96
W. L.		18.44	17.32	18.60	18.07	16.62	50.8	55.9	53.4	51.1	58.3	120	108	108	90	108
J. M.		25.75	30.03	27.93	31.57	30.84	41.2	30.7	35.0	38.8	33.9	132	117	114	114	114
P. W.		22.34	26.49	26.88	24.43	27.27	41.4	36.2	42.1	42.2	38.6	105	120	100	102	102
Mean		22.22	22.53	23.22	23.00	23.92	44.1	43.0	44.7	44.3	43.5	119	106	103	101	105
D. J.	900 kg-M/min	35.18	34.60	36.25	36.23	33.40	41.2	43.6	43.3	42.4	44.2	154	144	132	156	126
W. L.		32.34	29.11	30.14	26.47	32.36	52.6	60.9	55.5	58.4	55.3	148	144	138	132	150
J. M.		49.96	60.79	59.65	53.60	56.67	37.0	27.5	31.4	33.2	32.0	168	166	162	156	168
P. W.		47.08	51.80	55.60	54.76	53.56	39.3	34.2	39.3	39.8	46.6	150	150	150	152	150
Mean		41.14	44.08	45.41	42.77	44.00	42.5	41.6	42.4	43.5	44.5	155	151	146	149	149

[a] The changes in alveolar ventilation and in heart rate with increasing depth are statistically significant: $P < 0.05$.

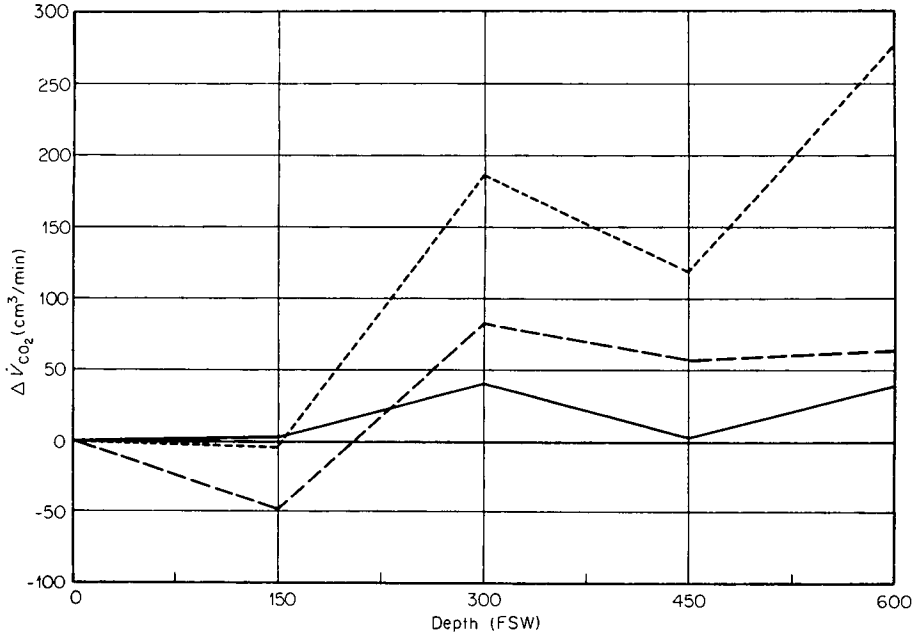

FIG. 2. Change with increasing depth in the CO_2 production of four divers during rest and exercise. (——) Rest; (— —) 450 kg-M/min; (- - -) 900 kg-M/min.

both during rest and exercise, but these increases were not statistically significant. Three subjects showed distinct increases in O_2 consumption during heavy work at the 600 ft depth, while the remaining subject showed a small decrease under the same conditions.

In this study, increasing pressure to a simulated depth of 600 FSW did not appear to influence the respiratory minute volume of subjects breathing He-O_2 (Fig. 3). One of the most striking findings of this study was the wide variation in the ventilatory response to exercise, both at sea level and at depth. One subject respired 35–40 L/min while exercising at 900 kg-M/min from the surface to the 600 FSW depth. Under the same conditions, another subject breathed twice that volume.

Respiratory frequency and tidal volume also varied considerably from one subject to the next. One subject's resting respiratory frequency ranged from 2.5 to 4.5 breaths/min from sea level to 600 FSW. During exercise at the moderate work level, it increased only to 4–8 breaths/min. Another subject's frequency tended to be about 12 breaths/min at rest and 24 breaths/min during moderate work. As depth increased, however, all of the subjects had progressively lower respiratory frequencies (Fig. 4) and progressively larger tidal volumes (Fig. 5). The largest change in respiratory frequency and tidal volume took place between sea level and 150 FSW. These changes were statistically significant at the 5% probability level.

The effect of the constant respiratory minute volume and alteration in the respiratory pattern with increasing depth was a significant increase ($P < 0.05$) in the calculated alveolar ventilation ($\dot{V}A$) (Fig. 6). This increase in $\dot{V}A$ did not completely correlate with the concurrently higher metabolic CO_2 production. As a result, computed alveolar CO_2 tension (P_{ACO_2})

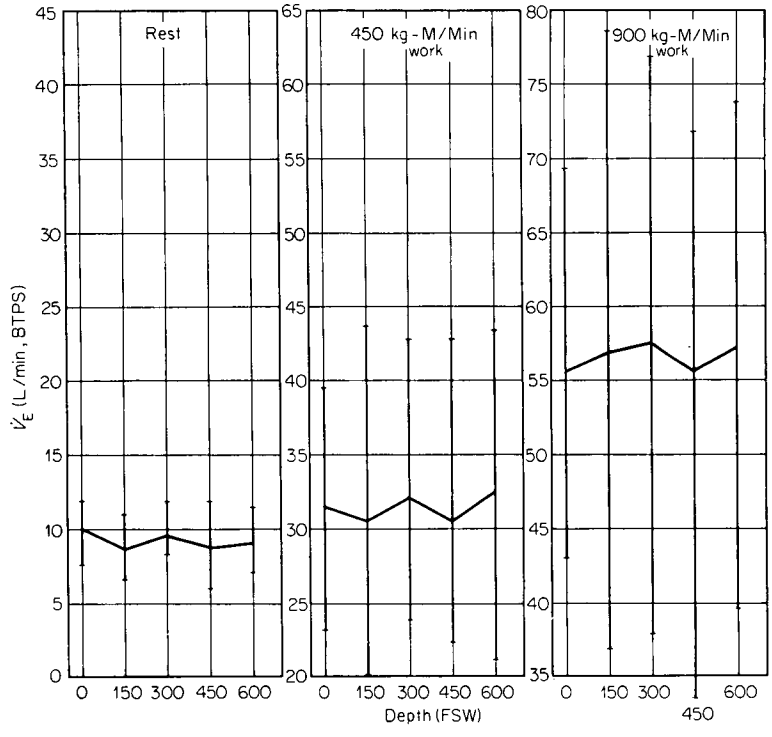

FIG. 3. Pulmonary ventilation during rest and exercise from sea level to 600 FSW.

appeared to rise very slightly as depth increased (Fig. 7). However, these slight increases in PA_{CO_2} were not statistically significant for any activity level.

As depth increased, there was a progressive bradycardia both at rest and during work that was statistically significant ($P < 0.05$) (Fig. 8). This finding was not consistent for all of the subjects, as one (P.W.) maintained the same heart rate within each activity level from sea level to the 600 FSW depth.

Discussion

The progressively larger O_2 consumptions and CO_2 productions with increasing depth are most likely the result of the increased work of breathing a progressively more dense gas mixture. In our study the O_2 cost of breathing 30 L/min of a He–O_2 mixture 3.7 times as dense as air at sea level was about 2 ml/L-min. Glauser *et al.* (4) reported the O_2 cost of breathing 36 L/min of a gas mixture 4.1 times as dense as air to be 5 ml/L-min.

Salzano *et al.* (17) measured the O_2 consumption of exercising men who were breathing 100% O_2 at 1 and 2 atm abs. The respiratory minute volumes of these subjects during work were about 35 L/min. At 2 atm abs their O_2 consumptions were 272 cm^3/min greater than they were at 1 atm abs. If increased work of breathing were solely responsible for this increase, the O_2

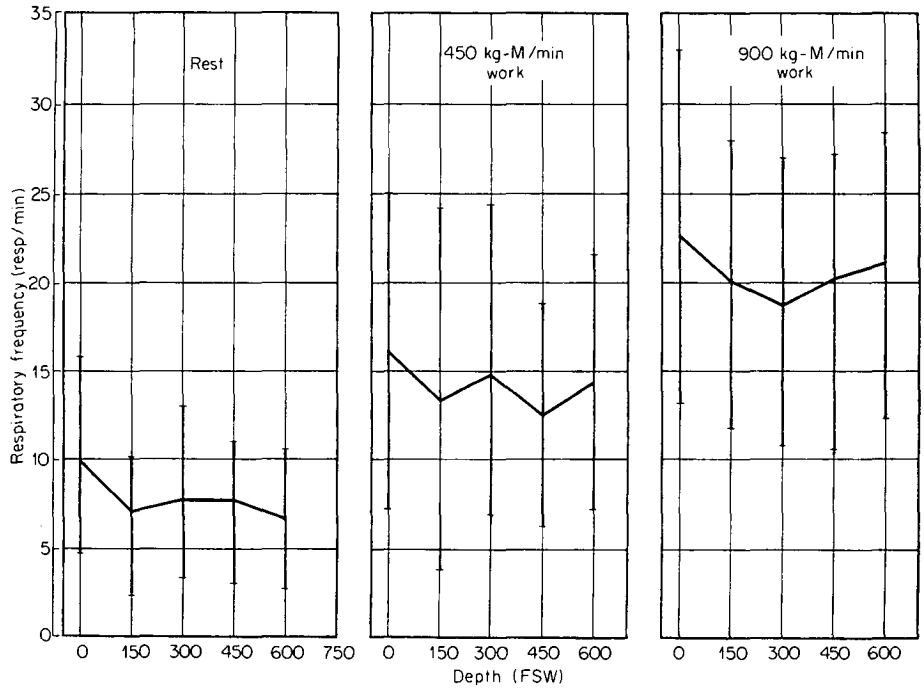

FIG. 4. Effect of increasing depth on the respiratory frequency of four subjects during rest and exercise.

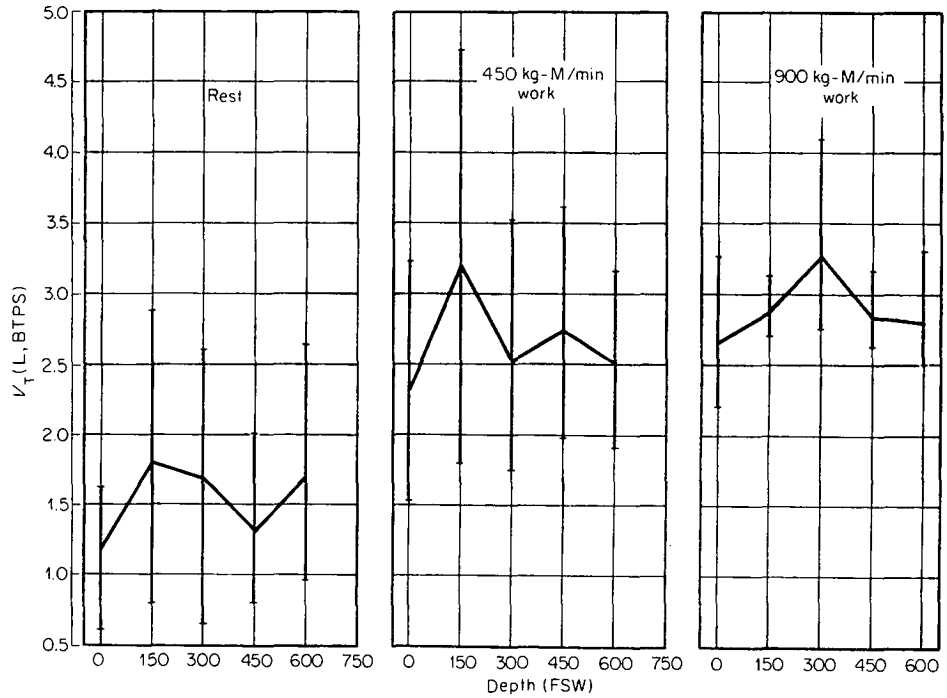

FIG. 5. Effect of increasing depth on the tidal volume of four subjects during rest and exercise.

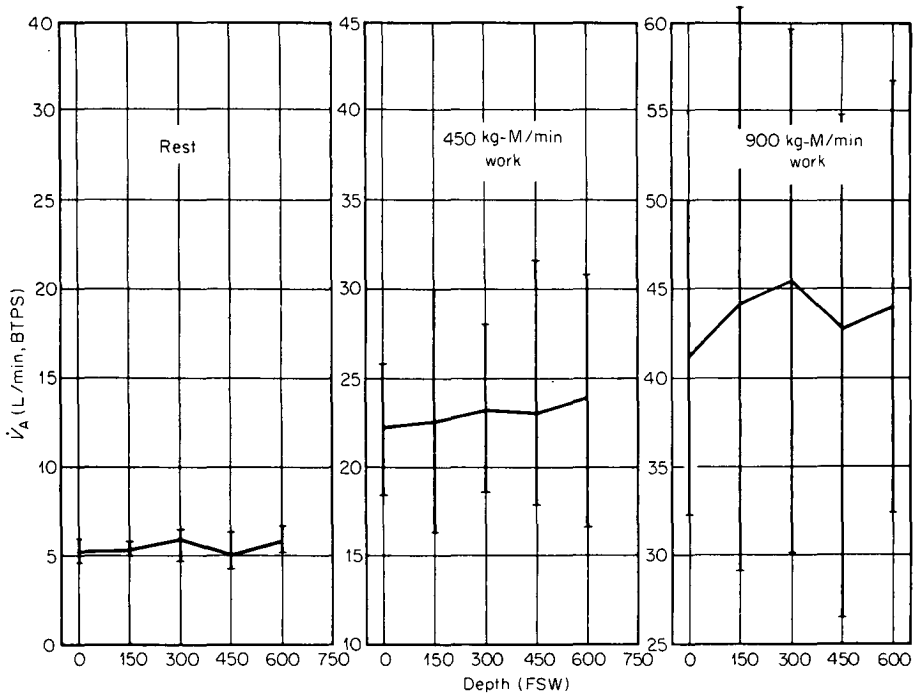

FIG. 6. Effect of increasing depth on the alveolar ventilation of four subjects during rest and exercise.

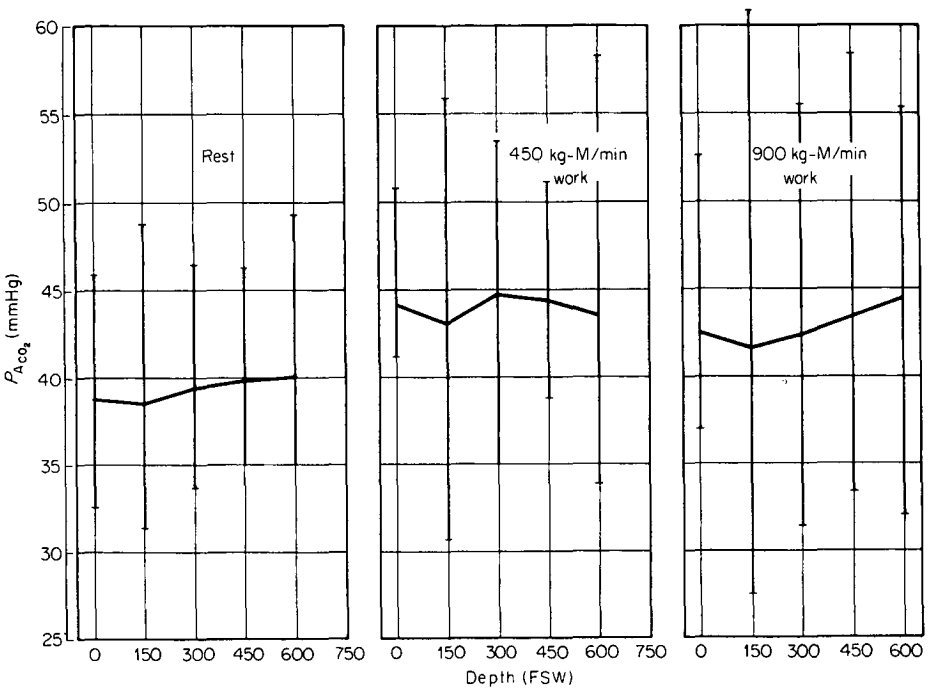

FIG. 7. Alveolar CO_2 tension of subjects during exercise from sea level to 600 FSW.

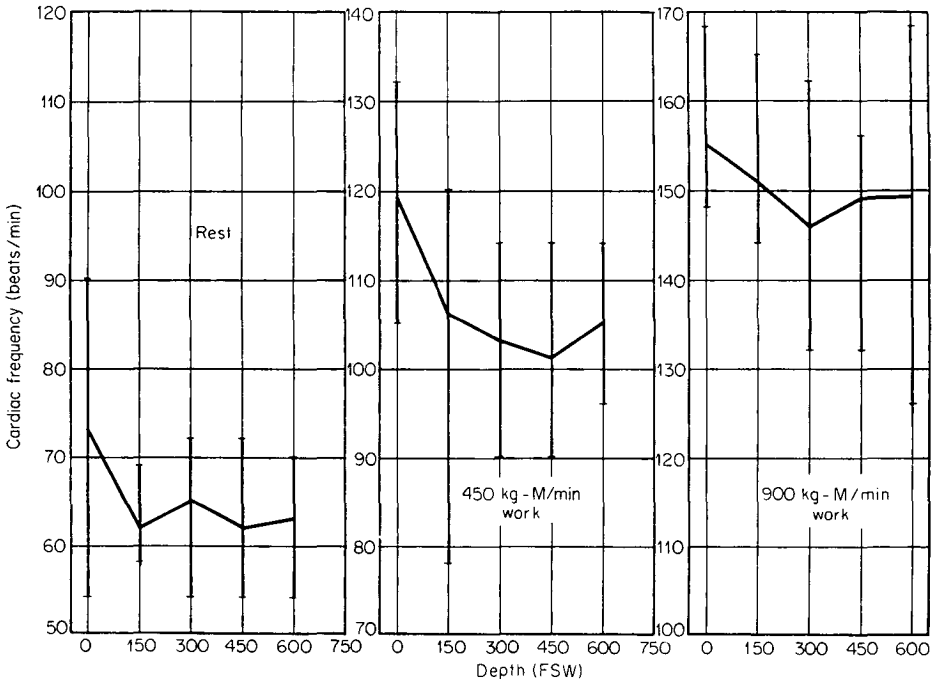

FIG. 8. Cardiac rate of subjects during rest and exercise from sea level to 600 FSW.

cost of breathing a gas only twice as dense as air would be 8.2 cm³/L-min. Salzano suggested that when high P_{O_2} is breathed during exercise, there may be a shift in metabolism from anaerobic to aerobic pathways. Our results lend support to this contention.

It is well recognized that within the diver population there exists wide variation in ventilatory response to exercise. That some divers hypoventilate and retain CO_2 during exertion has been reported by Lanphier (12) and other investigators (5). Why the ventilatory response of these divers to exertion is inadequate, whereas the response of others is "normal," is simply not clear at this time.

Many divers have been shown to be markedly insensitive to hypercapnia and the acid products of metabolism as stimuli to respiration (11, 18). Some investigators consider that this insensitivity represents an adaptive response to the conditions of diving and is responsible for the hypoventilation and CO_2 retention of divers during exercise (18). An imperfect correlation has been made between the length of diving experience and the tendency to retain CO_2 during work (11). Our two subjects, who would be classified as "CO_2 retainers," actually had less diving experience than did the other two subjects whose ventilatory response to exertion was more appropriate.

In divers whose ventilatory response to exercise is inadequate, breathing air at increasing depths markedly worsens the degree of hypoventilation and CO_2 retention (12). In other, more normal, subjects, breathing air (9) and $He–O_2$ mixtures (6) at depth has been reported to produce lesser degrees of hypoventilation and CO_2 retention. Inspired O_2 tension, narcotic

depression of respiratory centers, and increased work of breathing are factors that have been suggested as being responsible for this phenomenon (9, 12).

In our study there was no clear-cut evidence that hypoventilation and CO_2 retention were significantly greater at 600 FSW when a He–O_2 mixture was breathed than they were at sea level. In fact, both during rest and exercise, $\dot{V}A$ was noted to increase with depth.

Elevations in inspired O_2 tension have been shown to increase $\dot{V}A$ in the resting subject (8), but to cause hypoventilation during exercise (10, 17). Since inspired O_2 tension was kept constant in this study, the changes in $\dot{V}A$ observed cannot be explained on this basis.

Previous studies have failed to demonstrate that increased P_{N_2} induces narcotic depression of respiratory centers (7, 12). There is no evidence in our study that He at pressures up to 19.2 atm exerts a depressant effect on the respiratory centers.

Increased work of breathing has been shown to cause hypoventilation and CO_2 retention (3, 19). Although the most obvious variable in our study was the progressive increase in gas density, we were unable to demonstrate this phenomenon. In fact, the increases in $\dot{V}A$ appear to offset the greater CO_2 production at depth, thereby maintaining PA_{CO_2} at fairly constant levels.

In our calculation of $\dot{V}A$ and PA_{CO_2}, assumed values for the subject's respiratory dead space volume were used (2). This method has been shown to give lower and generally more accurate values for PA_{CO_2} during exercise than those obtained by end-tidal sampling (1). Whether this method is valid in subjects breathing dense gas mixtures has not been proven. Diffusion dead space (13) and alterations in the ventilation-perfusion ratio caused by dense gas breathing (15) may tend to increase physiological dead space. Alveolar ventilation would then be overestimated and PA_{CO_2} values erroneously low.

The increases in $\dot{V}A$ of our subjects were effected by progressive alterations in their breathing patterns. A breathing pattern of lower respiratory frequencies and larger tidal volumes has been noted to occur at depth in numerous studies (8, 9, 16, 17). This pattern occurs both during rest and exercise, and during He–O_2, N_2–O_2, and 100% O_2 breathing. This change in respiratory pattern appears to be primarily the result of breathing a dense gas.

It is teleologically satisfying to think that the observed changes in respiratory pattern represent an adaptive mechanism to minimize the work of breathing. It can be predicted theoretically that when flow resistance and respiratory dead space volume are increased, the optimal respiratory frequency becomes lower in order to minimize the energy expenditure required for breathing (14). Cain and Otis (3) and Zechman *et al.* (19) have reported a decrease in the respiratory frequency of human subjects who breathed through imposed resistance.

The largest changes in respiratory frequency and tidal volume in this study occurred between sea level and a pressure of 150 FSW. When the subjects breathed a 30% O_2–70% N_2 mixture at sea level, resting and exercise respiratory frequencies were only slightly lower than those obtained at sea level while they breathed a He–O_2 mixture. The density of the N_2–O_2 mixture at sea level was about three-fourths that of the He–O_2 mixture at 150 FSW, and therefore the subjects were expected to have respiratory frequencies closer to those found at 150 FSW than to those at sea level. It is not apparent whether small increases in gas density or some other factor is responsible for the large change that occurs between the surface and the 150-ft depth.

Bradycardia both during rest and exercise has been reported in subjects breathing air, a He–O_2 mixture, and 100% O_2 at depth (6, 9, 16, 17). Elevated inspired O_2 tensions accentuate

this phenomenon (17). Bradycardia at depth has been an inconstant finding among the subjects in this and other studies. We have monitored divers' pulse rates every 4 hr during 10-day, 450-FSW dives without finding evidence of slower heart rates at depth. Bradycardia at depth does occur in some individuals and is apparently a pressure-related phenomenon. The mechanism by which it occurs and its physiological significance remain unexplained.

Summary

The results of this study indicate that man breathing He–O_2 mixtures can perform heavy work for sustained periods to depths of 600 FSW without undue physiological stress. Hypoventilation and CO_2 retention resulting from increased airway resistance were not demonstrated. During performance of heavy work, there was a progressive increase in O_2 consumption with the increase in depth, most likely reflecting the increased work of breathing. A respiratory pattern of increased tidal volume and decreased respiratory frequency was present during both rest and exercise as depth was increased. This change probably represents an adaptive mechanism to minimize the work of breathing dense gas mixtures.

ACKNOWLEDGMENT

The authors gratefully acknowledge the technical assistance of LCDR M. Lafontaine, RCN, Chief Hospitalman T. Reedy, USN, and Mr. T. James.

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MECHANICS OF BREATHING WITH HELIUM–OXYGEN AND NEON–OXYGEN MIXTURES IN DEEP SATURATION DIVING

N. R. Anthonisen, M. E. Bradley, J. Vorosmarti, and P. G. Linaweaver

Breathing resistance at increased ambient pressure has been a subject of some concern (1, 2). As gas becomes more dense, its flow resistance increases, thereby possibly limiting the depth at which divers can successfully work. An opportunity to extend earlier experimentation was afforded us by SeaLab III practice dives, which were performed at the U.S. Navy Experimental Diving Unit in Washington, D. C.

Materials and Methods

Studies were made on four U.S. Navy divers who made a saturation dive in a dry chamber to a pressure of 825 FSW. Table I indicates the depth at which measurements were made (and on which day), the gas mixtures used, and their density relative to that of air. Control measurements were made at the surface before and after the dive; studies were conducted after compression to 825 FSW and during decompression at intervals of approximately 150 FSW. Gas density was varied, not only by gas compression, but also by varying the inert gas in the inspired breathing mixture. At the surface He, N_2, and SF_6 were used, and at depth He and Ne were used. By breathing Ne at 825 FSW, inspired gas densities were attained that were equal to 15 times that of room air, or to the appropriate O_2–He mixture for use at 2500–3000 FSW. Inspired O_2 tension was constant at 0.3 atm throughout all experiments.

Ventilation and gas flow were measured with a wedge spirometer (frequency response = 10 cps). A differential strain gauge measured the difference between mouth and pleural (esophageal) pressures. The outputs of the gauge and spirometer were recorded on an oscillograph outside the chamber. Pleural pressure was measured with He-filled latex balloons 7 cm long with a circumference of 35 mm. The balloons were fitted over polyethylene catheters with an internal diameter of 1.3 mm; the balloon volume was 0.5 cm^3. The balloons were positioned in the esophagus in the manner recommended by Milic-Emili *et al.* (4). The correct balloon position was ascertained in each subject before the dive, and this position was kept constant during subsequent experiments.

TABLE I

PARTICULARS OF STUDIES DURING EXPOSURE TO PRESSURE EQUIVALENT TO 825 FSW
(Compression Began on Day 1; Decompression Ended on Day 14)

Day	Depth (FSW)	Inspired mixture[a]	Density (rel. to air)
0	0	N_2–O_2	1.00
		He–O_2	0.43
		SF_6–O_2	3.75
4	825	He–O_2	4.25
		Ne–O_2	15.00
5	750	He–O_2	3.90
6	600	Ne–O_2	9.50
		He–O_2	3.20
8	450	He–O_2	2.52
10	300	He–O_2	1.83
11	155	He–O_2	1.13
		Ne–O_2	3.21
13	0	N_2–O_2	1.00
		He–O_2	0.43

[a] P_{O_2} constant at 0.3 atm abs.

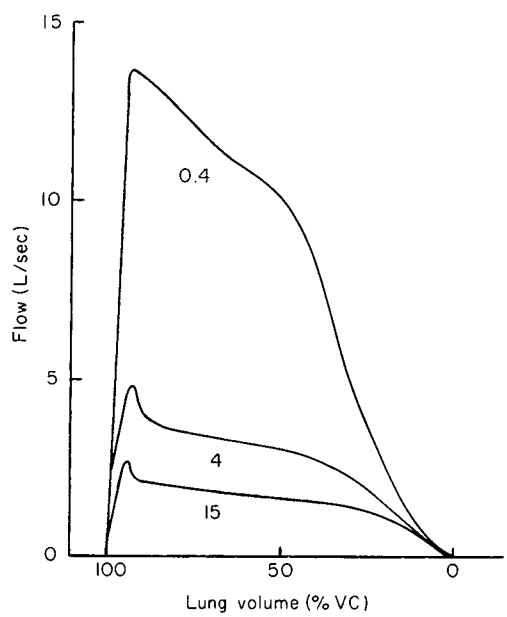

FIG. 1. Flow–volume curves during VC expirations performed with maximal rapidity at relative densities to air of 0.4 (He breathed at 1 atm); of 4 (He breathed at 825 FSW); and of 15 (Ne breathed at 825 FSW).

The subjects remained seated while they were studied. If the inspired gas differed from the chamber gas, the subject washed the foreign gas into his lungs by means of five consecutive vital capacity (VC) maneuvers, inspiring the foreign gas and expiring to the chamber atmosphere. The subject then began to breathe from the wedge spirometer; measurements were made during normal tidal breathing, forced hyperventilation, and a series of forced expiratory VC maneuvers. Throughout this sequence the subjects rebreathed from the spirometer and, at the end of each sequence, gas was sampled from the mouthpiece. This gas was later analyzed by chromatography for accurate determination of density.

Using the method of von Neergaard and Wirz (7), we measured lung resistance during tidal breathing, and inspiratory resistance during hyperventilation. From the forced VC data, flow–volume curves were constructed, as shown in Fig. 1.

Results and Discussion

In examining flow–volume curves, one must recall that during forced expiration, flow depends on lung volume and is, at most lung volumes, independent of pleural pressure; at these volumes the pressure driving flow may be regarded as constant (3, 5). Because of this we have compared gas density to maximum expiratory air flow at fixed lung volumes. Representative results are shown in Fig. 2. At high lung volumes, expiratory flow and gas density showed a linear relationship when plotted on log–log coordinates, suggesting an exponential relationship between these variables. At low lung volumes (Fig. 2), there was more scatter in the data; and if an exponential relationship existed, the exponent would have been close to zero.

Figure 3 plots the exponents (slopes of lines of Fig. 2) relating gas density to maximum expiratory air flow against the lung volume at which the flows were observed. Above 40% VC, the exponent was relatively constant between −0.4 and −0.5; at lower lung volumes, it declined. These data may be interpreted according to the relationships between gas flow and density, which depend on the flow conditions. These conditions have been summarized as

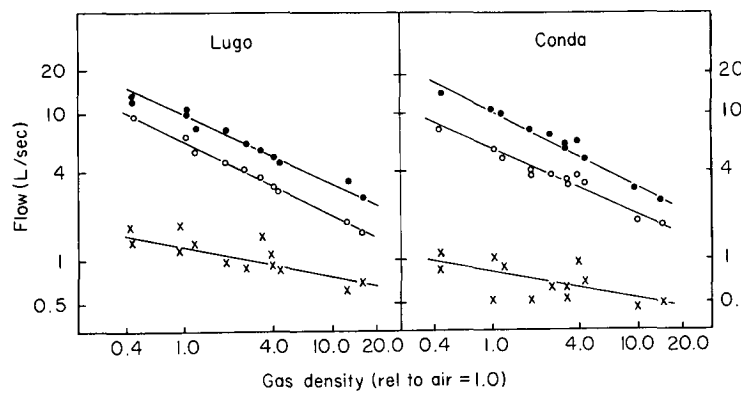

FIG. 2. Relationship between gas density and maximum expiratory air flow, at 90% (●), 50% (○), and 15% (×) VC, in two subjects.

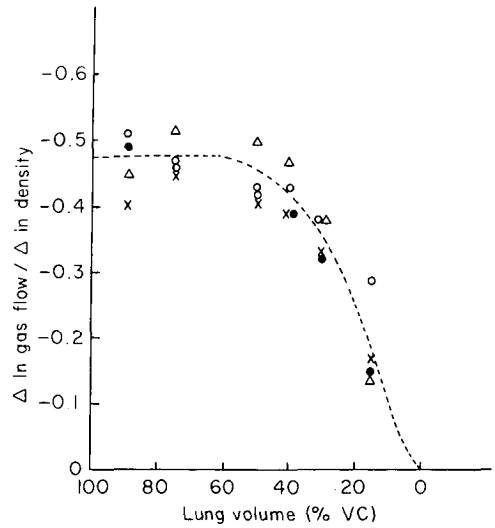

FIG. 3. Maximum expiratory gas flow–lung volume relationship in four subjects. Lung volume (% VC) refers to the volume at which a given flow–density relationship occurred. (●) Conda; (△) Lugo; (×) Risk; (○) Winters.

follows (4):

When flow is turbulent:

$$\dot{V} = \frac{1}{\rho^{0.43}} \left(\frac{\Delta P^{0.57} D^{2.71}}{L^{0.57} \mu^{0.14}} \right) \tag{1}$$

when flow is governed by forces related to convective acceleration:

$$\dot{V} = \frac{1}{\rho^{0.5}} (D^2 \Delta P^{0.5}) \tag{2}$$

and when flow is laminar:

$$\dot{V} = \frac{\Delta P D^4}{\mu L} \tag{3}$$

In these equations, $\dot{V}$ is gas flow, ρ is gas density, μ is gas viscosity, D is the diameter of the airway considered, L is airway length, and ΔP is the pressure that drives the gas flow.

During maximum expiratory effort at any fixed lung volume, airway geometry and driving pressure are constant. Although gas viscosity did vary somewhat in our experiments (Ne is a viscous gas), viscosity was relatively constant when compared to gas density.

Thus, if flow were turbulent and/or governed by forces related to convective acceleration, the exponent relating flow to density should be between -0.4 and -0.5, which is what we found at lung volumes greater than 40% VC. At lower lung volumes the exponent approached zero, as would be expected if a greater proportion of flow became laminar or was less density-dependent. Since flow decreases at low lung volumes, it is therefore more likely to be laminar.

TABLE II

EXPONENTS RELATING FLOW (AS CONDUCTANCE) TO GAS DENSITY

Subjects	Exponents			
	$G\dot{V}_1$[a]	$G\dot{V}_2$[b]	$\dot{V}m$[c]	$\dot{V}_{50}$[d]
Risk	−0.30	−0.43	−0.40	−0.41
Lugo	−0.48	−0.43	−0.45	−0.50
Winters	−0.39	−0.55	−0.51	−0.43
Conda	−0.38	−0.47	−0.49	−0.42
Mean	−0.39	−0.47	−0.46	−0.44

[a] $G\dot{V}_1$ = Conductance during tidal breathing.
[b] $G\dot{V}_2$ = Inspiratory conductance during hyperventilation.
[c] $\dot{V}m$ = Peak flow, expiratory.
[d] $\dot{V}_{50}$ = Maximum expiratory flow at 50% VC.

These results and interpretations agree with those of Schilder *et al.* (6), and are similar to those of Wood and Bryan (8).

Lung conductance (the reciprocal of resistance) during tidal breathing, and inspiratory conductance during hyperventilation, also appeared to relate to density in exponential fashion (Fig. 4). Indeed, these exponents did not differ greatly from those observed at high lung volumes during forced expiration (see Table II). Before these data can be interpreted in the same way as the data derived from forced expiration, however, we must clarify the meaning of the term *conductance.* Conductance is the ratio of flow to driving pressure (ΔP). To interpret conductance as gas flow, therefore, it is first necessary to show that driving pressure did not change with density whereas flow did. This was approximately the case in volun-

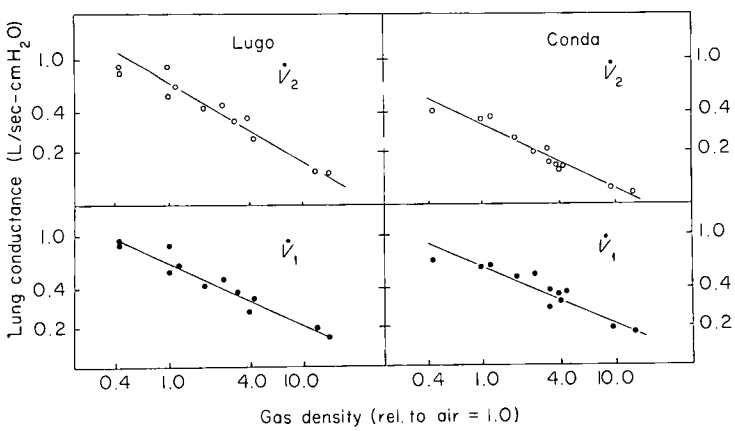

FIG. 4. Relationship of conductance to gas density in two subjects; $\dot{V}_1$ represents conductance during tidal breathing, and $\dot{V}_2$ represents inspiratory conductance during hyperventilation.

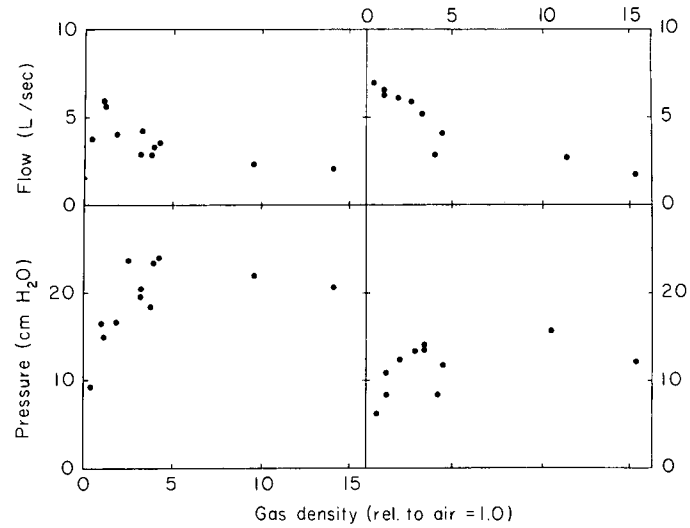

FIG. 5. Components of inspiratory conductance during hyperventilation in two subjects.

tary hyperventilation, as indicated by Fig. 5. Inspiratory driving pressure tended to be fixed, whereas inspiratory gas flow declined with increasing density. Thus the exponents relating inspiratory conductance to density during hyperventilation may be interpreted as indicating that flow was turbulent during this maneuver.

During normal tidal breathing, on the other hand, driving pressure increased while flow remained constant (Fig. 6), so that changes in conductance must have been due to changes in driving pressure. In a turbulent system at constant flow, the exponent relating density and driving pressure should be about twice as large as those shown in Table II. Air flow cannot therefore be regarded as turbulent during tidal breathing. However, since conductance was clearly density-dependent to some degree, neither can flow be regarded as entirely laminar.

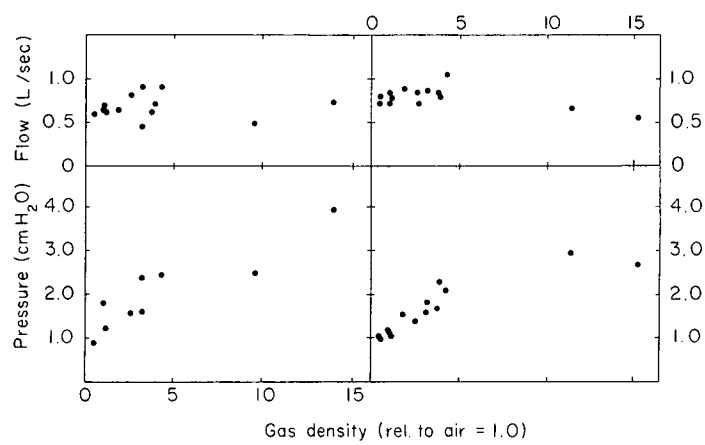

FIG. 6. Components of conductance during tidal breathing in two subjects.

During tidal breathing both laminar and turbulent flow are probably present to an important extent.

It should be noted that these interpretations are almost certainly oversimplifications. The pulmonary airway is an extremely complicated system that probably cannot be characterized as simply as we have done here. However, the relationships summarized in Table II were observed over a wide range of densities and have practical predictive value.

Finally, airway resistance at 825 FSW during Ne breathing was on the order of 8 cm/L-sec. These were obviously not normal values, but neither are they the kind of values seen in patients with respiratory insufficiency. All our subjects were able to move 40–50 L/min at this extreme gas density. Insofar as their lung resistance is concerned, therefore, divers should be capable of performing some useful activity even at 2,000 FSW in an O_2–He atmosphere.

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ARTERIAL BLOOD GASES, HEART RATE, AND GAS EXCHANGE DURING REST AND EXERCISE IN MEN SATURATED AT A SIMULATED SEAWATER DEPTH OF 1000 FEET

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A simulated dive to an equivalent depth of 1000 FSW (31.3 atm abs) in Duke University's hyperbaric chambers—a dive undertaken in collaboration with the U.S. Navy—provided the opportunity to investigate several intriguing questions. Can P_{O_2} and P_{CO_2} in blood be measured at 1000 FSW with currently available laboratory equipment? How does breathing a He–O_2 atmosphere, which is 4.4 times as dense as air is at sea-level pressure, influence gas exchange in normal divers? Does breathing almost pure inert gas alter the difference between the mean partial pressures of oxygen in alveolar gas and arterial blood? Can moderately heavy exercise be performed at 1000 FSW? Finally, how does the physiologic response to exercise differ at this pressure from the response at sea level?

Methods

Physiological measurements were obtained from each of three healthy male volunteers during alternating periods of exercise and rest. Studies were performed on two or more occasions at the surface, once when the subjects were saturated at a simulated seawater depth of 320 ft (10.7 atm abs), and once when they were saturated at a simulated depth of 1000 FSW (31.3 atm abs), within a dry compression chamber.

The atmosphere in the chamber was monitored continuously with equipment, located outside the chamber, that was sensitive to CO_2 concentrations of 1.25 ppm and to O_2 concentrations of 250 ppm. Except for the inspired P_{CO_2}, all analyses were performed inside the compression chamber at ambient pressure.

The data were collected while each subject, seated on a Fleisch bicycle ergometer, breathed ambient gas through a low-resistance respiratory valve having a dead space of 160 ml. The work load of the ergometer was not affected by changes in the density of the gaseous environment.

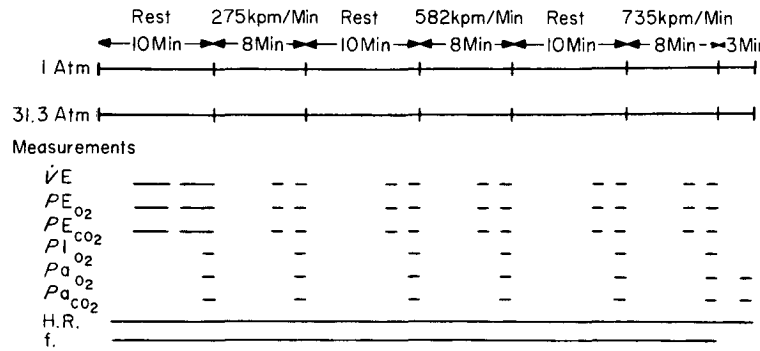

FIG. 1. Diagrammatic representation of experimental sequence used in this study. Continuous lines at the top of the figure indicate that the periods of alternating rest and work were uninterrupted. The time and duration of each measurement are indicated by the position and length of each line in relation to the timing sequence above.

The experimental sequence is shown in Fig. 1. At 31.3 atm abs, the final period of work at 735 kpm/min (kilopond) was extended for 3 min without the respiratory valve to evaluate how the external resistance of the breathing assembly influenced the steady state pressure of CO_2 in arterial blood (Pa_{CO_2}).

Samples of arterial blood, mixed expired gas, and inspired gas were collected simultaneously at specific times, both under conditions of rest and exercise, according to the schedule shown in Fig. 1. Arterial blood was collected anaerobically in iced glass syringes. Expired gas was collected in Douglas bags, and volumes per unit time ($\dot{V}_E$) were measured by use of a modified 120-L Tissot gasometer. Inspired gas was sampled from a site immediately upstream to the respiratory valve. The times of sample collection were the same at depth and at surface so that it could be determined if the interval required to reach a steady state during work and the rate of recovery from work were the same under both conditions. Heart rate (HR) and respiratory frequency (f) were recorded continuously during each experimental sequence on a polygraph positioned outside the chamber.

Measurements of P_{O_2} and P_{CO_2} in the respired gas and arterial blood were made electrochemically with commercially available electrodes. The electrodes and three rotating flask-type tonometers were all contained in the same water bath, which was maintained thermostatically at 37°C (verified with a hydrostatically pressure-calibrated mercury thermometer).

Specially prepared gas mixtures, certified to be accurate within 5 ppm, were used for calibration (42 ppm equals a partial pressure of 1 mmHg at 1000 FSW). The electrodes were calibrated with at least four different gas mixtures. Oxygen tensions in blood measured at depth were corrected by multiplication with the tonometer factor, also determined at depth. Body temperature was measured sublingually with a thermistor probe, which had been previously calibrated in the water bath at 37°C.

Identical studies also were performed at a simulated depth of 320 FSW (10.7 atm abs) while the subjects breathed a gas consisting of 97% He and 3% O_2. At this depth, however, the only valid measurements obtained were those of pulse and respiratory rate.

In addition to the three subjects, a physician and a technician participated in the simulated dives. Each participant was assigned and trained to make specific measurements during the

TABLE I

MEAN VALUES[a] OF PULMONARY VENTILATION ($\dot{V}_E$), O_2 CONSUMPTION ($\dot{V}_{O_2}$) AND CO_2 ELIMINATION ($\dot{V}_{CO_2}$) FOR THREE SUBJECTS AT REST AND DURING EXERCISE AT SEA LEVEL (1 ATM ABS) AND SATURATED AT A SIMULATED DEPTH OF 1000 FSW (31.3 ATM ABS)

Condition (kpm/min)	Pressure (atm abs)	No.	$\dot{V}_E$, (L/min, BTPS)		$\dot{V}_{O_2}$ (ml/min, STPD)		$\dot{V}_{CO_2}$ (ml/min, STPD)	
			Mean	Range	Mean	Range	Mean	Range
Rest	1	21	11.9	9.1–16.4	336	286–404	300	253–393
	31.3	6	11.0	9.2–13.1	381	293–469	310	227–387
275	1	18	23.7	22.1–26.9	930	774–1036	828	744–967
	31.3	6	25.1	22.0–28.6	1109	1043–1228	928	826–1093
582	1	18	41.7	34.7–47.6	1454	1293–1588	1499	1167–1677
	31.3	4	40.7	38.5–51.1	1791	1654–1950	1664	1535–1791
735	1	16	53.4	47.1–72.1	1799	1552–2101	1870	1666–2107
	31.3	6	51.1	47.0–58.7	2177	1830–2244	1929	1720–2228

[a] Mean values at rest were calculated from pooled data of the two measurement intervals of the initial rest period. Mean values during exercise were calculated from pooled data of the two measurement intervals of each work load (Fig. 1). Data were pooled because of their similarity.

surface experiments, and to repeat the same measurements during the dives. The sequence and timing of these rehearsed experiments were supervised continuously by the investigators (who were outside the chamber) through visual and audio contacts with the men inside. Detailed physical examinations of the subjects, prior to and following the dive, including measurements of respiratory function, revealed no abnormalities.

TABLE II

MEAN VALUES[a] OF RESPIRATORY EXCHANGE RATIO AND MEAN ALVEOLAR–ARTERIAL O_2 PRESSURE DIFFERENCE [$(A-a)\Delta P_{O_2}$] FOR THREE SUBJECTS AT REST AND DURING EXERCISE AT SEA LEVEL (1 ATM ABS) AND SATURATED AT A SIMULATED DEPTH OF 1000 FSW (31.3 ATM ABS)

Condition (kpm/min)	Pressure (atm abs)	Respiratory exchange ratio			$(A-a)\Delta P_{O_2}$ (mmHg),		
		No.	Mean	Range	No.	Mean	Range
Rest	1	21	0.88	0.80–0.98	9	16	4–21
	31.3	6	0.81	0.78–0.89	5	31	26–37
275	1	18	0.88	0.76–1.00	4	19	10–28
	31.3	6	0.83	0.79–1.00	3	11	3–24
582	1	18	1.03	0.81–1.20	3	20	17–24
	31.3	6	0.89	0.80–1.00	2	24	23–25
735	1	16	1.04	0.87–1.16	3	19	13–28
	31.3	6	0.96	0.90–1.05	3	25	12–35

[a] Mean values were calculated as explained in footnote to Table I.

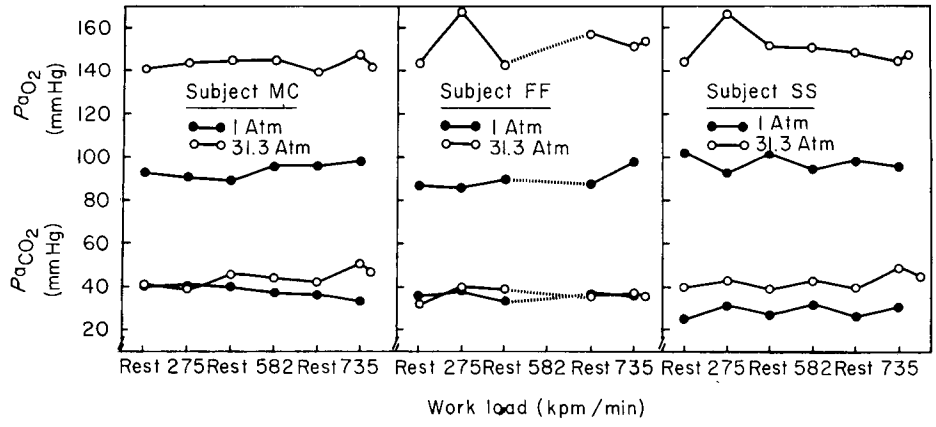

FIG. 2. Arterial P_{CO_2} and P_{O_2} values during rest and work at normal and elevated ambient pressure. Each point represents a single value. The broken line indicates that the second work load value for subject FF at 31.3 atm abs is missing. In subject MC, the Pa_{CO_2} rose from a resting level of 41 mmHg to a maximal value of 51 mmHg at a work load of 735 kpm/min at 31.3 atm abs, but decreased to 47 mmHg while he continued the exercise but no longer breathed through the respiratory assembly. For subject SS, under similar conditions, the values for Pa_{CO_2} were 40, 49, and 45 mmHg, respectively. The Pa_{CO_2} did not increase significantly from a resting value of 32 mmHg under similar conditions in subject FF.

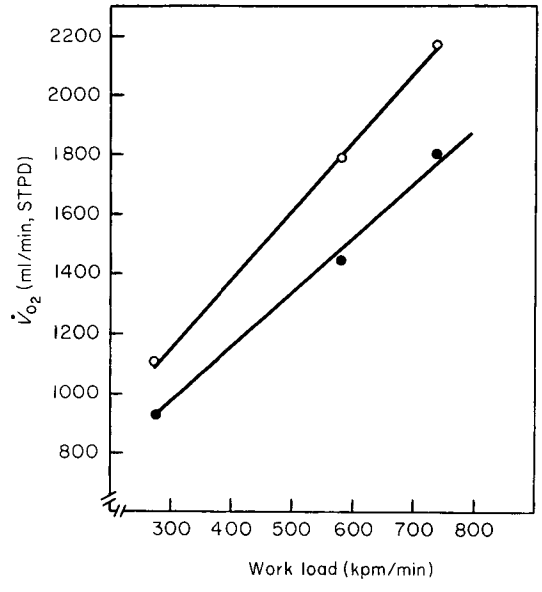

FIG. 3. Oxygen consumption ($\dot{V}_{O_2}$) at various work loads at sea level and elevated ambient pressure. Each point is the mean value from three subjects. Means were calculated as explained in footnote to Table I. (●) 1 atm; (○) 31.3 atm.

TABLE III

MEAN VALUES[a] OF RESPIRATORY RATE AND HEART RATE FOR THREE SUBJECTS AT REST AND DURING EXERCISE AT SEA LEVEL (1 ATM ABS), AND SATURATED AT SIMULATED DEPTHS OF 320 FSW (10.7 ATM ABS) AND 1000 FSW (31.3 ATM ABS)

Condition (kpm/min)	Pressure (atm abs)	No.	Respiratory rate (breaths/min)		Heart rate (beats/min)	
			Mean	Range	Mean	Range
Rest	1	21	14.4	12.0–17.5	90	78–101
	10.7	6	15.0	13.0–16.3	85	67–94
	31.3	6	12.3	10.0–14.0	79	69–89
275	1	18	18.7	16.0–21.0	108	98–118
	10.7	6	15.9	13.0–21.3	97	80–104
	31.3	6	15.3	13.5–17.0	94	87–97
582	1	18	24.4	15.5–30	138	124–162
	10.7	6	20.6	15.0–26.5	123	106–138
	31.3	6	17.8	15.0–20.0	122	116–129
735	1	16	27.3	22.0–30.0	158	136–172
	10.7	6	20.9	15.0–25.0	139	129–152
	31.3	6	22.9	17.0–30.0	144	126–160

[a] Mean values were calculated as explained in footnote to Table I.

Results

At 31.3 atm abs the inspired O_2 pressure ranged from 216 to 221 mmHg, and the inspired P_{CO_2} was never more than 0.5 mmHg (21 ppm). There did not appear to be any consistent difference between pulmonary-gas-exchange values obtained during rest at surface pressure and those obtained at depth, except for the higher arterial P_{O_2} and larger alveolar–arterial P_{O_2} differences [(A–a) ΔP_{O_2}] (Tables I, II; Fig. 2). (In one subject at 14.6 atm abs, the inspired O_2 tension was virtually identical to what it had been at the surface when he breathed air, but the mean difference in alveolar–arterial O_2 tension was still greater than it was at surface pressure.)

In general, the patterns of responses to work at 31.3 atm abs resembled those of healthy individuals working at 1 atm abs pressure. In comparison with work performed at sea-level pressure, each work load at depth was performed with a greater $\dot{V}_{O_2}$ (Fig. 3; Table I), unchanged or greater $\dot{V}_{CO_2}$ (Table I), lower heart rate (Table III; Fig. 4), lower respiratory rate (Table III), larger tidal volume, lower ventilatory equivalent for O_2 ($\dot{V}_E/\dot{V}_{O_2}$) (Fig. 5), and greater O_2 pulse ($\dot{V}_{O_2}$/heart beat) (Fig. 4). Moderate arterial hypercapnia occurred in two subjects during exercise at depth (Fig. 2).

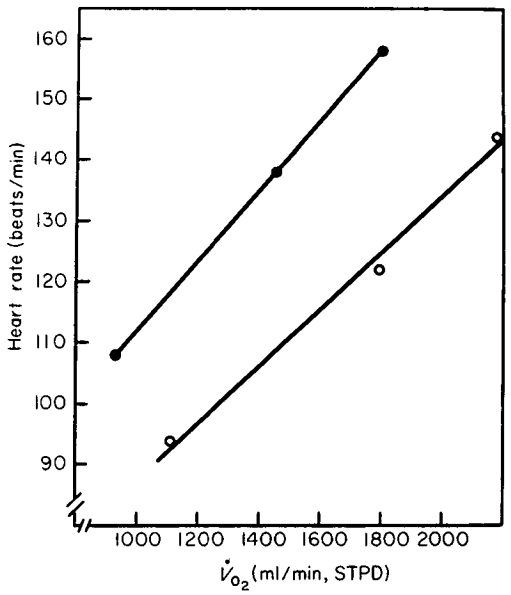

FIG. 4. Relationship of heart rate to O_2 consumption ($\dot{V}_{O_2}$) at normal and elevated ambient pressure. Each point is the mean value from three subjects. Means were calculated as explained in footnote to Table I. Oxygen pulse can be computed from the $\dot{V}_{O_2}$/heart rate ratio. (●) 1 atm; (○) 31.3 atm.

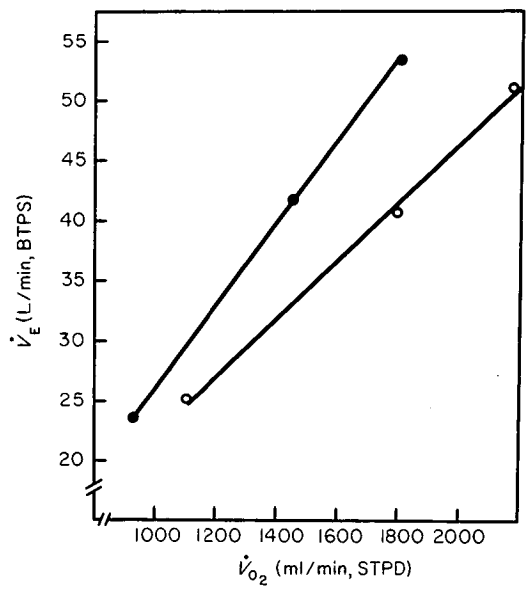

FIG. 5. Relationship of pulmonary ventilation ($\dot{V}_E$) to O_2 consumption ($\dot{V}_{O_2}$) at sea level and elevated ambient pressure. Each point is the mean value from three subjects. Means were calculated as explained in footnote to Table I. The O_2 ventilatory equivalent can be computed from the $\dot{V}_E/\dot{V}_{O_2}$ ratio. (●) 1 atm; (○) 31.3 atm.

Discussion

Respiratory gas exchange at 1000 FSW while the subjects were at rest appeared in general to be normal except for a larger (A–a) ΔP_{O_2} difference. These findings confirm those of Hamilton (10) who studied minute ventilation, O_2 consumption, and end-tidal P_{CO_2} in men at 660 FSW.

If ventilation and perfusion are not uniformly balanced, as is the case when humans stand erect (26), then breathing a gas mixture with a greater than normal inert gas fraction should result in an increased (A–a) ΔP_{O_2}. The results of experiments by Lenfant (15) and Cole and Bishop (4, 5) have provided evidence that the lungs of normal men contain a small number of alveoli with extremely low ventilation–perfusion ratios. Thus a larger than normal inert gas fraction in the inspired gas might well result in an increased (A–a) ΔP_{O_2} in normal men (21). When 99.1% inert gas was breathed at 1000 FSW, the (A–a) ΔP_{O_2} was indeed greater than at surface pressure during air breathing. However, a variety of other factors may be involved: (*1*) an increased PI_{O_2} could increase the (A–a) ΔP_{O_2} as a result of any true shunts; (*2*) increased gas density could impair diffusion in the airway; and (*3*) increased gas density might cause a redistribution of ventilation. To test these possibilities, we repeated the studies at 450 FSW, using one subject who breathed 98.6% inert gas with a density 2.08 times greater than air at sea level pressure. The PI_{O_2} was 148.5–150 mmHg, yet the (A–a) ΔP_{O_2} was still greater than it was at sea level. The second and third possibilities above cannot be ruled out, but it seems likely that the increased (A–a) ΔP_{O_2} was due at least in part to the greater inert gas fraction of the respired medium.

The increased $\dot{V}_{O_2}$ for a given ergometric load at 31.3 atm abs (Fig. 3) can be attributed in large part, or entirely, to the increased work of breathing the denser gas (1, 17, 18). To provide a convenient, valid estimate of the O_2 cost of ventilation during moderate exercise at sea level pressure while air is breathed, a value of 2 ml O_2/L $\dot{V}_E$ has been selected (3, 16, 19, 20). If the observed $\dot{V}_{O_2}$ increase at depth was due entirely to the greater effort necessary to ventilate a gas that is 4.4 times as dense as air at surface pressure, then the cost of breathing during exercise at 31.3 atm abs for two of the three subjects can be calculated to have been 8 to 10 ml O_2/L $\dot{V}_E$. This estimated four- to fivefold increase in O_2 cost of breathing is similar to that observed by Glauser *et al.* (7) when normal subjects were induced to hyperventilate with a 7% CO_2–73% SF_6–20% O_2 mixture. An 80% SF_6–20% O_2 mixture at 1 atm has a density equivalent to that of an He–O_2 mixture containing one-fifth of an atm of O_2 at 30 atm. Although each work load required a greater O_2 uptake at 31.3 atm abs (Fig. 3), there was no convincing evidence that work performance was limited by the O_2 cost of breathing.

The reduction of heart rate observed in this study at 10.7 atm abs indicates that the extent of bradycardia does not correlate in a linear manner with increases either in hydrostatic pressure or in the inspired pressure of He (Table III). At the same time, the occurrence of bradycardia at 10.7 atm abs when the gas density is similar to that of air at sea level, and when there is no marked increase in PI_{O_2}, rules out significant negative chronotropic contributions by these factors at greater depths unless other interactions take place.

Previous reports have indicated that bradycardia occurs when man is exposed to a hyperbaric environment. Hamilton (10) found that the heart rates of two subjects exercising at 620 FSW in a He–O_2 environment were slower than they were at an equivalent $\dot{V}_{O_2}$ at the surface. Hesser *et al.* (11) showed that elevated PI_{O_2} was only partially responsible for rela-

tive bradycardia during exercise while subjects breathed air at 4.5 atm abs. They found that increased N_2 pressure and gas density were partially responsible as well. Other reports (6, 23, 27) document the occurrence of bradycardia in man breathing O_2 at pressures of 1 atm abs or greater. In our study, the PI_{O_2} was below the level that would cause the observed bradycardia. Another possible explanation for this condition in a hyperbaric He environment is a cardiovascular response to peripheral vasoconstriction that must accompany the known decrease in skin temperature in the He environment (22). From the available evidence, a valid single explanation for bradycardia at depth is lacking.

The net result of the increased O_2 pulse (that is, the volume of O_2 transported per heart beat) at depth is that O_2 transport to tissues is maintained despite a decrease in heart rate (Fig. 4). Hesser *et al.* found that O_2 pulse increased with a rise in PI_{O_2} to 1 atm abs, and that it increased still further with a rise in P_{N_2} during exercise with air at 4.5 atm abs. Thus, the evidence from previous and present studies is that the increase of O_2 pulse at depth may be a response to the same causative factors involved in bradycardia.

Slower respiratory rates at depth (Table III) may represent a mechanism whereby the work required to overcome the increased nonelastic resistance to breathing the denser gas is less than it would be if the respiratory rates observed at the surface had been maintained. Hesser *et al.* have recently reported similar findings in subjects exercising at 4.5 atm abs while breathing air. They showed that the slower respiratory rates and larger tidal volumes under these conditions were related to the density of the respired gas.

In the present study, minute ventilation ($\dot{V}_E$) in response to a given ergometric load was similar at the surface and at depth (Table I), but was less for a given $\dot{V}_{O_2}$ at depth (Fig. 5). This reduction in the ventilatory equivalent for O_2 ($\dot{V}_E/\dot{V}_{O_2}$) implies a greater efficiency of O_2 transfer from the atmosphere to the blood if all other conditions of gas exchange remain constant. In our study, however, the conditions were dissimilar in that the PI_{O_2} at the simulated depth of 1000 FSW was higher, $\dot{V}_{O_2}$ was greater, and significant arterial hypercapnia occurred in two subjects during performance of the heaviest work load.

Reductions in the ventilatory equivalent for O_2 have been reported under a variety of conditions in which the common factor was increased breathing resistance because of airway obstruction or increased gas density that led to a lower level of ventilation (8, 9, 11, 25).

That relative alveolar hypoventilation did occur during exercise at depth in this study is indicated by the occurrence of hypercapnia in two subjects who exercised at the highest work load (Fig. 2), but whose Pa_{CO_2} in the intervening rest periods returned to or toward baseline values. These findings cannot be explained by an elevated PI_{CO_2}, for its valves did not exceed 0.5 mmHg at 31.3 atm abs.

Alveolar hypoventilation with concomitant elevations in Pa_{CO_2} has been reported during exercise in hyperbaric conditions (10–14). This response has been shown to be related in part to an increased PI_{O_2} and in part to the increased resistance of breathing a denser gas. In the present investigation, however, the increase in PI_{O_2} was comparatively small and should not have influenced ventilation significantly (Table III).

It has been suggested that alveolar hypoventilation in response to an increase in airway resistance is an adaptive reaction in man—i.e., hypercapnia is better tolerated than the increased work of breathing required to maintain normal CO_2 levels (2). This response has been shown to be especially characteristic of divers (13, 14, 24). It is of interest that the two subjects (MC and SS) who manifested hypercapnia during exercise at depth were (and are

currently) active divers, whereas the third subject, who maintained normal CO_2 levels, had not worked as a diver in recent years.

Breathing resistance in the present study was elevated by the increased density of the gas and the inherent resistance of the breathing assembly. At least part of the hypercapnia during exercise in subjects MC and SS can be attributed to the resistance of the breathing valve, since Pa_{CO_2} fell in both subjects when they exercised at a 735 kpm/min work load without breathing through the respiratory assembly (Fig. 2). It is possible that any substantial increase in external breathing resistance may exceed the adaptive capabilities of man working at a relative density of 4.4. That this limitation may be serious is apparent when one compares the much greater resistance of underwater breathing valves with the low resistance of the breathing assembly used in the present physiological studies.

The conclusions from this study are that work at a pressure equivalent to 1000 FSW is not detectably limited. Gas exchange in divers breathing 99.1% He and 0.9% O_2 during rest at 31.3 atm abs is essentially normal. The slight increase in (A–a) ΔP_{O_2} at 1000 FSW, which was probably caused in part by the high fraction of inspired inert gas, is not of practical significance in normal divers. Normal men can perform moderately heavy exercise, for brief periods at least, at increased environmental pressures simulating those found at depths in the sea of up to 1000 ft. The anticipated relationships among the uptake of O_2 by the body, ergometric work, pulse, and ventilation prevailed at depth. The rate of recovery from exercise was similar to that at surface pressure. Impairment of the capacity to perform work could not be discerned by the physiological methods used, and there was no evidence indicating that the physiological limits of performance had been attained.

Acknowledgments

This work was supported in part by grants HE 07896, He 5662, HE 5663, and NBO 3897, in addition to grants from the Office of Naval Research, the North Carolina Heart Association, and the North Carolina Tuberculosis Association.

We gratefully acknowledge the contributions of Captain Eugene Mitchell, Commander of the U.S. Navy Experimental Diving Unit; Captain Tor Richter, Deputy Commander of the U.S. Navy Bureau of Medicine and Surgery; Captain Robert Workman, Head of the Environmental Stress Division, Naval Medical Research Institute; W. W. Smith, M.D., Associate Professor of Experimental Surgery, Duke University Medical Center; S. L. Linderoth, Jr., M.E., Professor of Mechanical Engineering, Duke University; and Mr. William L. Greeman, Chief Chamber Operator.

Last, but certainly not least, we thank the five men inside the chamber.

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PULMONARY FUNCTION AND RESPIRATORY GAS EXCHANGE DURING SATURATION–EXCURSION DIVING TO PRESSURES EQUIVALENT TO 1000 FEET OF SEAWATER

K. E. Schaefer, C. R. Carey, and J. H. Dougherty, Jr.

Capacity to work under high pressure is mainly determined by the limitations of the respiratory system. Little is known about cardiopulmonary functions during prolonged exposure to great depth. To study lung functions and pulmonary gas exchange under high pressure, therefore, saturation–excursion dives to pressures equivalent to 800 and 1000 FSW were carried out as a joint project among the International Underwater Contractors, Inc. (IUC), of College Point, N.Y., the Advanced Engineering Laboratories of Air Reduction Company (AIRCO) at Murray Hill, N.J. and the Submarine Medical Research Laboratory (SMRL), Groton, Conn.

Material and Methods

These decompression schedules were developed by Mr. André Galerne, President of IUC. In the saturation and excursion periods of these experiments, O_2 tensions varied from 300 to 450 mmHg.

During decompression following the dives, the subjects breathed a special gas mixture (3.5 atm N_2, 1.4 atm O_2) and He through a respiratory mask for 10 min at every 100-ft level starting at 600 ft. During the last 50 ft of decompression, however, 100% O_2 was inhaled by mask, alternating with 21% O_2 in N_2 every 20 min (P_{O_2} = 760–1912 mmHg).

Our investigation involved two dives during which the subjects breathed primarily a He–O_2 mixture with a small amount of N_2 remaining from the air of the chamber prior to compression. The first was a saturation dive by two subjects to 600 FSW with excursions to 800 FSW. The rate of compression was 2 ft/min. The divers spent a total of 7 days under pressure.

The second was a saturation dive, also made by two subjects, to 800 FSW. The compression rate was 3.5 ft/min. Subject DF made a 30-min excursion dive to 1050 FSW, and Subject CD, a 5-min excursion dive to 1112 FSW. Both divers later spent 2¾ hr at 1000 ft. Their total time under pressure was 13 days.

The life support system, developed by AIRCO (9), during both dives provided accurate environmental control. The ambient CO_2 partial pressure was kept under 2 mmHg at all times during the dives.

Pulmonary Function Tests

Lung volumes and flow rates were determined by the maximal inspiratory–expiratory velocity–volume technique through the use of a wedge spirometer, an oscilloscope, and an oscilloscope camera.

Pulmonary Gas Exchange

Measurements of O_2 consumption, CO_2 excretion, and alveolar gas tension were made under resting conditions and at the end of 10 min of exercise (100-W work load). The divers, who were lying supine while the resting samples were taken, exhaled into a Douglas bag for 10 min. The samples of expired air, collected in evacuated steel cylinders, were later analyzed by mass spectrometry at the Naval Research Laboratory (NRL) and at AIRCO. The volumes were measured by a dry gas meter immediately after the chamber test. Alveolar gas samples were exhaled into a rubber Haldane sample bag through a rubber tube of 1 m length and 2 in. in diameter. The last portion of the exhaled sample was collected in evacuated steel cylinders.

The divers exercised on a Fleisch bicycle ergometer while breathing ambient gas through a low-resistance Otis-McKerrow respiratory valve. During the last 2 min of exercise, exhaled gas was collected in Douglas bags and alveolar samples were obtained in the same manner as

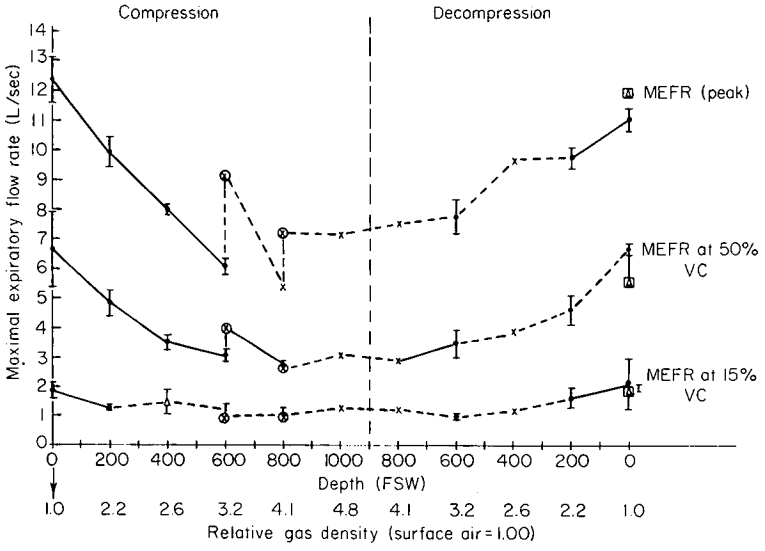

Fig. 1. Effect of pressure (equivalent FSW) and density of ambient gas on maximal expiratory flow rate at three lung volumes during two chamber dives (two subjects each). Values marked by a circle were obtained after saturation at 600 and 800 FSW; values marked by a square were obtained at surface after periods of 1 day and after 1 month following the dive. (●) 4 subjects; (△) 3 subjects; (×) 2 subjects.

they were under resting conditions. Samples of inspired gas were collected by opening evacuated steel cylinders near the inspiratory inlet of the respiratory valve.

In all instances, respiratory rate was recorded with a thermistor placed in the mouthpiece of the respiratory valve. Furthermore, EKG and EEG were recorded on a polygraph located outside the chamber while the divers rested and exercised. A skullcap prepared in cooperation with Dr. Proctor's group at the Henry Ford Hospital in Detroit, Mich., was used in obtaining the EEG's.

Twenty-four-hour urine specimens were collected in polyethylene bottles. A cover of liquid silicone (Dow Corning 200) (instead of toluene) was used to prevent CO_2 from escaping from the urine into the chamber atmosphere. Aliquots were frozen until analyzed. Total urine CO_2 was determined by the manometric method of Van Slyke. Urine pH and titratable acidity were measured. Ammonia could not be determined because of insufficient quantity. A number of urine samples were analyzed immediately after collection at the Overlook Hospital, Summit, N.J., and the results agreed well with our own analyses performed later.

Clinical examinations and lung X-rays revealed no abnormalities in the subjects prior to and following the dive.

Results and Discussion

The effects of pressure on peak expiratory flow rates are shown in Fig. 1.

During the compression there was a regular decrease in maximum expiratory flow rate (MEFR) at vital capacity (VC). This observation agrees with earlier findings that demonstrated the reduction in flow rates at increased pressures (3, 6, 19) as a consequence of the increased airway resistance (3). However, a 44% recovery in MEFR was observed during the

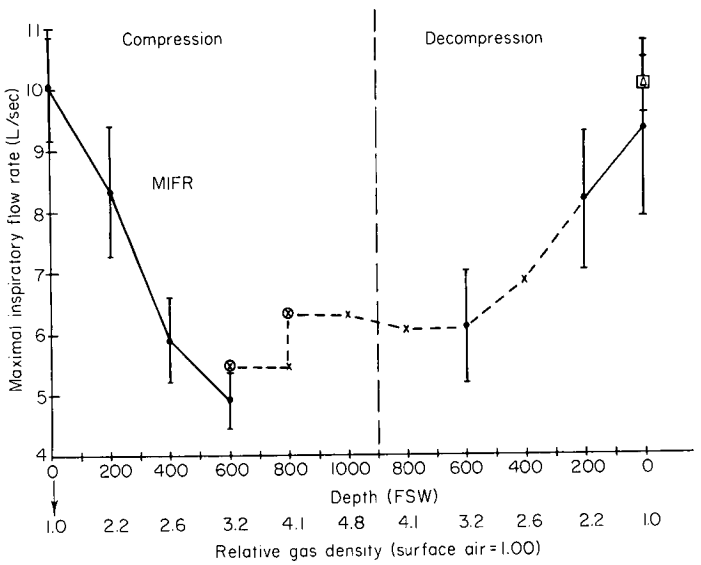

FIG. 2. Effect of pressure (equivalent FSW) and density of ambient gas on maximal inspiratory flow rate during two chamber dives (two subjects each). See Fig. 1 for explanation of symbols.

TABLE I

CHANGE IN VC, MEFR, AND MIFR AFTER SATURATION PERIODS OF 12 TO 14 HR AND 35 TO 36 HR AT 600 AND 800 FSW (FOUR SUBJECTS)

Measurement		Saturation period		
		0 hr	12–14 hr	35–36 hr
VC (L/BTPS)	Mean	4.77	4.88	5.08
	S.E.M.	±.48	±.45	±.36
% change		0	+2.3	+6.4
MEFR (L/sec)	Mean	5.71	7.18	8.21
	S.E.M.	±.20	±.56	±.69
% change		0	+26.	+44.
MIFR(L/sec)	Mean	4.83	4.94	5.88
	S.E.M.	±.50	±.37	±.41
% change		0	+7.	+23.

saturation periods at 600 and 800 FSW. The MEFR at 50% VC showed a similar response. The average increase in maximum inspiratory flow rates (MIFR) was 23% during the saturation period (see Fig. 2). This astonishing recovery of inspiratory and expiratory flow rates during the saturation period increased with time. The values obtained after 35–36 hr of saturation were larger than those obtained after 12–14 hr.

It might be argued that the improvement in the flow rates could be attributed to the effects of training during the dive. We discount this argument, however, since our divers were well trained prior to these experiments, and the average increase (44%) in their MEFR during the saturation period far exceeded the effects of training.

We also took into account the role of circadian cycles of lung functions. We observed an average daily MEFR variation of up to 10.0 ± 4%, and in MIFR, of up to 20 ± 10% (25). Considering the times at which the MEFR and MIFR measurements were made—at the beginning, the 12th to 14th hour, and 35th to 36th hour of the saturation periods—one would expect the circadian cycle effect to be toward a decrease rather than an increase in the flow rates. The measurements showing the largest recovery values were made at the end of the 35- to 36-hr saturation period, shortly after the four subjects awoke. This is ordinarily the time at which the lowest flow rates occur.

The divers' VC decreased during compression from 400 FSW onward. A steady rise in VC was noted throughout the 36-hr saturation period in conjunction with the increase in MEFR and MIFR (Table I). This finding suggests that air had been trapped in the collapsed airway during the rapid compression and was slowly released with the opening of the airway during the saturation period. This explanation is supported by the differences observed between our results and those obtained in a similar saturation diving experiment to 825 FSW carried out at the Experimental Diving Unit (EDU), Washington, D.C., in which a much slower compression rate (0.66 ft/min) was used (2).

In Fig. 3 the measurements of VC obtained in the EDU and our own experiments are compared. There is a slight but consistent increase of about 6% in the EDU dive. Their postdive controls were somewhat higher than their predive surface controls were. In our experiment,

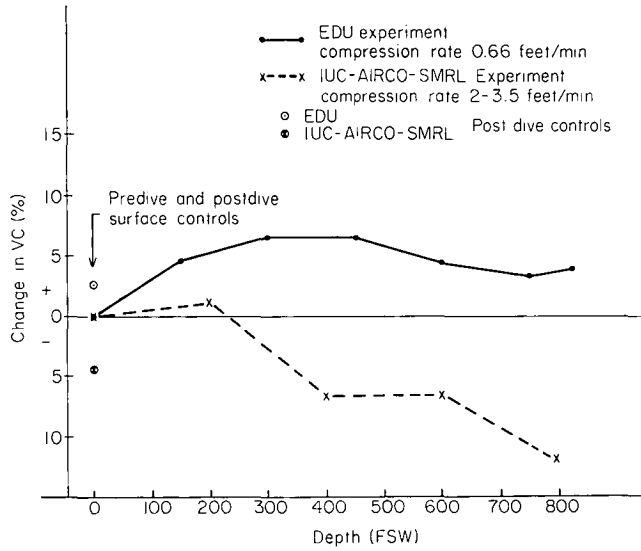

FIG. 3. Effect of different compression rates on vital capacity at depth while subjects breathed He–O_2 mixture.

by contrast, VC was decreased from 200 FSW onward, and the initial postdive control values were lower than the predive surface controls were.

Figure 4 illustrates the effects that two different compression schedules have on MEFR. The MEFR control values in our subjects were slightly greater (0.5 L/sec) than those of the

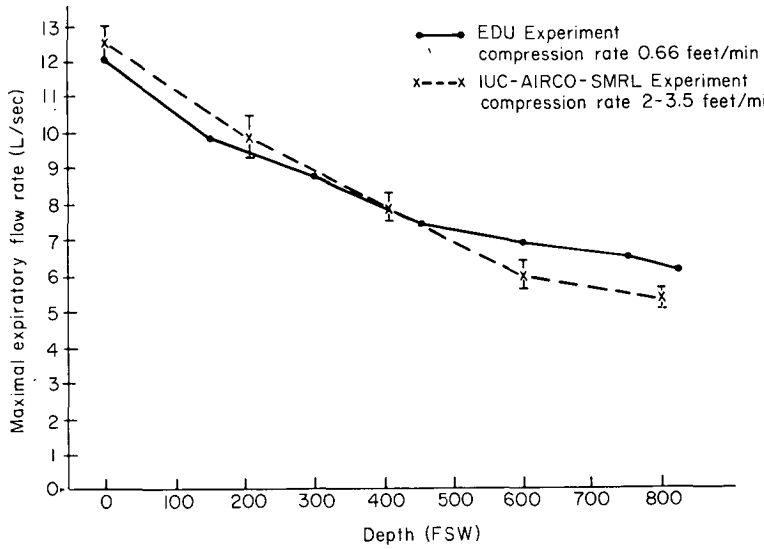

FIG. 4. Effect of different rates of compression on maximum expiratory flow rates (MEFR) at depth while subjects breathed He–O_2 mixture.

EDU subjects; at 400 FSW the curves cross each other, and at 800 FSW, our subjects' MEFR rates were 1 L/sec lower.

These findings suggest that fast and slow compression rates exert different effects on pulmonary functions—differences that to our knowledge have not been reported on in the literature. The compression rate of about 2.0 to 3.5 ft/min (3.3 ft/min = 0.10 atm/min) used in our experiment produced a greater depression of MEFR at depths exceeding 400 FSW in a He–O_2 atmosphere than the compression rate of 0.66 ft/min (0.02 atm/min) used in the EDU experiment did. The larger decrease in MEFR was associated with a decrease in VC that did not occur with slower compression rates. It is therefore logical to assume that forced expiration under our experimental conditions—compression rate of 0.1 atm/min and a relative gas density of 2.6 (at 400 FSW)—can result in a "dynamic collapse" of some air passages. [This collapse may subsequently be maintained by surface forces, as Leith and Mead (18) reported to have occurred in the normal lung under certain circumstances.] The remarkable recovery of flow rates during the 35-hr saturation period and the concomitant increase in VC provide strong support for this conclusion, since they indicate that the airway slowly reopens during a saturation period.

The airway collapse theory has been discussed by Maio and Farhi (20) and A. DuBois (7) in their studies of the various effects of increased gas density on pulmonary functions. There is a higher pressure gradient between the alveolus and the bronchus during a high-velocity flow as air density increases. The pleural pressure exerted on the bronchus tends to collapse it, causing air to be trapped. Burger and Macklem (4) demonstrated that airway closure occurred in some normal human subjects at low lung volumes during pure O_2 breathing. These findings correspond to those of DuBois *et al.* (8), who suggested that airway closure, causing air to be trapped, occurred during quiet O_2 breathing in certain human subjects. In our experiments, P_{O_2} ranged between 300 and 350 mmHg during compression. It is therefore likely that the increased O_2 tension contributed to the airway collapse. However, it is possible to offer another explanation for the observed changes in respiratory flow rates during the compression and saturation periods. The marked decrease in heart rate during rest and exercise at depth indicates a strong vagotonic stimulation. It is conceivable that the decrease in VC and the low values of MEFR and MIFR at the end of the compression period are caused by a bronchoconstriction due to vagal stimulation, in addition to the effects of rapidly increasing gas density. During the saturation period at depth at which gas density remained constant, this bronchoconstrictor effect could subside with time, resulting in a recovery of VC and respiratory flow rates.

Chouteau (5) underscored the significance of the rate of compression for the survival of animals in high pressure experiments. He exposed goats for several days to 81 atm abs (2624 FSW). He used a compression rate identical to our own, 0.10 atm/min, but found it necessary to introduce equilibration periods of from 1.5 to 4 hr at different stages. His overall compression rate—0.02 atm/min corresponded to that used in the EDU experiment. Chouteau's reason for using stage compression was the better survival rate that he had observed among animals exposed to high pressure in this manner.

During the decompression phase, our subjects had 10-min periods of breathing mixtures of increased P_{N_2} and P_{O_2} (P_{O_2} = 1000 mmHg), which could have produced some alterations in the ventilation–perfusion relationships in the lungs. To determine if such alterations occurred, radiographic scans of the lungs were performed by Dr. A. D. Crosett, Jr., of Overlook Hospital,

TABLE II

EFFECT OF EXPOSURE TO HIGH PRESSURE ON PULMONARY VENTILATION AND GAS EXCHANGE IN RESTING CONDITIONS (FOUR SUBJECTS) (24)[a]

Condition		$\dot{V}_E$ (L/min) BTPS	Resp. rate (breaths/ min)	Tidal vol. (L)	Pulse rate (beats/ min)	$P_{A_{CO_2}}$ (mmHg)	$\dot{V}_{CO_2}$ (L/min)	$\dot{V}_{O_2}$ (L/min)	RQ
Control, predive	Mean	6.88	11.4	0.617	80.3	36.5	0.225	0.254	0.88
	S.E.	0.60	1.56	0.041	5.0	1.03	0.015	0.011	0.04
Compression at 800 FSW	Mean	8.27	11.1	0.788	64.8[b]	35.9	0.289	0.371	0.78
	S.E.	0.62	1.3	0.132	3.0	2.1	0.044	0.068	0.01
Decompression at 400 FSW	Mean	9.26[b]	9.8	0.961[b]	60.3[b]	32.9	0.225	0.285	0.79
	S.E.	0.44	0.9	0.069	4.5	1.8	0.098	0.074	0.03
Surface, postdive	Mean	8.62	10.6	0.858	76.3	37.9	0.289	0.278	0.89
	S.E.	1.02	1.7	0.167	5.1	2.6	0.042	0.028	0.02

[a] By permission of the publishers.

[b] Statistically significant difference from control, at the 5% level and better.

Summit, N.J. The scans were made by injecting the divers with 290 μCi of ^{131}I labeled human serum albumin. They were made of the two subjects in the 800-FSW dive 3 hr after they left the pressure chamber and again 1 week later. Scans were made of the two subjects in the 1000-FSW exposure before and after their dive.

In all instances, there was a slight increase in peripheral perfusion of the lungs immediately after the dive. These findings are in keeping with our own observations that VC decreased significantly throughout the decompression phase and was still below control values when the subjects reached surface pressure. Vital capacity returned to normal values during the recovery period following the dive.

PULMONARY GAS EXCHANGE

Data on respiratory minute volume (BTPS), respiratory rate, tidal volume, pulse rate, $P_{A_{CO_2}}$, CO_2 excretion, O_2 consumption, and respiratory exchange ratio obtained on all four subjects at 800 FSW and during decompression at 400 FSW are presented, together with predive and postdive surface values, in Table II. Respiratory minute volume increased under pressure based on an increase in tidal volume. There was a pronounced fall in pulse rate.

The four subjects' $P_{A_{CO_2}}$ varied considerably at depth. Each of the two divers participating in the saturation–excursion dive from 600 to 800 FSW exhibited one peak in $P_{A_{CO_2}}$, one at 57 and the other at 64 mmHg (Fig. 5). Otherwise, $P_{A_{CO_2}}$ remained within the normal range. Although we took alveolar samples at more frequent intervals in the 1000-ft dive, we obtained no evidence of elevated $P_{A_{CO_2}}$ in the two subjects; to the contrary, the $P_{A_{CO_2}}$ tended to decrease both during rest and exercise. If taken at comparable periods at depths and lumped together, the data on the four subjects revealed no significant change in $P_{A_{CO_2}}$. Oxygen consumption increased slightly more than CO_2 excretion did at depth under resting conditions.

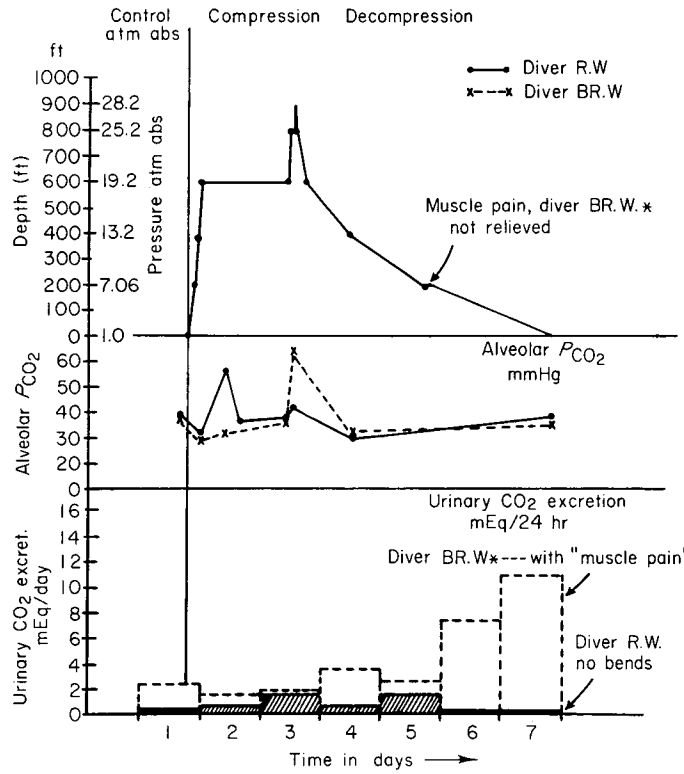

FIG. 5. Diving profile, showing alveolar CO_2 tension and urinary CO_2 excretion of two subjects during saturation dive at 600 FSW with an excursion dive to 800 FSW (24). (By permission of the publishers.)

The first two subjects, who showed higher PA_{CO_2} at depth, also exhibited larger CO_2 excretion at depth than the other two subjects did.

Table III shows similar data collected on two subjects during moderate exercise (100 W) breathing $He–O_2$ at depths of 800 FSW and 1000 FSW, as well as pre- and postdive control values breathing air. In addition, data were collected during moderate exercise nine months later, breathing air and a mixture of 60% O_2–40% N_2. Respiratory minute volume was amazingly constant under all conditions, whereas tidal volume increased and respiratory rate declined under pressure. Breathing 60% O_2–40% N_2 resulted in a very slight increase in tidal volume and small decreases in respiratory and pulse rates. Although the changes were in the same direction, their magnitude was much larger under pressure than when the subjects breathed 60% O_2–40% N_2. The PA_{CO_2} did not change significantly, but tended to decrease as it did when the subjects rested. Excretion of CO_2 decreased slightly when the subjects exercised, whereas O_2 consumption increased, causing the respiratory exchange ratio to decrease.

The change we observed in breathing patterns at great depth—an increase in tidal volume, and a decrease in respiratory frequency at rest and during exercise—is consistent with the findings of earlier investigators (12, 13, 21, 22). This change is probably caused by breathing

TABLE III

EFFECT OF EXPOSURE TO HIGH PRESSURE ON PULMONARY VENTILATION AND GAS EXCHANGE DURING EXERCISE (100 W, TWO SUBJECTS) (24)[a]

Condition		$\dot{V}_E$ (L/min) BTPS	Resp. rate (breaths/ min)	Tidal vol. (L)	Pulse rate (beats/ min)	$P_{A_{CO_2}}$ (mmHg)	$\dot{V}_{CO_2}$ (L/min)	$\dot{V}_{O_2}$ (L/min)	RQ
Control predive on air	Mean	33.6	23.4	1.407	135.6	35.7	1.205	1.306	0.93
800 feet on $He–O_2$	Mean	30.05	19.2	1.567	101.8	34.4	1.085	1.406	0.77
1000 feet on $He–O_2$	Mean	30.3	17.5	1.720	94.8	33.2	1.030	1.446	0.78
Immediate postdive on air	Mean	33.6	22.7	1.476	115.8	33.5	1.078	1.272	0.85
9 months postdive on air	Mean	31.6	23.0	1.360	126.5	37.5	1.146	1.169	0.89
9 months postdive on 60% O_2–40% N_2	Mean	31.6	21.8	1.470	124.6	36.8	1.093	1.250	0.87

[a] By permission of the publishers.

gases of high density, rather than by the increased P_{O_2} at depth. This conclusion is supported by comparing the changes occurring in tidal volume and respiratory frequency during exercise at 1000 FSW with an inspired P_{O_2} of 450 mmHg with the changes occurring under the same exercise load at 1 atm abs at a corresponding inspired P_{O_2} of 459 mmHg. At depth, tidal volume increased 23%; at sea level pressure, 8%. Respiratory frequency decreased 25 and 2.2%, respectively.

The 100-W work load with a minute ventilation of 30 L imposed no ventilatory restrictions at 1000 FSW when $He–O_2$ was breathed. Consequently, there was no evidence of CO_2 excretion when environmental CO_2 was below measurable concentrations (1–2 ppm)—observations that stand in contrast to our previous findings that $P_{A_{CO_2}}$ increases during prolonged exposure to a $He–O_2–N_2$ atmosphere, at 200-FSW pressure with an equivalent sea level CO_2 content of 1.1% (23). The importance of maintaining adequate environmental control in high pressure experiments is obvious.

The rather large individual variations both in $P_{A_{CO_2}}$ and CO_2 excretion that we observed in our four subjects (all trained divers) under high pressure have also been reported by other investigators (2). However, the differences observed in the subjects of the first and second dives cannot be related to their training in diving, which has been previously shown to influence the alveolar ventilation in response to an increase in airway resistance (15–17, 22).

Since the minute ventilation showed no response to the 100-W work load at depth while the O_2 consumption increased, the ventilation equivalent for O_2 ($\dot{V}_E/\dot{V}_{O_2}$) decreased, suggesting

an improved O_2 transfer at depth from the atmosphere to the blood. The elevated PI_{O_2} at depth could not have caused this decrease, since the ventilation equivalent did not change markedly when the same exercise was performed at a corresponding higher PI_{O_2} at 1 atm abs pressure. Similar reductions in the ventilatory equivalent of O_2 under pressure have been reported by Hamilton (10).

There was a pronounced reduction in heart rate whether the subjects rested or exercised under pressure—findings similar to those of Hamilton concerning a He–O_2 saturation dive to 620 FSW (10). Two conditions could have caused this bradycardia: (*1*) increased P_{O_2} (300–450 mmHg), or (*2*) increased gas density. We were able to differentiate in our exercise tests between the effects of these conditions since we repeated the tests with the same work load at 1 atm abs pressure with a PI_{O_2} of 459 mmHg corresponding to 1000 FSW (450 mmHg). Increased P_{O_2} at 1 atm abs pressure caused a 1.5% fall in pulse rate, compared with a 30% fall at 1000 FSW. Hesser *et al.* (11) likewise found that bradycardia during exercise at 4.5 atm while the subjects breathed air was only partially related to increased PI_{O_2}—that it was primarily the result of increased gas density.

Bradycardia at depth caused the O_2 pulse (ratio of O_2 consumption/min: pulse rate) to increase from a predive value of 9.6 to 15.3 during exercise at 1000 FSW. There is only a slight increase (from 9.2 to 10.0) during exercise at an elevated PI_{O_2} at 1 atm abs pressure.

The increase in O_2 pulse at depth, which helps to maintain O_2 supply to the tissues at a lower heart rate, appears to be part of the regulatory process in persons having increased parasympathetic activity (e.g., well-trained athletes). Not only bradycardia at depth, but also the respiratory pattern of increased tidal volume and decreased frequency are quite likely the results of a vagal stimulation produced by exposure to high pressure.

TABLE IV

EFFECT OF EXPOSURE TO HIGH PRESSURE ON URINARY ELECTROLYTE EXCRETION (FOUR SUBJECTS) (24)[a]

Condition		Urine volume (L/24 hr)	Urinary pH	Total CO_2 (mEq/24 hr)	HCO_3 (mEq/24 hr)	Chloride (mEq/24 hr)	Titratable acidity (mEq/24 hr)
Two-day control period on air prior to dive	Mean	0.930	6.480	1.18	1.08	278.0	37.8
	S.E.	0.125	0.06	0.25	0.31	14.5	5.2
Three-day saturation-excursion dives	Mean	1.260	6.695[b]	3.54[b]	2.92[b]	223.5[b]	34.2
	S.E.	0.097	0.059	0.98	0.46	18.1	5.1
Five-day decompression	Mean	1.173	6.65[b]	4.71[b]	3.52[b]	241.6	30.2
	S.E.	0.090	0.03	0.98	0.77	17.0	3.5

[a] By permission of the publishers.

[b] Differences from controls statistically significant at the 5% level and better.

Urinary CO_2 Excretion

Urinary excretion data are shown in Table IV. Urinary volume tended to increase both during the saturation–excursion dives and the decompression periods. Urinary pH, CO_2, and bicarbonate increased significantly during exposure to high pressure; titratable acidity changed little, and chloride excretion decreased. Unfortunately, ammonia excretion could not be determined because of insufficient samples; no estimate of net H_2 ion excretion could therefore be made.

Daily changes in urinary CO_2 excretion and PA_{CO_2} observed in the 800-FSW dive are shown in Fig. 5. The PA_{CO_2} of both divers remained within a normal range, with the exception of one

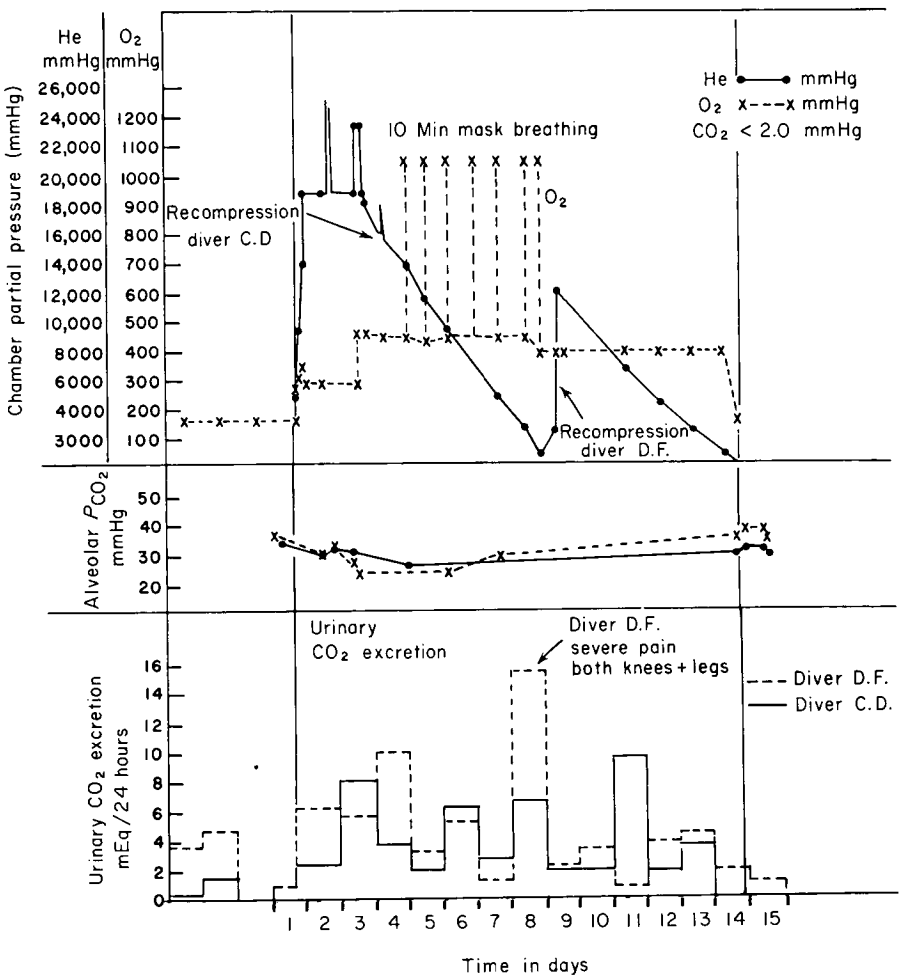

FIG. 6. Diving profile, showing partial pressure of He and O_2, alveolar CO_2 tension, and urinary CO_2 excretion of two subjects during saturation–excursion dive to 1000 FSW (30.3 atm abs) and recompression of divers (24). (By permission of the publishers.)

peak in each diver following compression to 600 and 800 FSW. Urinary CO_2 excretion rose only slightly in one diver, but greatly in the other. The latter developed pains in both knees and legs at 180 FSW during decompression. The pain was not relieved by a 2-hr recompression to 210 FSW during which O_2 at 1.4 atm was breathed through a face mask for 30-min periods. Pain persisted for 2 weeks after the experiment.

Similar urinary measurements were made during the second dive to 1000 FSW (Fig. 6). The PA_{CO_2} tended to decrease slightly both during the saturation–excursion dive and decompression. Urinary CO_2 excretion of both subjects was elevated throughout the exposure. One subject exhibited a high peak on the day that he suffered severe pain in both knees and legs at 50 FSW during decompression. Attempts to relieve the pain through recompression were unsuccessful, even when the pressure was raised to 527 FSW. At this point, recompression was halted, and the diver was given one Bufferin (buffered aspirin) tablet. After 3 hr of sleep, he was symptom-free.

These two cases of bends-like symptoms developed, of course, during decompression, when at 10-min intervals the divers breathed O_2 by mask at 1000 mmHg in N_2. It appears, therefore, that symptoms consisting of muscle pain and vague stiffness that do not seem to respond to recompression are related to the combined effects of high pressure of He and increased P_{O_2}, as reported by Bennett (1).

Since the titratable acidity declined rather than increased during saturation and decompression, it appears that renal compensation of an increased acid or CO_2 load is not needed. This assumption is supported by the fact that alveolar CO_2 tension remained normal. The hypothesis advanced by Kylstra *et al.* (14) that osmotic gradients are produced by dissolved gases therefore appears pertinent. If during rapid compression the partial pressure of inert gases in the blood exceeds the partial pressure in the poorly perfused tissues, osmotic gas gradients would cause water shifts from poorly perfused tissues to the blood and better perfused tissues. The extracellular volume might thereby increase, causing the more profuse urinary excretion that we observed in our subjects during the saturation–excursion period.

Since increased bicarbonate excretion is associated with decreased chloride excretion, it appears that the former acts to conserve chloride. This mechanism is a defense of the chloride component of the plasma under conditions of fluid volume shifts. The excessive amount of bicarbonate excreted during decompression by the divers whose muscle pains did not respond to recompression suggests that greater fluid shifts contributed to the development of their symptoms.

Summary

The reduction of maximal expiratory flow rate and maximal inspiratory flow rate produced by the rapid compression of four subjects to depths of 600 and 800 FSW at a rate of 2–3.5 ft/min was found to be associated with decreased vital capacity. During the 35- to 36-hr saturation periods at depth, MEFR rose 44%; MIFR and VC increased 23% and 6%, respectively. Airway collapse during rapid compression and airway reopening during the subsequent saturation period are suggested as the most likely explanation of the observed changes. However, it is conceivable that phasic changes in vagal stimulation resulting in bronchoconstriction during compression and recovery of a more normal bronchial tone during the saturation period could have caused the observed decrease and subsequent increase in VC and respiratory flow rates.

In rest and exercise, PA_{CO_2} did not change significantly at depth, but showed considerable individual variations. Pulmonary gas exchange at rest remained within normal limits at depths of 800 and 1000 FSW. Exercise performed by two subjects at a moderate work load (100 W) at 800 and 1000 FSW resulted in an increase of O_2 consumption, whereas CO_2 excretion tended to decrease.

Tidal volume increased and respiratory rate decreased with increasing pressure in rest and exercise. A pronounced bradycardia was observed under both conditions, which we attributed primarily to the effect of inert gas pressure rather than to the effects of elevated P_{O_2}.

Urinary CO_2 excretion was significantly elevated during the saturation–excursion and decompression periods. The two subjects who complained about muscle pains in the leg, which did not respond immediately to recompression, showed peak urinary CO_2 excretion either at the time the symptoms occurred or during the following days.

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PART VIII. RESPIRATORY LIMITATIONS OF HIGH AMBIENT PRESSURES*

DISCUSSION

C. J. Lambertsen, Chairman

Chairman Lambertsen: I am going to invite discussion from the panel to see if we can arrive at a consensus on progress from here on. Not too many years ago we had concern about what was called a "sound barrier" in aviation and great worry generated out of thinking about what would happen to individuals if ever they flew faster than the speed of sound. We are not worried about that any longer.

Over the past few years, though, there has been similar, largely psychological fear of crossing a 1000-ft diving depth barrier. The men who have crossed it are here. They seem to have put their concerns aside and now are glibly talking about moving on to depths of 2200, 2500, or 3000 FSW. We should not let them get away without expressing their reasons for new confidence. We will direct our attention not just to what they have done (which I think is extremely important) but to why they are so confident now that they are not going to be stopped by some of the mysterious troubles that have been hinted at in the past.

We can call first upon Dr. Miller, who has used the approach which will continue to be an extremely important one—simulation of very deep diving by use of a denser gas to open the way to what is likely to happen when one goes on and on to greater and greater depths. Do you think there are any real difficulties in the simulation procedure or should we extend its use?

Dr. Miller: You ask a very difficult question. First, one can make predictions on the basis of gas density alone. Dr. Wood and Dr. Anthonisen showed that the pressure drop of breathing results mainly from turbulence or convective acceleration or both for maximum ventilatory flow situations.

When we consider how much physical work a person may do at certain depths, we really are considering maximum ventilatory flow. We are considering the point where on expiration flow is getting very close to effort independence, and on inspiration we are concerned with the sort of situation where perhaps the work of inspiration is becoming almost more than the subject is prepared to endure. Consequently, there is a certain amount of validity in making such predictions down to a given depth—let's call it density—relative to an air depth.

For estimating ventilatory limitations this is reasonable. For other types of limitations that one may well get into, such as the CNS effects that Dr. Brauer talks about, we are not really discussing that sort of thing. And our predictions are purely hypothetical. We do not wish to discourage ourselves or to confidently leap ahead into the future.

Chairman Lambertsen: I hoped you would take that attitude because it is quite evident that there is not enough opportunity to conduct experiments at extreme pressures and there will not be improved opportunity for a long time. If we depend only upon doing work at 2000 or 3000 ft, and not using simulation, we will never be ready when the time comes.

Dr. Wood: Several papers this morning dealt with the feasibility of performing various levels of exercise at simulated depths. I can add that the available data would seem to indicate that at a depth of 300 ft breathing air our subjects are encountering limiting effects at an exercise ventilation in the range of 45–60 L/min. There have been predictions made today that divers doing their usual kind of work at these depths can easily handle this, but in fact that is a light working ventilation and, in view of the very severe symptoms that occur when one gets over the plateau and starts to close airways (as we have seen today), this would be a bad situation to be in the water at 1000 ft and experience. So I would be cautious. The limits are clear at 10 atm, and we

* *Panelists:* L. D. H. Wood, J. N. Miller, M. E. Bradley, N. R. Anthonisen, J. Salzano, K. E. Schaefer, J. Chouteau, E. H. Lanphier.

can extend this to simulated He depths. These limits are a direct function of the diver's maximum breathing capacity at ground level and can be predicted.

Chairman Lambertsen: You would at least agree that we must continue the simulation efforts in order to explore the probabilities.

Dr. Wood: That is certainly the easiest way to get the necessary information.

Dr. Hamilton: Several years ago when we did a 600-ft He–O_2 saturation dive, we were surprised by the bradycardia which now everybody sees at exercise. Dr. Schaefer's experiment showed it very clearly, even at rest. We also found it at rest, but not everybody has, probably because standard conditions are not used for the resting measurements.

Chairman Lambertsen: If I understand you, there have been changes at high pressure which indicate a decrease in certain functions relative to oxygen consumption. At increased O_2 consumption there is a relatively slower pulse rate, for example.

Dr. Salzano: We too did find some variability. The response was most pronounced in two of the subjects, who also happened to be the two divers. Also, we may be neglecting another factor, namely, thermoregulation; and I would like to at least start thinking about the possibility that this decrease in heart rate may be related to some sort of thermoregulation that is going on at the same time whereby blood is being shunted from the skin to the core. Finally, our subjects also had a pronounced decrease in respiratory rate; and in regulation of the respiratory and circulatory systems, respiration and pulse rate may be coupled in some common mechanism.

What is more important, we are starting to examine factors that Dr. Lanphier mentioned at the last symposium, factors that by themselves do not exert physiological effect, but when the effects of three or four of these factors are pooled, we may then have some interaction and generate detectable physiological effects from the combination.

Chairman Lambertsen: Whenever there are multiple correlations such as this, with decreases in respiratory rate and cardiac rate all correlating with change in one variable, O_2 consumption, the first thing to do (and I'm sure it has been done) is to find out whether the O_2 consumption measurements were caused to be systematically in error because of pressure-related gas measurement difficulties or changes in characteristics of the ergometer used. Could you reassure us that these changes are not measurement artifacts which make everything seem to be controlled at a different level at high pressure?

Dr. Salzano: Of all the measurements I have the most confidence in the O_2 consumption measurements. The measurements, I think, are valid and are what you would expect. An increased work of breathing with a gas density of 4 times that of air at sea level will increase overall O_2 consumption unless we have some other factor which is simultaneously reducing it below what it was at sea level. Moreover, the beautiful interrelationship we have between work rate and O_2 consumption would indicate that if error exists it is acting in a peculiar way.

Dr. D'Aoust: Does anyone know of work that has used the well-known bradycardia of breathhold diving response under hyperbaric conditions to try to understand this decrease in bradycardia that everybody sees?

Dr. Lanphier: I have not been able to visualize in my own mind just how the two effects would be related.

Dr. Smith: You asked about the limits of simulation of very dense atmospheres. There is one pertinent experiment, done by Dr. Eger, at San Francisco Medical Center, in which large mammals have been exposed to an extremely dense gas. Dogs were kept alive at 30 atm with carbon tetrafluoride. The dogs were apparently reasonably comfortable, though not very active. And this represents exactly 100 times greater density than air at 1 atm or equivalent to 20,000 ft of He with a reasonably large mammal. This would suggest (to answer the question raised by Dr. Lambertsen earlier) that experiments simulating high pulmonary work could show that one could go to quite considerable depths without at least producing fatal results in a mammal the size of a dog.

Dr. Wood: We were talking earlier about simulation in terms of depth and now about simulation in terms of using another mammal. Leith and Mead have pointed out that the dog is peculiar inasmuch as its maximum pulmonary flow rate per kilogram is at least 10 times that of man per kilogram. This makes a great difference when considering maximum flow rates limited by airway compression. Therefore the estimates which Dr. Smith has just offered must be reduced accordingly in terms of ventilation per kilogram body weight.

Chairman Lambertsen: The increase in maximum flow rate per unit of body size in going from man to dog would extend to smaller and smaller animals as well, would it not?

Dr. Wood: The correlations of vital capacity to flow rate per body size do extend to animals of smaller size, but the dog is peculiar in this. It seems to be as a result of the very large size of his smaller airways.

Dr. Bryan: There is a pleasant measure of agreement in the facts here, but we are in trouble with our extrapolation from air diving to depth. I would like to ask you to back away from the figure of 2000 ft or more that you said we could attain without any serious difficulty even while working.

Our data suggest that we are getting airway compression during exercise—in other words, ventilation is being limited—at 4 atm. Furthermore, the work of inspiration is very high at 4 atm and therefore CO_2 buildup is high—not very high, but it is increasing at 4 atm. This would mean that on He we could start thinking of an equivalent situation at 800–900 ft, which is substantially short of the extrapolation you have made.

This seems to fit data that Duke has produced because they found CO_2 retention during work at 120 W, which really is not much.

Therefore I think you are being optimistic as to how deep you can go and do useful work. I stress the point that Dr. Wood just made: when you get into airway compression during heavy exercise, the sensation of dyspnea is entirely different than normal breathlessness. This is a choking dyspnea and really rather frightening because it won't go away. Situations we encounter in a dry chamber are very different from the situation of a man diving in water and depending upon a mouthpiece. I therefore ask whether you really believe we will be able to do heavy work to depths in excess of 2000 ft.

Dr. Miller: Let us consider heavy work first. I did not say we would expect heavy work to be possible down to depths in excess of 2000 ft. I extrapolated down to the equivalent He depth where we found expiratory flow limitation, and this was between 6 and 7 atm abs on N_2–O_2. The equivalent He depth, using a 99% He–1% O_2 mixture is between 1400 and 1500 ft. This is still a good deal short of 2000 ft.

For moderate workload, of 100 W or 1.2 liters of O_2 consumption per minute, we have not been able to demonstrate any significant degree of expiratory flow limitation. We have not been able to demonstrate this work as becoming expiratory flow-limited on N_2–O_2 at any depth down to our maximum chamber pressure, which is 7.8 atm.

So far in these studies we have not yet encountered choking dyspnea. Dr. Lanphier a couple of years ago reported the problems that he ran into in pedaling himself to unconsciousness. So the implied warning in what you said is certainly something that we are very conscious of.

Dr. Anthonisen: We also used the number 2000 ft as an estimate of attainable depth. Nobody will argue with the fact that if you breathe very dense gas you will not be able to breathe as hard as if you did not breathe dense gas. Therefore if we insist that a diver maintain ventilation constant at great depth with an O_2 uptake of 3000 cm³/min, then we will produce physiological derangements in this individual. If we are willing to permit a lower O_2 uptake, we will produce physiological derangements at a deeper depth, and so on.

I think what we have said from our data is that, at gas densities equivalent to a depth of 2000 ft or more, normal subjects can move enough gas to increase their resting metabolism somewhat—to double it, or something like that. This is consonant with performance of some useful work.

Dr. Saltzman: There are two findings from the Duke dives that I think bear on this very question. The first is that in one of the three exercising subjects a change in the respiratory pattern did occur during the last 5 min of maximal exercise, when his respiratory rate became higher and tidal volume smaller in contrast to the usual pattern. This was the same subject who exhibited the highest arterial P_{CO_2} (51 mmHg) during exercise. He was not aware of choking dyspnea. The second point is that as part of the experiment the exercise was continued while not breathing through the low-resistance respiratory valve. The P_{CO_2} dropped rather significantly in both the individuals who exhibited hypercapnia on removing what we would ordinarily consider a very small external resistance.

Chairman Lambertsen: We must put these many important comments into a perspective which relates to extending useful undersea activity. Man is useful in other ways than as a performer of maximal physical work. All of us do constructive work during the course of our own normal week and it does not involve our maximum physical output. Very few of us use more than about 600–800 cm³ O_2/min when we are working hardest in laboratory or chamber experiments. Let us recognize that the diver has several functions when he is working under water. One is to do physical work, and the less this limits his other functions the better. If he can use his mind and his eye and his thumb, he will be using the main assets man has. These do not require a high pulmonary ventilation.

We therefore should take seriously into account what Dr. Anthonisen was hinting at, namely, that the limits of practical depth depend upon how hard the diver must work. The limits are not going to be determined by maximal work excepting in one respect: The diver must be able to get out of an emergency situation.

There is another important aspect, and that is that during chronic stress, such as sustained increase in

work of breathing or chronic exposure to moderately elevated CO_2 tensions there will occur an accumulation of physiological adjustments which, at the most extreme depth, may lead to dyspnea, respiratory fatigue, or narcosis—even at rest and even in sleep.

We therefore have the two extremes to consider. One is the man working at his hardest, and he will in fact be driven unconscious by his respiratory limitations as depth becomes great enough. So will he also have this happen at rest when the depth is extended enough for him to be driven to the same stage. Dr. Chouteau, what can we say about the limits of the resting individual? You have exposed goats to very high pressures. Can you describe what you think will be the depth limits for adequate pulmonary function in the nonexercising human?

Dr. Chouteau: I do not know about man. It is difficult to compare the problem of respiration in animals and in man. We have not measured ventilatory disturbances in the goat. The only parameter we can observe in our system is respiratory frequency. I am sure that in the next series of experiments we will make ventilatory measurements, but until now we have only counted frequency and we have found no significant change, even in our animals exposed for 10 days to 70–80 atm.

Dr. Linaweaver: One answer to your question, Dr. Lambertsen, might have been given to us rather subtly and I want to bring it out lest it get buried in the statistics. This was the Duke group's discovery of the disparity between the alveolar and arterial O_2 tension. Perhaps as we go deeper we may find that this disparity increases.

In Dr. Chouteau's studies with goats he indicated that he had to increase the inspired P_{O_2} level in order to keep the animals from being hypoxic. Therefore gas density may not be our biggest problem; providing enough O_2 may be.

Dr. Lundgren: I have information concerning the dogs that Dr. Smith mentioned since these experiments were done in our chamber. The chamber pressure was actually close to 40 atm. The alveolar CO_2 tensions varied between 60 and 90 mm of mercury even though the dogs appeared to be sufficiently ventilated.

Dr. Craig: The panel this morning indicated that ventilation at increased ambient pressures is going to be limited by flow, and we have heard several speakers describe the interactions of ventilation and exercise. These studies are simulations of diving, but have we simulated the problem faced by the diver who is actually in water? Perhaps the work of breathing would be increased further by having the subject immersed in water, and I wonder if ventilation with the subject exposed to water plus high gas density might not be effort-limited, rather than flow-limited as it is when the subject is out of water.

Chairman Lambertsen: I'm glad you raised the question of immersion, Dr. Craig, because there are fluid shifts in the immersed subject which tend to increase the pulmonary blood volume as well and might further exaggerate the ventilatory problem.

Dr. Raymond: With regard to ventilatory limitation, we have taken X-ray motion pictures of humans at 1 atm to determine the difference in two types of respiration: the assisted type of intermittent positive pressure breathing (IPPB) and the extrathoracic type of respiratory assistance. We noted that in the IPPB there is a decrease in the cardiac output, which perhaps may account for the decreased flow and the increase in CO_2 retention. There is also a very definite change in the appearance of the heart, with compression of the arterial sides. Perhaps with the decrease in flow there may be a decrease in blood supply to the tissues and resulting decrease in work effort.

We found, on the other hand, by using the extrathoracic type of artificial respiration that there was an increase in cardiac output by as much as 15%. We could increase the tidal volume by four and a half times with the method, which appeared to be more physiological than IPPB. It might pay to investigate this as a means of assisting breathing while using dense gas media, decreasing the work effort on the part of the diver.

Dr. Schaefer: We have not discussed enough the point raised concerning fluid shifts, the occurrence of which is indicated by increased urinary volume and composition. Together with other possible blood volume shifts related to pressure changes, these might affect the overall performance independent of effects on the respiratory system itself.

Dr. Bradley: I also want to stress that there is a tremendous difference between a diver in the chamber and a diver in the water. There appears not to be any great change in people in chambers doing relatively hard work down to 1000 ft. Yet there is a difference in a diver 600 ft deep in water who is breathing with scuba equipment, whose flow resistance characteristics are poor, who is thermally unsupported and who does experience the choking dyspnea which Dr. Lanphier and other people have experienced. Man appears to be

relatively adaptable to the depths we have studied. However, the quality of the supportive equipment with which he breathes is far from what it should be.

Chairman Lambertsen: To summarize this session, consider that we have been discussing step by step the kind of variables that have to be studied separately. As we proceed to study the ultimate limits of pulmonary function, man will be able to do practical and useful physical work in water without encountering an important pulmonary load by simply limiting overall exertion. It also appears that he will be able to do intellectually useful work as well because the He does not narcotize him to an important degree.

These positive findings obtained by relatively pure experimental approaches, by simulation at sea level or by studies in chambers, are vital and very distinct from the question of equipment. It is also evident that it is possible to incapacitate the diver by poor equipment alone or by additive stress. We can easily make it impossible to do useful work. What we most require now is to demonstrate whether or not normal function is possible at great depths. We can then bring the general technical and equipment support up to the level of physiological capability.

Part IX. CARBON DIOXIDE, EXERCISE, AND ACCLIMATIZATION TO HYPERCARBIA

THE EFFECTS OF BREATHING A HIGH DENSITY GAS UPON CARBON DIOXIDE ELIMINATION

Donald C. Parker and Eugene Nagel

The problems encountered when humans breathe a dense gaseous medium are of great interest to underwater physiologists, since these difficulties arise whenever man dives below the surface of the water and breathes air at a pressure greater than 1 atm abs. One of the problems concerns the role that gas density plays in inert gas narcosis. It has been postulated (2) that at high ambient pressures the increased work of breathing a dense gas produces hypercarbia, which, in turn, is responsible for the narcosis. While it is unlikely that CO_2 retention is the primary cause of inert gas narcosis (1), its role in potentiating the narcosis cannot be overlooked (4, 7).

It has been demonstrated (3, 6, 9) that SF_6 possesses narcotic qualities similar to those of other inert gases. Since SF_6 is nearly five times as dense as air, the possibility suggests itself that the narcotic action of SF_6 is caused, or at least aggravated, by CO_2 retention. The present study was therefore undertaken to determine whether CO_2 elimination is affected by breathing SF_6.

Methods

Six mongrel dogs were lightly anesthetized with intravenous pentobarbital. After orotracheal intubation of the animals with a saline-inflated cuffed Portex tube, each animal's femoral artery was cannulated with a nylon cardiac catheter. Each dog was then placed in a 7 ft $\times$ 3 ft steel hyperbaric chamber and allowed to breathe the desired gas mixtures spontaneously through a low-resistance demand regulator attached to the orotracheal tube that had been inserted. Duplicate samples of arterial blood were collected in siliconized, matched glass syringes. Immediately after the samples were collected, the Pa_{CO_2} was measured by means of a blood gas analyzer.

When the Pa_{CO_2} of each animal breathing air at 1 atm abs had stabilized, the inspired gas was switched to a mixture of 80% SF_6 and 20% O_2. When 5–10 min had elapsed, the Pa_{CO_2} was again determined and the breathing mixture switched back to air. After this sequence had been repeated two or three times, the chamber was slowly pressurized with air to 3.5 atm abs, and Pa_{CO_2} determinations were made while the animals breathed first air and then the SF_6–O_2 mixture, through the use of the same techniques described above.

TABLE I

EFFECTS ON ARTERIAL P_{CO_2} OF BREATHING AIR AND SF_6–O_2 AT 1 ATM ABS

Dog	Inspired gas	Duration gas breathed (min)	Arterial P_{CO_2} (mmHg)
1	Air	—	43.0
	SF_6–O_2	5	45.5
	Air	8	47.0
	SF_6–O_2	6	45.5
2	Air	—	39.5
	SF_6–O_2	5	40.5
	Air	5	41.0
	SF_6–O_2	4	40.5
3	Air	—	47.5
	SF_6–O_2	6	47.0
	Air	6	47.0
	SF_6–O_2	5	53.5
	Air	9	53.0
4	Air	—	45.0
	SF_6–O_2	8	43.0
	Air	5	44.0
	SF_6–O_2	5	42.0
5	Air	—	46.0
	SF_6–O_2	5	43.0
	Air	6	45.5
6	Air	—	41.0
	SF_6–O_2	6	39.5
	Air	6	40.0
	SF_6–O_2	5	39.5
	Air	6	38.5

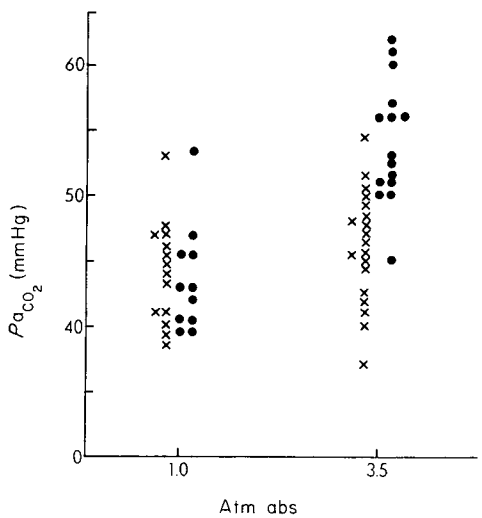

FIG. 1. Changes in Pa_{CO_2} in dogs breathing air (×) and a SF_6–O_2 mixture (●) at 1 and 3.5 atm abs.

TABLE II

EFFECTS ON ARTERIAL P_{CO_2} OF BREATHING AIR AND SF_6–O_2 AT 3.5 ATM ABS

Dog	Inspired gas	Duration gas breathed (min)	Arterial P_{CO_2} (mmHg)
1	Air	—	47.0
	SF_6–O_2	5	51.0
	Air	7	51.5
	SF_6–O_2	6	53.0
	Air	13	48.0
	SF_6–O_2	5	56.0
2	Air	—	45.5
	SF_6–O_2	7	50.0
	Air	6	48.5
	SF_6–O_2	6	56.0
	Air	4	50.0
	SF_6–O_2	5	57.0
	Air	5	47.5
	SF_6–O_2	5	52.5
3	Air	—	50.5
	SF_6–O_2	5	61.0
	Air	5	54.5
	SF_6–O_2	4	62.0
4	Air	—	41.0
	SF_6–O_2	5	50.0
	Air	7	42.0
	SF_6–O_2	5	51.0
	Air	5	48.0
5	Air	—	45.5
	Air	7	46.0
	SF_6–O_2	4	56.0
	SF_6–O_2	9	60.0
	Air	6	49.5
6	Air	—	37.0
	Air	5	40.0
	SF_6–O_2	5	45.0
	SF_6–O_2	9	51.5
	Air	5	45.0
	Air	5	42.5

Results

The Pa_{CO_2} values for dogs breathing air and SF_6–O_2 at 1 atm abs and at 3.5 atm abs are listed in Tables I and II, respectively. At 1 atm abs no significant differences in the arterial P_{CO_2} levels were observed when the SF_6–O_2 mixture was breathed instead of air (Table III; Figs. 1 and 2).

When the SF_6–O_2 mixture was breathed at 3.5 atm abs, there was a mean rise of 7.8 mmHg in the Pa_{CO_2}. The difference between Pa_{CO_2} values during air breathing and during SF_6–O_2 breathing at this pressure is highly significant ($P = < 0.01$).

TABLE III

STATISTICAL ANALYSIS OF DATA PRESENTED IN TABLES I AND II

Inspired gas	Pressure (atm abs)	No. subjects	Mean Pa_{CO_2} (mmHg)	Standard deviation (mmHg)
Air	1.0	14	44.1	±4.0
SF_6–O_2	1.0	11	43.6	±4.2
Air	3.5	19	46.3	±4.3
SF_6–O_2	3.5	15	54.1[a]	±4.7

[a] Significant difference from air breathing control at same ambient pressure.

During these experiments no consistent changes in ventilatory frequency were noted, nor was there a significant difference between the Pa_{CO_2} levels measured during air breathing at 1 and 3.5 atm abs.

Discussion

At 1 atm abs, a mixture of 80% SF_6–20% O_2 has a density approximately four times greater than that of air, and its kinematic viscosity is one-fifth that of air. Therefore, a subject breathing this mixture at 1 atm abs would have a pulmonary airway resistance approximately equal to that experienced when air is breathed at 5 atm abs. That no CO_2 retention occurred when the animals breathed SF_6–O_2 at 1 atm abs is not surprising.

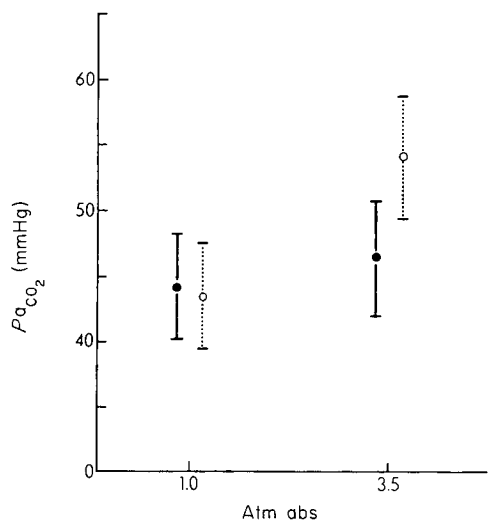

FIG. 2. Mean Pa_{CO_2} values and standard deviations in dogs breathing air (●) and a SF_6–O_2 mixture (○) at 1 and 3.5 atm abs.

In another experiment, however, we did find that SF_6 exerts a narcotic effect even at 1 atm abs. Five volunteers, who thought that they were breathing O_2 or air, were switched to a SF_6–O_2 breathing mixture. Each terminated the experiment within 3 min, complaining of light-headedness, numbness, and disorientation. Another five volunteers, all trained anesthesiologists, breathed a mixture of 75% SF_6 and 25% O_2 for 5–10 min. In addition to experiencing the paresthesias and light-headedness noted by the previous group, the second group also detected some loss of proprioception and light tactile sensation. These effects correlated with those experienced when each subject later breathed 25% N_2O in O_2. The senior author's personal experience is that breathing SF_6 causes a vague sensation of unpleasantness and a feeling of fullness in the head that are not experienced when N_2O is breathed. These symptoms may be due to the high density of the SF_6–O_2 mixture. It appears, therefore, that SF_6 breathed at partial pressures of 0.75 to 0.80 atm abs has a pronounced narcotic effect, but that CO_2 retention plays no part in the narcosis.

There are at least three possible explanations for the mild hypercapnia observed in the dogs during SF_6–O_2 breathing at 3.5 atm abs. Since the kinematic viscosity of the mixture at this pressure is equivalent to that of air at 16.5 atm abs, Pa_{CO_2} may rise as a result of the hypoventilation caused by increased resistance to breathing. Furthermore, CO_2 diffusion across the alveolar membrane may be impaired by the high density of SF_6 (8). Finally, the narcotic effect of SF_6 may be sufficient to alter the ventilatory response to CO_2 and thus produce hypercarbia.

Eger *et al.* (5) have recently found that the alveolar tension of SF_6 producing light surgical anesthesia in dogs is 4.9 atm abs. This minimal alveolar concentration is 2.6 times greater than that required to produce light N_2O anesthesia in the dog. Eger's findings agree with the results of our own earlier experimentation with volunteers—i.e., that SF_6 has about one-third the narcotic potency of N_2O. Since SF_6 has a very low blood solubility, the alveolar concentration approaches the inspired concentration quite rapidly. Therefore the dogs breathing the SF_6–O_2 mixture at 3.5 atm abs had an alveolar SF_6 tension of approximately 2.8 atm abs, or 0.57 minimal alveolar concentration. It is doubtful that this concentration, even when combined with light pentobarbital anesthesia, would produce enough CNS and respiratory depression to cause a rise of nearly 8 mmHg in the Pa_{CO_2}.

Whatever its etiology, hypercarbia did occur in our animal subjects when SF_6 was breathed at high ambient pressures. Although CO_2 retention most probably does not cause inert gas narcosis, it must still be considered a concomitant and aggravating factor as ambient pressures rise.

Acknowledgments

The authors gratefully acknowledge the assistance of David W. Cugell, M.D., Department of Medicine, Northwestern University Medical School, and of Magnus H. Agustsson, M.D., Department of Cardiology, Augustana Hospital, Chicago, Illinois.

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RESPIRATORY GAS EXCHANGE IN ANIMALS DURING EXPOSURE TO EXTREME AMBIENT PRESSURES

J. Chouteau

The work of Workman *et al.* (19) and of Barthélèmy (2) on animals formed a solid base for the first human experiments in saturation diving: Conshelf I and II (4), Man in the Sea (14), and SeaLab I and II (17, 18). Since all but one of these studies were limited to depths of 230 FSW, we had to do a new series of animal experiments down to 656 FSW (1, 6, 7) in preparation for the Conshelf III dives.

We have therefore tried to determine the limits in the use of O_2–He breathing mixtures and, more recently, O_2–H_2 mixtures in deep saturation diving. The immediate goal of this work was to determine the safe operational depth for the submarine Argyronète, at present being prepared by the Centre d'Etudes Marines Avancées (CEMA) for use by the National Center for Ocean Exploitation (CNEXO) and the French Institute of Petroleum (IFP). This submarine will be the carrier of an undersea habitat, designed by J. Y. Cousteau, that will be used for the future Conshelf IV experiments.

Our investigations have revealed a new phenomenon, which might be identified as hypoxic difficulties that occur when subjects breathe normoxic mixtures under pressure. (By *normoxic* we mean a P_{O_2} of 159 mmHg in the breathing mixture.) Aside from their implications in general physiology, the results of our experimentation can perhaps shed new light on some problems encountered during recent years in human experiments in deep saturation diving.

The present research was carried out in the main at CEMA and also at Groupe d'Etudes et de Recherches Sous Marine (GERS) and Commission d'Etudes Pratiques des Sous-Marins (CERTSM, French Navy, Toulon).

Hypoxia Caused by Breathing Normoxic Gas Mixtures under Pressure

In one experiment (code name, *Ursula*), previously reported (6, 9, 10), we compressed four goats in a normoxic O_2–He atmosphere (see Table I).

We pointed out that although the mixture was tolerated perfectly during a 5-day exposure at 1280 FSW (39.71 atm abs),* after 1600 FSW (49.8 atm abs) the animals showed behavioral disturbances and progressive paralysis. These disturbances disappeared completely and

* As used in this paper, one atmosphere absolute is considered equal to one Bar (= 750.2 mmHg). It is also approximately equal to a pressure exerted by 10 m of sea water.

TABLE I

PHYSICAL PROPERTIES OF BREATHING MIXTURES USED IN PROJECT *Ursula*

Depth (FSW)	Pressure (atm abs)	Duration (days)	PI_{O_2} (mmHg)	PI_{N_2} (mmHg)	PI_{CO_2} (mmHg)	Absolute density (gm/L, STPD)	Density relative to air	Depth with equivalent density of air (FSW)
1280	39.71	7	154±29	22±3.5	0.74	7.440	5.757	156
1820	56.17	3[a]	191±22	29±3.5	0.74	10.741	8.312	240

[a] Includes some time spent at 1600 FSW (49.8 atm abs).

quickly when the PI_{O_2} was raised to about 191 mmHg (0.25 atm), and reoccurred when PI_{O_2} fell to 154 mmHg (0.20 atm). During the remainder of the exposure—two days at 1820 FSW (56.2 atm abs) with a PI_{O_2} of 194 mmHg (0.25 atm)—the animals displayed perfectly normal behavior. Clinical, biochemical, and anatomical examinations conducted on two of the animals killed shortly after the experimentation revealed no specific pathology, except the aftereffects of anoxic lesions at the pulmonary and muscular levels.

We undertook a similar experiment using an O_2–N_2 normoxic mixture (8, 11) and saw identical phenomena at 320 FSW (10.67 atm abs). Raising PI_{O_2} to 250 mmHg (0.33 atm) resulted in a return to normal activity within 5 min.

Using rabbits fitted with permanently implanted cortical electrodes, we have demonstrated in previous experiments (5) that EEG disturbances took place when the animals breathed normoxic O_2–Ar, O_2–N_2, and O_2–He mixtures under pressure. At the beginning, these disturbances consisted of a replacement of the α rhythm with slow-wave θ rhythm (narcosis), followed by a flattening-out of the EEG (hypoxia). These phenomena appeared with Ar between 4 and 10 atm abs, with N_2 between 11 and 20 atm abs, and with He beyond 50–60 atm abs, and all proved reversible when the PI_{O_2} was raised. It seems, therefore, that when the specific mass of a normoxic mixture exceeds a certain value, hypoxic signs and symptoms appear.

The hypoxic symptoms may be due to alveolar hypoventilation resulting from the increase in the specific mass of the inspired mixture, or to an increase in the coefficients of intrapulmonary diffusion (15). In either case, hypercapnia should occur, the signs of which would not disappear with the late slight increase in PI_{O_2}; however, the animals never showed any hyperpnea. Furthermore, a fall in PI_{O_2} to around 110 mmHg (0.14 atm) in the O_2–He experiments at 1280 FSW (39.71 atm abs) did not cause any trouble. And in several anoxia experiments at atmospheric pressure, it was necessary to use PI_{O_2} levels as low as 75 mmHg (0.10 atm) to confirm the hypoxic nature of the signs and symptoms.

Alternatively, the hypoxic signs and symptoms may have resulted from a cellular hypoxia of a histotoxic nature caused by increased inert gas partial pressure. Such a mechanism has been described by Bennett (3) as a possible mechanism of narcosis at the synapse. However,

this cause cannot be the only one, because the slight change in PIO_2 associated with the reversibility of these phenomena cannot alter the tissue P_{O2} to a significant degree.

The most likely cause of the hypoxic signs and symptoms is a disturbance in the alveolar–capillary exchange. There might be either a decrease in the pulmonary diffusion of O_2 or an alveolar–capillary block altering the ventilation–perfusion ratio; or both conditions might occur simultaneously.

Workman *et al.* (19) and Barthélèmy (2) have observed similar paresis during their animal experiments at 193 FSW (6.8 atm abs) with O_2–N_2. In animals killed immediately after the experiments, they found pulmonary atelectasis and pneumonia, which appeared to be reversible changes since they were not evident in the animals killed later. Whatever the cause of these lesions, we can postulate that they might be at least partially responsible for the disturbance of alveolar–capillary exchange discussed here.

An Approach to Determining Limits in the Use of Oxygen–Helium Breathing Mixtures

The facts and hypotheses mentioned above led us to consider the limit of the use of O_2 in breathing mixtures in a different light. Let us assume that if the PIO_2 of a gas mixture is limited during prolonged exposure diving by the phenomenon of chronic hyperoxia (2, 19), then the possible increase in PIO_2 would be limited to a value of 0.42 atm (line 1 of Fig. 1). The assumption can also be made that if disturbances in O_2 diffusion exist, they may increase the threshold of onset of chronic hyperoxic lesions, the evolution of PIO_2 taking place according to lines 2 or 3 of Fig. 1.

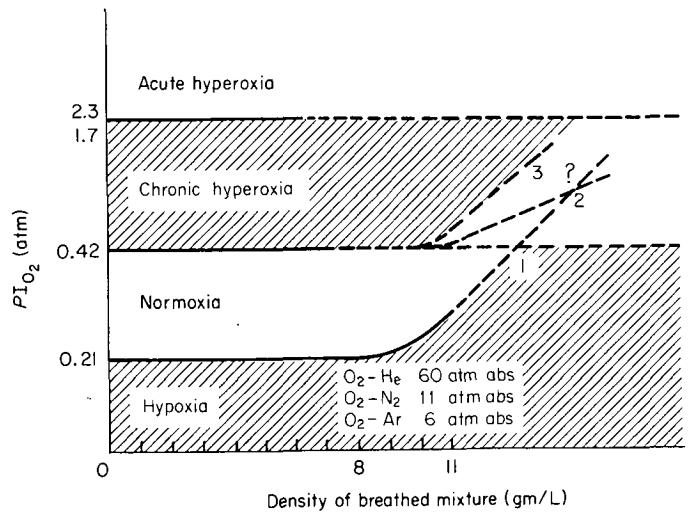

FIG. 1. Schematic representation of possibilities of raising PIO_2 of a respiratory mixture as a function of the specific gravity.

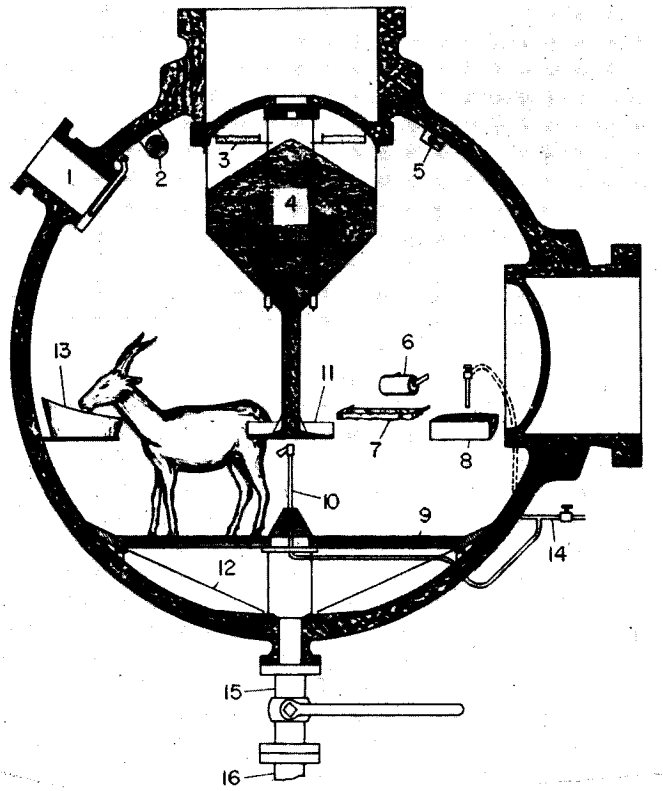

FIG. 2. Installation for goats in the spherical high pressure chamber. Lock (1) used to introduce food into auxiliary manger (13) in case of malfunction of main device (11). Main manger continuously supplied with granulated food by feeding box (4). Silica gel (3) prevents moisture in food supply. Ventilator (2), light (5), salt block (6), piece of wood (7), water trough (8); floor grating (9) washed by rotating water jet nozzle (10); circular neoprene cone (12) collects excrement passing through 3-in. valve (15) to evacuating lock (16); high pressure water supply for 8 and 10 (14).

In order to verify these assumptions, we carried out in late 1968 an experiment involving progressive saturation dives, using three goats in improved experimental conditions (code name, *Boucabloc*) (13). The animals were compressed in a chamber that was more spacious than usual (diameter, 7.87 ft; volume, 247 ft^3) at a maximum pressure of 151 atm abs (Figs. 2, 3, 4).

Figure 5 shows the pressurization schedule used—the rate of ascent between decompression stops being 3.28 ft/min—and also the variation in PI_{O_2}. Table II presents the mean values of physical constants in the atmosphere breathed at each stop.

The following data were obtained:

To 71 atm abs (2296 FSW), the animals tolerated the normoxic mixture O_2–He (PI_{O_2} = 0.22 atm) perfectly.

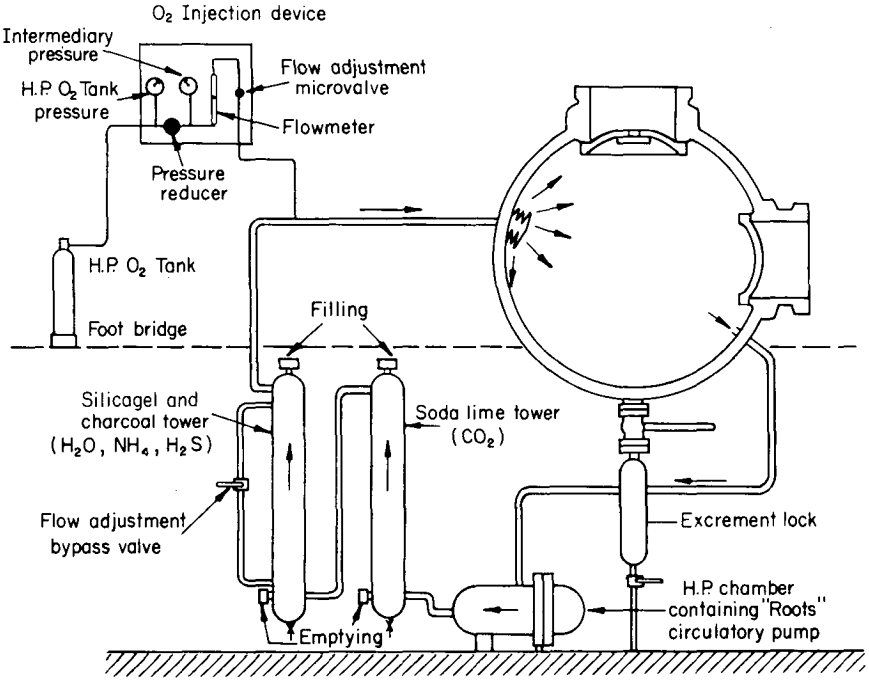

FIG. 3. High pressure regeneration circuit.

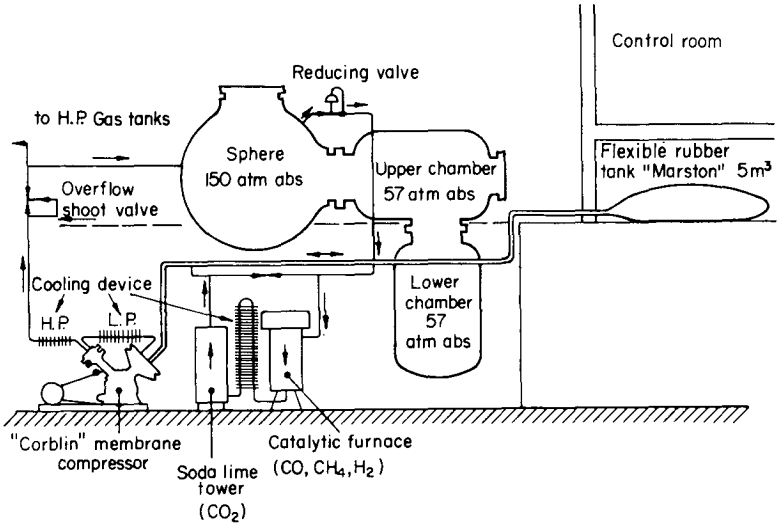

FIG. 4. Low pressure regeneration circuit.

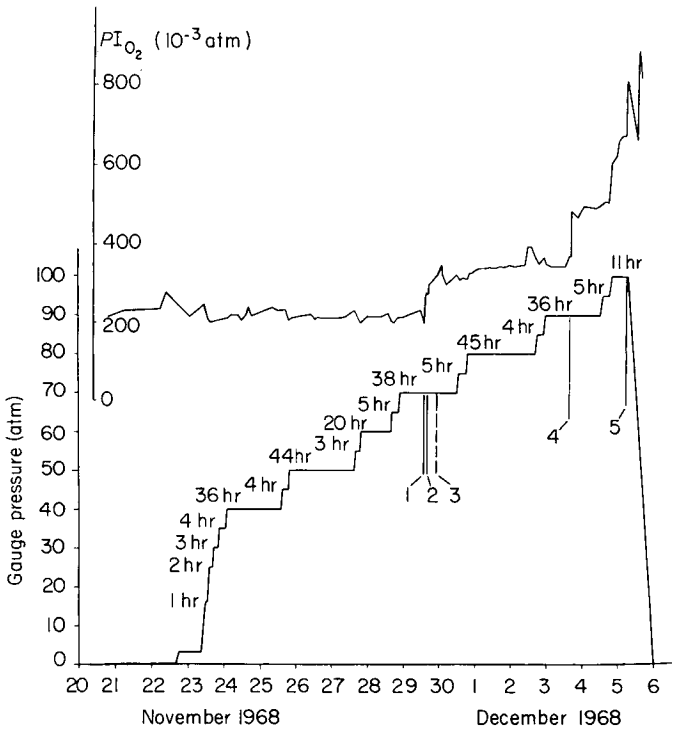

FIG. 5. Lower curve, dive profile of goats exposed to O_2–He breathing mixture at 100 atm abs. The numbered vertical lines indicate the order and time of hypoxic crisis. Upper curve, PI_{O_2} evolution.

At 71 atm abs (2296 FSW), after a 10-hr exposure, one animal (point 1, Fig. 5) developed such pathological signs as cessation of all activity and hindquarter paresis, with paroxysmal attempts to maintain posture and equilibrium through use of the forelimbs, followed by falling and total paralysis. The animal finally lay on its side with its four legs and neck extended (see Fig. 6).

When PI_{O_2} was raised to 0.28 atm, progressive return to normal activity occurred, which became total in 30 min, leaving no apparent aftereffects.

A second crisis (point 2, Fig. 5) overcame another animal following an accidental lowering of PI_{O_2} to 0.27 atm, but the symptoms disappeared when PI_{O_2} was raised to 0.29 atm.

At 81 atm abs (2624 FSW), PI_{O_2} was maintained at 0.35 atm, and the 45-hr exposure was tolerated perfectly by the animals.

At 91 atm abs (2952 FSW) at the same PI_{O_2}, after 10 hr the third animal suffered an identical crisis (point 4, Fig. 5), which was reversed by raising PI_{O_2} to about 0.50 atm. The remainder of this stay was perfectly tolerated.

At 101 atm abs (3280 FSW), the animals were tired and we raised PI_{O_2} gradually to about 0.65 atm. In spite of this, a crisis (point 5, Fig. 5) developed in the animal that had suffered one at 71 atm abs. A rapid rise in PI_{O_2} to 0.80 –0.90 atm did not cause the symptoms to remit, and the animal died.

TABLE II

MEAN VALUES OF PHYSICAL CONSTANTS OF THE ATMOSPHERE BREATHED AT EACH PRESSURE LEVEL DURING PRESSURE EXPOSURE PROFILED IN FIG. 5

Depth (FSW)	Pressure (atm abs)[a]	PI_{O_2} (atm)	PI_{N_2} (atm)	PI_{CO_2} (atm)	PI_{CH_4} (atm)[b]	Absolute density (gm/L, STPD)	Density relative to air	Depth with equivalent density of air (FSW)
1312	41	0.218	0.042	0.004	0.012	7.647	5.914	161
1640	51	0.217	0.055	0.005	0.024	9.454	7.312	207
1968	61	0.217	0.061	0.005	0.022	11.240	8.693	253
2296	71*b*	0.220	0.066	0.005	0.026	13.037	10.082	295
2296	71*a*	0.309	0.068	0.003	0.024	13.148	10.168	295
2624	81	0.349	0.068	0.008	0.015	14.990	11.593	348
2952	91*b*	0.351	0.074	0.010	0.013	16.782	12.979	394
2952	91*a*	0.491	0.074	0.009	0.012	16.954	13.112	397
3280	101	0.648	0.080	0.013	0.012	18.948	14.654	449

[a] Figures with *b* and *a* are values before and after, respectively, the hypoxic crises were treated by raising the PI_{O_2}.

[b] Methane is removed by forcing breathing mixture through a catalytic furnace heated to 300°C.

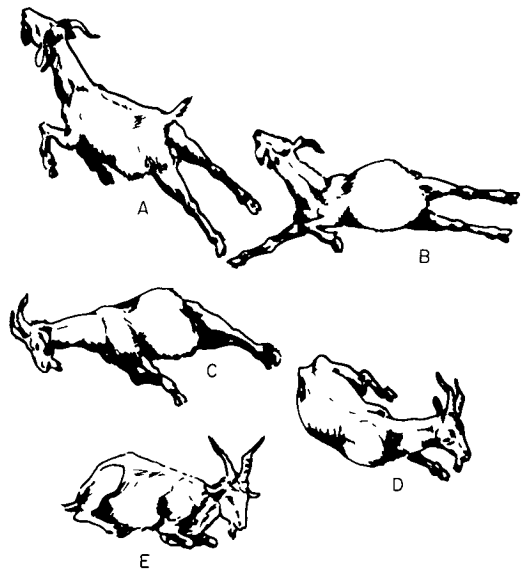

FIG. 6. Typical evolution of hypoxic crisis in goat breathing normoxic mixture under pressure. (A) hindquarter paralysis and attempt to regulate posture equilibrium with the forelimbs; (B) animal falls, with hind leg extension; (C) flaccid paralysis, leg extended, no reactions; (D) beginning of recuperation; (E) animal lying down normally, awake, shortly after which it stands up and eats.

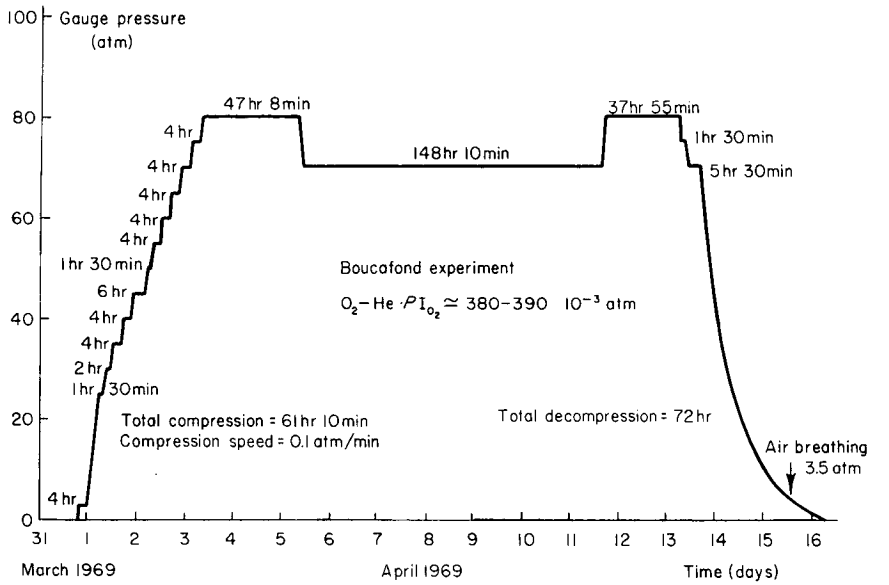

FIG. 7. Dive profile of O_2-He long-duration exposure at 81 and 71 atm abs (PI_{O_2} = 0.39 atm abs).

We tried decompressing the other two, but as one animal was obviously dying at about 66 atm abs, we decided to sacrifice both animals in the chamber by increasing the CO_2 content of their breathing mixture.

Rapid postmortem decompression with the consequent gaseous distention of the animals did not permit valid anatomicopathological examinations.

This more precise experimentation, in which we allowed a longer time for hypoxic disturbances to develop, confirms the results of our previous work. The symptoms we observed in our earlier experiment, *Ursula*, appeared at 50.4 atm abs (1600 FSW), and in a less definitive form, whereas in the present experimentation they appeared at 71 atm abs (2296 FSW) after a longer and fairly constant delay (10 hr).

The difference in results, in our thinking, is the better control of atmospheric humidity during the present experimentation. Humidity was maintained, on the average, at between 50 and 80%, whereas during *Ursula* it was always 100%. A chance verification of the effect of humidity was made during one animal's stay at 71 atm abs, when it suffered a second attack of symptoms (point 3, Fig. 5) at a time when the humidity rose rapidly to 100% while the PI_{O_2} level remained unchanged. Simply replacing the silica gel in the chamber caused the humidity to lower quickly to 40%, which brought about total remission of symptoms without raising the PI_{O_2} level. One might conclude that a factor relating to high humidity has a bearing on the development of the lesions responsible for alveolar–capillary diffusion disturbances.

In April 1969 we carried out another saturation exposure (*Boucafond* experiment) of long duration at 81 atm abs (2624 FSW), once more using goats. Three animals were compressed progressively at a constant PI_{O_2} of 0.39 atm (see Fig. 7).

After remaining 47 hr at that pressure, one animal began a generalized trembling, then

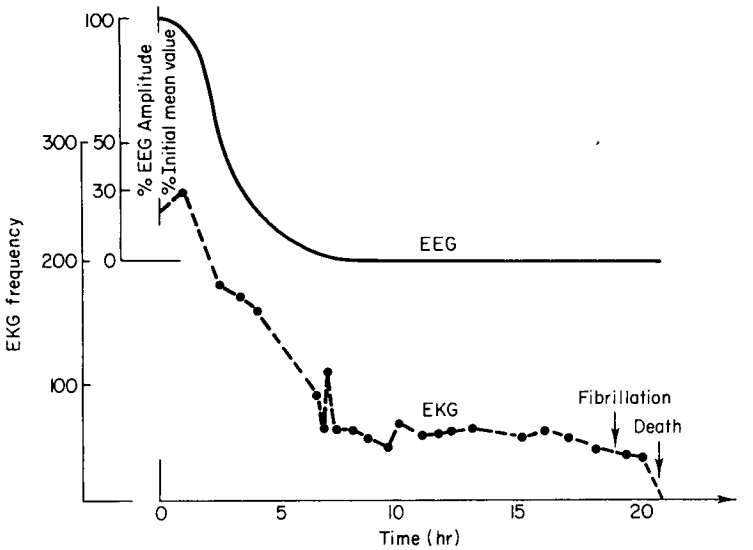

FIG. 8. EEG and EKG findings in a rabbit breathing O_2–H_2 mixture at 29 atm abs.

lay down. It kept its head lifted, however, and stayed awake. This symptom is difficult to compare with hypoxic crises. The other two animals behaved normally. As planned, we decompressed the animals to 71 atm abs (2296 FSW) and kept them 148 hr at that pressure. The sick animal became normal after 1½ hr. This stop was marked only by diarrhea caused by the animals' difficulty in adjusting to their feed.

The goats were then compressed once more to 81 atm abs, which they tolerated well in the beginning. After about 38 hr, however, the same animal began a generalized trembling anew, and we therefore decided to decompress to 71 atm abs once more. After 6 hr at this pressure, the animal's condition did not improve despite our raising the PI_{O_2} to 0.55 atm, and we therefore decided on final decompression. Furthermore, a technical difficulty prevented our using the silica gel and charcoal regeneration tower.

The sick animal died at 46 atm abs pressure, and the two survivors left the chamber after 72 hr of exponential decompression. They were tired and thinner, but exhibited no evidence of physical or behavioral difficulties as a result of their pressure exposure.

Clinical and biological examination of the surviving goats revealed some difficulties of nutritional origin but no specific pathological changes. The autopsy on the dead animal in the chamber, and on one of the other two, which was destroyed the next day, revealed no further evidence of gross pathology. At present, the third animal is alive and well. We are awaiting histological examination results.

It is difficult to draw conclusions from such an experiment (which, in any event, we plan to repeat). The death of the animal is hard to explain; it was perhaps due to its feebler constitution. Certainly the other two did not appear affected by their approximate 10-day stay at between 71 and 81 atm abs, or by the decompression that followed. On this basis, saturation exposures to depths between 1600 and 2300 FSW can be considered reasonable.

Preliminary Results of Tests to Determine Limits in the Use of Oxygen–Hydrogen Breathing Mixtures

We shall report briefly on the results of a series of experiments that are being carried out at GERS in collaboration with Michaud, Parc, Barthélèmy, Le Chuiton, Corriol, and Le Boucher (16). Tests involving rabbits in a succession of five individual exposures were conducted in a heated chamber immersed for safety reasons. The animals, with permanently implanted electrodes, were compressed in an O_2–H_2 atmosphere to 29 atm abs (908 FSW) with an average PI_{O_2} of 0.43 atm. The results on one animal in this series are presented in Figs. 8 and 9. The general results on all the animals in the series are as follows:

1. A progressive reduction in the amplitude of EEG potentials, leading to cortical electric silence in 7–12 hr. The flattening-out of the traces was broken by brief periods of revival that came farther and farther apart.

2. A reduction in cardiac frequency that followed a time pattern similar to that of the EEG in the beginning. The EKG activity, however, survived 6–13 hr longer than the EEG did (35 hr in one animal). During this period the animals showed a progressive reduction in respiratory and motor activities (the latter having been stimulated by a vibrator in the chamber). The EKG silence was preceded by extrasystoles and ventricular fibrillation.

Great prudence is obviously required in using an O_2–H_2 breathing mixture. Although these experiments appear, at first impression, to condemn its use for long-duration saturation dives, it does not follow that its use would be dangerous in short-duration "excursion" dives. It can perhaps be considered comparable to the example of hyperbaric oxygenation, which is innocuous for short and intermittent exposures.

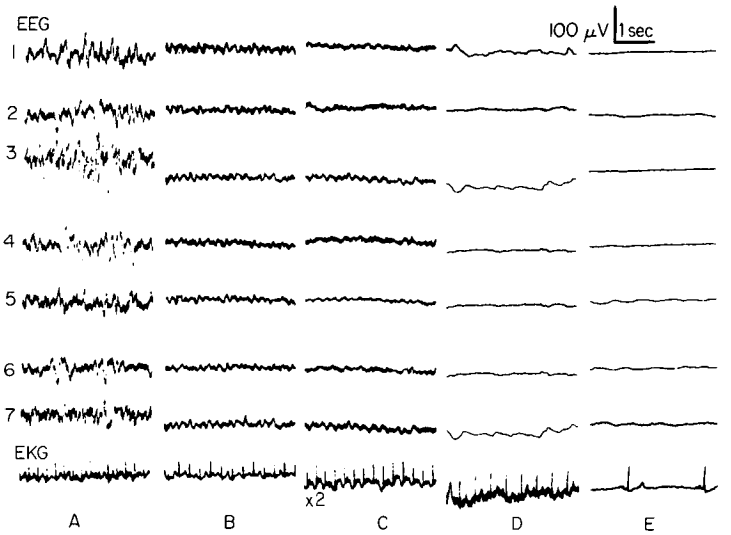

FIG. 9. Evolution of EEG and EKG during exposure of a rabbit in O_2–H_2 atmosphere at 29 atm abs. (A) before compression; (B, C, D, and E) after 2, 4, 6, and 8 hr, respectively, of exposure. Note the very clear diminution of EEG amplitude and the disappearance of all organized activity before the sixth hour, and the important terminal bradycardia.

Discussion

We conclude from our animal experimentation that the limits for human saturation diving in which O_2–He mixtures are breathed have not been reached. Perhaps depths of 1640–2300 FSW are feasible, but much research remains to be done. We ourselves are planning to start a new series of experiments using goats with permanently implanted electrodes to record EEG, EKG, EMG, body and cutaneous temperatures, respiratory rhythm, and Pa_{O_2}. Simple visual observation and recording of the total behavior of the animal subjects, even when continuously and carefully done, certainly provide insufficient evidence from which to draw valid conclusions.

Our present investigation demonstrated the importance of rigorous monitoring of the atmospheric parameters—e.g., PI_{CO_2}, humidity, and miscellaneous pollution. The importance of the breathing mixture's specific gravity caused us to maintain the N_2 level systematically as low as possible.

The discovery of hypoxic troubles during respiration of normoxic mixtures under pressure, and the role played by specific gravity at the hypoxic threshold, seem of special interest. Interpretation of these phenomena is not easy, but it is reasonable to think that the saturation of some structures by inert gases may result in the formation of O_2 diffusion barriers. Concerning the hypothesis of an alveolar–capillary block, we, together with Guillerm and Hee (12), have recently obtained experimental confirmation. We placed amperopolarographic electrodes in the aortic arch through the left carotid artery of anesthetized and heavily heparinized rabbits. The animals were then compressed to 21 atm abs in a small chamber in which PI_{O_2}

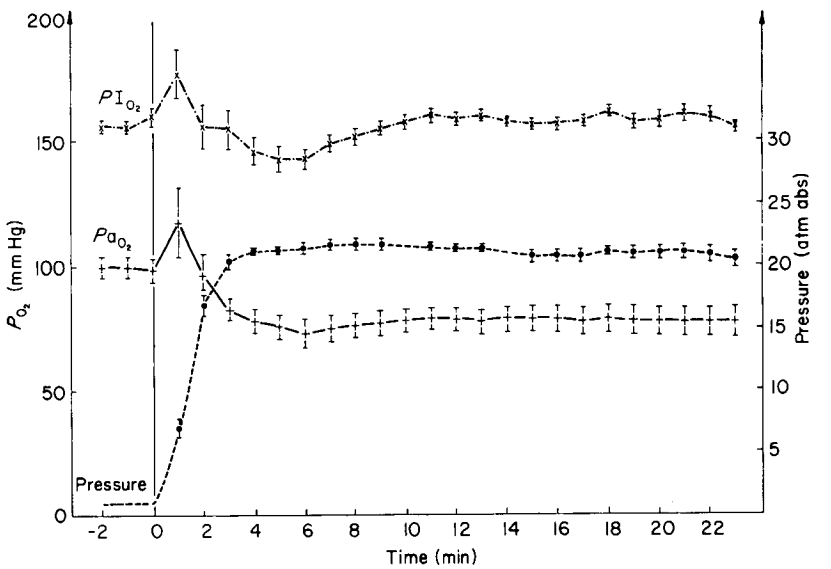

FIG. 10. Mean values for experiments on 12 rabbits exposed to 20–21 atm abs while they breathed an O_2–H_2 normoxic mixture. PI_{O_2} recorded with Beckman O_2 electrode; Pa_{O_2} recorded with Rybak-type O_2 electrode in aorta. (The PI_{O_2} and Pa_{O_2} fluctuation during the first minutes is due to an inhomogeneity of the gas mixture in the chamber.)

was maintained between 150 and 160 mmHg. The PI_{O_2}, Pa_{O_2}, and chamber pressure were recorded simultaneously (Fig. 10).

After the PI_{O_2} was stabilized, a significant change in Pa_{O_2} occurred: a fall of 21.4 ± 3.9 mmHg after 10 min (in 12 rabbits), and of 21.6 ± 5.7 mmHg after 20 min (in 10 rabbits). In five trials at 15 atm abs, no change was observed; a pressure threshold therefore appears to exist. In decompression, during which a normoxic PI_{O_2} was maintained, Pa_{O_2} returned to its original level measured at atmospheric pressure. The phenomenon thus proved to be completely reversible.

The first data obtained from experiments being carried out with an O_2–Ar mixture seem to correlate with those from an O_2–He mixture. But the results were obtained at a lower pressure, according to the ratio of the molecular weights of both inert gases.

In fact, the fall of Pa_{O_2} is of insufficient amplitude to explain all the phenomena we observed in our goat experimentation. But if we assume the existence of a change in alveolar–capillary diffusion, it is logical to suppose that the same change can be found amplified at the hematoencephalic barrier or at the cellular level. Nerve tissue hypoxia could therefore reach a high level, which would explain the behavioral and paralytic troubles in the goats. It remains to be proved that the hypoxia is really the result of a modification of the alveolar–capillary diffusion and not of a perturbation of the alveolar ventilation /perfusion ratio ($\dot{V}A/\dot{Q}$).

Finally, concerning the O_2–H_2 mixture, it is too soon to consider it a safe breathing medium. One can advance the hypothesis that changes occur slowly at the respiratory chain level and in the redox process as a result of the high partial pressures of H_2 that have been established. It is noteworthy that a slow perfusion with cyanide caused the same EEG and EKG changes in the rabbits that occurred when the animals breathed an O_2–H_2 mixture under pressure, and in the same length of time (Corriol, work in progress).

Acknowledgments

This paper was prepared with the collaboration of MM. Alinat, Imbert, Bianco, Coulboy, Oriol, Pironti, LePechon, Angeniol, and Aquadro, and Mme. Andrac of CEMA, Marseilles; of MM. Michaud, Barthélèmy, Parc, Le Chuiton, and Le Boucher of GERS, Toulon; of MM. Guillerm and Hee of CERTSM, Toulon; and of Professor Corriol of the Faculty of Medicine, Marseilles.

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RATE OF ACCLIMATIZATION TO CHRONIC HYPERCAPNIA IN MAN

J. M. Clark, R. D. Sinclair, and B. E. Welch

The logistics of manned undersea and space exploration require that life-support systems be contained in sealed environments from which the CO_2 produced by cellular metabolism must be continually removed. Partial or total failure of the CO_2-removal system will expose the inhabitants of the environment to increased P_{CO_2}. Because such a failure may occur at any time, the tolerance limits in normal man to acute and chronic hypercapnia must be determined.

It is well known that prolonged exposures to elevated Pa_{CO_2} generate a process of acclimatization that greatly increases tolerance to hypercapnia (1, 3, 9, 24, 26). This acclimatization includes reduction of the ventilatory response to hypercapnia (1, 6, 10, 20, 22) and partial or complete reversal of the initial increase in $[H^+]$ in body fluids (3, 5, 9, 23–26). Determining the rate of acclimatization is of particular importance, because CO_2 accumulation in a sealed environment will probably be gradual and may therefore allow sufficient time for compensatory mechanisms to increase CO_2 tolerance effectively.

Previous Studies

Previous studies providing data on the rate of acclimatization to chronic hypercapnia are summarized in Table I (6, 10, 20, 22–24). Although there are many indices of acclimatization to hypercapnia, ventilatory response to CO_2 and maximal compensation of arterial pH were selected as the bases of comparison in the table. There is general agreement that the duration of exposure to chronic hypercapnia required to produce maximal compensation of arterial pH is about 3–5 days. The single reported exception of 23 days was based upon venous pH measurements that probably did not reflect corresponding changes in the arterial blood. However, the duration of chronic hypercapnia observed to produce a significant reduction in the ventilatory response to inspired CO_2 ranged from 13 hr to 40 days. In the latter case, the ventilatory response to CO_2 was not specifically tested until after 40 days of exposure, and the

TABLE I

RATE OF ACCLIMATIZATION TO CHRONIC HYPERCAPNIA

Ambient P_{CO_2} (mmHg)	Species	Index of Acclimatization		Source
		Significant reduction in ventilatory response to CO_2	Maximal compensation of arterial pH	
11	Man	40 days	23 days (venous pH)	Schaefer, Hastings, Carey, & Nichols (22)
18–21	Man	3 days	—	Habisch (10)
21	Man	3 days	3 days	Schaefer (20)
21	Man	13 hr	—	Chapin, Otis, & Rahn (6)
107	Guinea pig	—	3 days	Schaefer, McCabe, & Withers (23)
50–121	Dog	—	3–5 days	Schwartz, Brackett, & Cohen (24)

change may therefore have occurred before that time. With the exception of the study by Chapin *et al.* (6), little attention has been given to the possibility that significant respiratory acclimatization may occur within the first 24 hr of exposure to hypercapnia.

Recent Experimentation

We conducted a series of experiments to study extensively the rate of respiratory acclimatization to chronic hypercapnia in normal man. Some of the data from three of these sets of experiments will be presented here. In the first experiment, four subjects were exposed to an ambient P_{CO_2} of 30 mmHg for 11 days; in the second experiment, four different subjects were exposed to the same ambient P_{CO_2} for 5 days; and the third experiment consisted of a 30-day exposure of four additional subjects to an ambient P_{CO_2} of 21 mmHg. Each experiment was designed to obtain repeated measurements of ventilation and to obtain acid–base indices of arterial blood and lumbar cerebrospinal fluid (CSF) during hypercapnia.

EXPOSURE TO AN AMBIENT P_{CO_2} OF 30 MMHG FOR 11 DAYS

Effects of the 11-day exposure to an ambient P_{CO_2} of 30 mmHg on ventilation, Pa_{CO_2}, and CSF P_{CO_2} are shown in Fig. 1. The exposure was preceded by a 5-day control period and followed by a 5-day recovery period. Average ventilation tripled at the start of CO_2 breathing and then decreased slightly after the first day, remaining essentially constant for the rest of the exposure. After an initial elevation of about 2 mmHg, the mean Pa_{CO_2} increased by an additional 3 mmHg during the first day of hypercapnia and was not changed after 5 and 9 days of exposure. Average valves of CSF P_{CO_2} measured after 1 and 10 days of exposure were elevated in both instances by about 6 mmHg. During the recovery period, ventilation rapidly returned to normal, and Pa_{CO_2} was normal when it was first measured after 3–4 days of recovery.

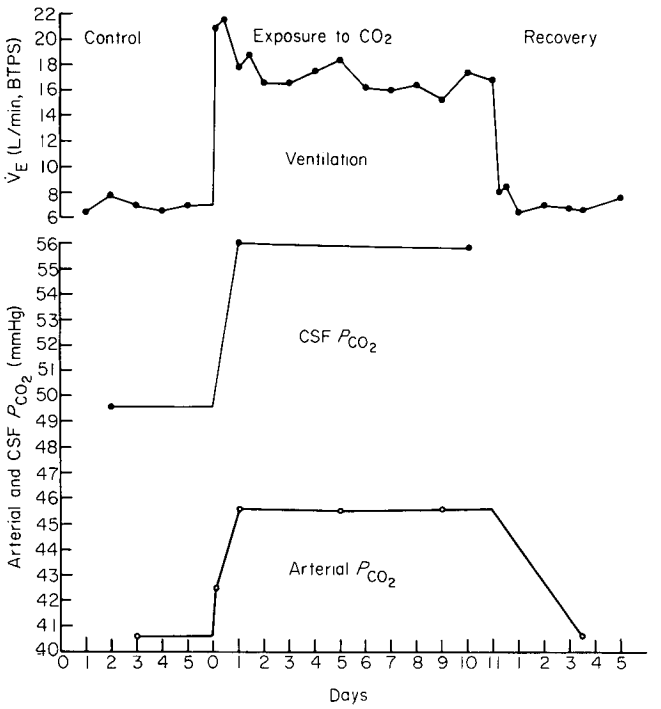

FIG. 1. Effect of an 11-day exposure to an ambient P_{CO_2} of 30 mmHg upon ventilation, Pa_{CO_2}, and CSF P_{CO_2} in man. The exposure period was preceded by a 5-day control period and was followed by a 5-day recovery period. Ambient O_2 concentration was maintained at about 19–21% throughout the experiment. Ambient P_{CO_2} was less than 2 mmHg during control and recovery periods. The data, which represent the average results of four subjects, were obtained from unpublished observations of S. J. Menn, R. D. Sinclair, J. M. Clark, and B. E. Welch.

EXPOSURE TO AN AMBIENT P_{CO_2} OF 30 MMHG FOR 5 DAYS

Because the results of the 11-day study indicated that respiratory acclimatization to hypercapnia was essentially complete after 1 day of exposure, a second experiment was designed to study more carefully the first 24 hr of hypercapnia and the first 24 hr of recovery (Fig. 2). This experiment involved a 5-day exposure to an ambient P_{CO_2} of 30 mmHg, which was preceded by a 3-day control period and followed by 2 days of recovery.

Complete data were obtained for three of the four subjects, and only these three were included in the average results. Ventilation and Pa_{CO_2} were measured simultaneously at 4-hour intervals during the first day of exposure and the first day of recovery. Average ventilation doubled at the start of CO_2 exposure and then continued to increase slightly during the first day of exposure. It decreased during the second day, and remained essentially constant for the remainder of the exposure.

Arterial P_{CO_2} increased immediately by almost 4 mmHg, reaching its maximum elevation after 20 hr of exposure. It then decreased during the next 4 hr to a level about 5 mmHg above the control value, and was unchanged after 2 and 5 days of exposure. The sustained Pa_{CO_2}

elevation of 5 mmHg agreed closely with the results of the previous 11-day exposure. Changes in average CSF P_{CO_2} appeared to parallel those in arterial blood; and CSF P_{CO_2} was only 0.5 mmHg less after 24 hr of hypercapnia than it was after 5 days of exposure. During the recovery period, ventilation returned promptly to normal. Arterial P_{CO_2} decreased initially to 2.5 mmHg above the control value, continued to decrease during the first 8 hr of recovery, increased after 16–20 hr of recovery, and finally returned to normal. After 2 days of recovery, CSF P_{CO_2} was about 1 mmHg above the control value.

Average pH and bicarbonate ($[HCO_3^-]$) data from the same experiment are summarized in Fig. 3. Arterial pH decreased immediately by 0.025 units, reached its lowest level after 20 hr of exposure, and then increased progressively until it returned to normal by the fifth day of exposure. Average CSF pH was lowest after 8 hr of exposure, and then increased by the end of exposure to a level about 0.01 units below the control value. Both arterial and CSF $[HCO_3^-]$ increased progressively in parallel fashion to reach their maximum elevations by the fifth day of exposure. During the recovery period, all parameters returned to normal with the exception of CSF pH, which was lower than the control value after 2 days of recovery; concomitantly, CSF P_{CO_2} was slightly elevated (Fig. 2).

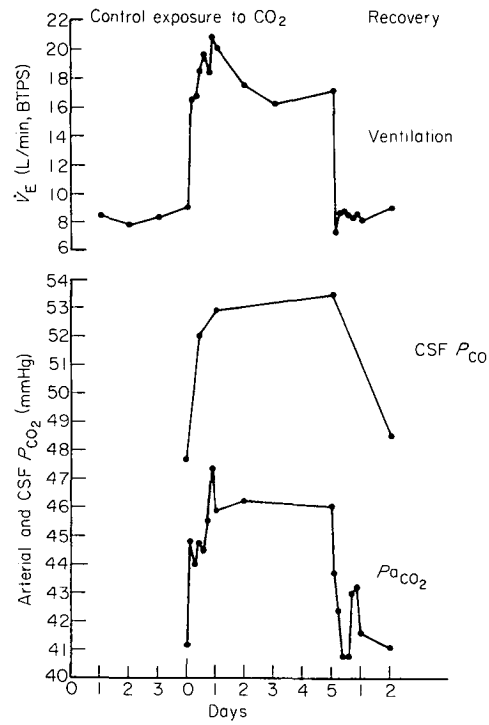

FIG. 2. Effect of a 5-day exposure to an ambient P_{CO_2} of 30 mmHg upon ventilation, Pa_{CO_2}, and CSF P_{CO_2} in man. Control data were obtained just before the start of the exposure period and on 3 previous days. The exposure period was followed by a 2-day recovery period. Ambient O_2 concentration was maintained at about 20–22% throughout the experiment. Ambient P_{CO_2} was less than 2 mmHg during control and recovery periods. The data represent the average results of three subjects.

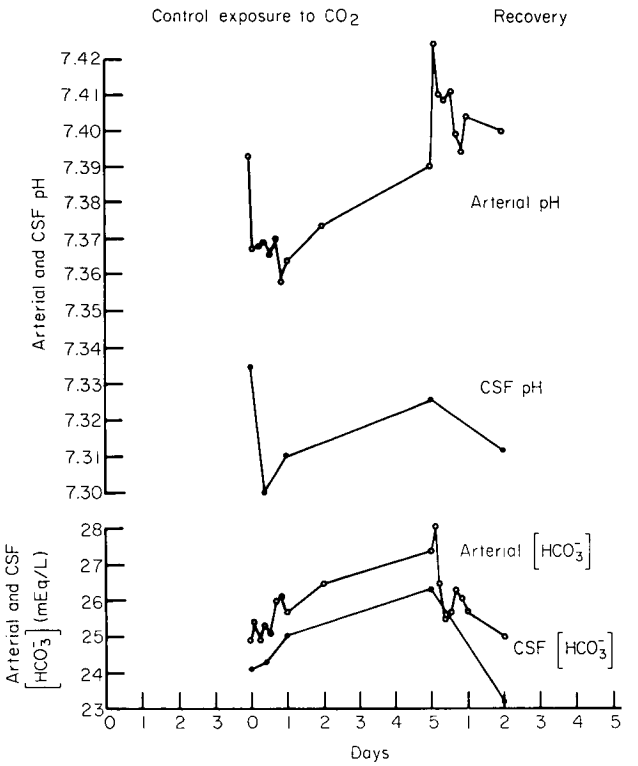

FIG. 3. Effect of a 5-day exposure to an ambient P_{CO_2} of 30 mmHg upon pH and $[HCO_3^-]$ of arterial blood and CSF in man. Data were obtained from the same experiment described in the legend for Fig. 2.

Data from the 5-day exposure showing the relationship of ventilation to arterial P_{CO_2} and pH are shown in Fig. 4. After 24 hr of exposure, the Pa_{CO_2}–ventilation response curve shifted to the right of the control curve. The curves obtained after 2 and 5 days of exposure indicate that there may have been a slight additional shift to the right along with the continued elevation of arterial and CSF $[HCO_3^-]$ (Fig. 3). After 24 hr of air breathing, the P_{CO_2}–ventilation response curve shifted back to the left to overlap the control curve and appeared to shift still further to the left after 2 days of recovery. Although the shifts in the Pa_{CO_2}–ventilation response curves appear to be small in magnitude, comparison of the ventilatory responses obtained at a constant Pa_{CO_2} shows that ventilation after 5 days of hypercapnia was about 10–12 L/min less than it was at the same Pa_{CO_2} during the exposure and recovery periods.

Schaefer (20) has reported a similar shift to the right of the PA_{CO_2}–ventilation response curve in normal men exposed to chronic hypercapnia, and Katsaros *et al.* (11) produced the same result by acute administration of $[HCO_3^-]$. The data of Katsaros *et al.* show that the shift to the right of the Pa_{CO_2}–ventilation response curve caused by acute elevation of arterial $[HCO_3^-]$ was accompanied by a shift of the arterial pH–ventilation response curve to the left toward higher pH levels. During prolonged hypercapnia, however, the initial shift of the Pa_{CO_2}–ventilation response curve to the right was accompanied by a similar shift of the pH–ventila-

tion response curve toward lower pH values. As arterial [HCO_3^-] increased, the pH–ventilation response curve moved in the direction of higher pH values to the left of the control curve. During the recovery period the pH–ventilation response curve moved to the right again, in conjunction with a decreasing arterial [HCO_3^-].

Exposure to an Ambient P_{CO_2} of 21 mmHg for 30 Days

Respiratory control data from the third experiment essentially confirm the findings in the two earlier experiments. Figure 5 shows the effects in four subjects of a 30-day exposure to an ambient P_{CO_2} of 21 mmHg on the relationship of ventilation to arterial P_{CO_2} and pH. After 24 hr of hypercapnia, the Pa_{CO_2}–ventilation respones curve shifted slightly to the right of two control curves obtained at the start of CO_2 exposure and 2 days previously. The corresponding arterial pH–ventilation response curve again shifted initially to the right toward lower pH levels. After four days of hypercapnia, arterial [HCO_3^-] reached maximum elevation, and the initial decrease in arterial pH was completely compensated. At this time, the Pa_{CO_2}–ventilation response curve was less than 1 mmHg to the right of the 24-hr exposure curve, whereas

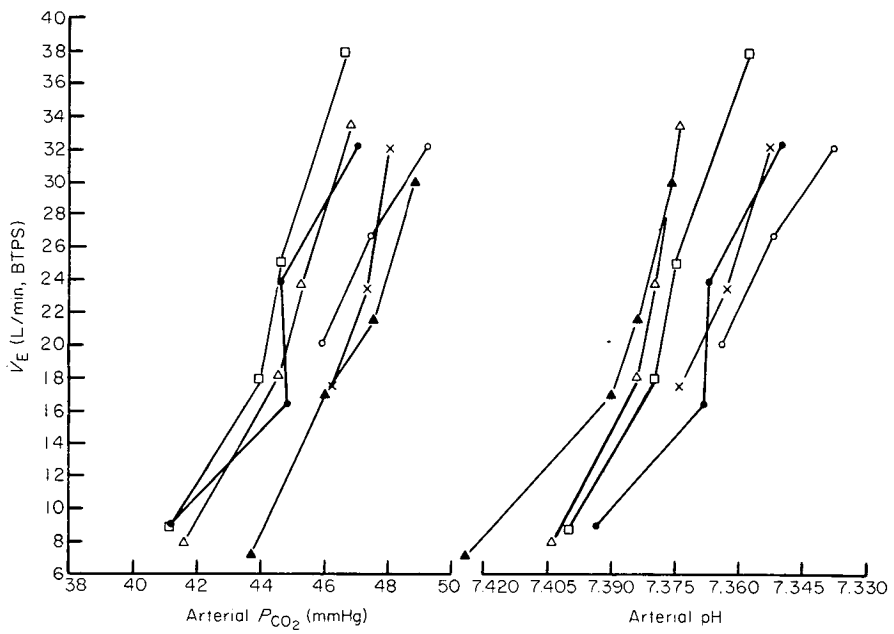

Fig. 4. Effect of a 5-day exposure to an ambient P_{CO_2} of 30 mmHg upon the relationship of ventilation to arterial P_{CO_2} and pH in man. Data were obtained from the same experiment described in the legend for Fig. 2. Measurements of ventilation, Pa_{CO_2}, and arterial pH were obtained at the times indicated in the figure. In the arterial blood of normal subjects during acute administration of CO_2, the relationship of change in P_{CO_2} to change in pH has a slope of −0.0075 pH units/1.0 mmHg P_{CO_2} (14). The abscissa was drawn to the same scale in order that a change on the P_{CO_2} axis would produce an identical change on the pH axis if arterial [HCO_3^-] remained constant. (●) Start exposure; (○) 1 day exposure; (×) 2 days exposure; (▲) 5 days exposure; (△) 1 day recovery; (□) 2 days recovery.

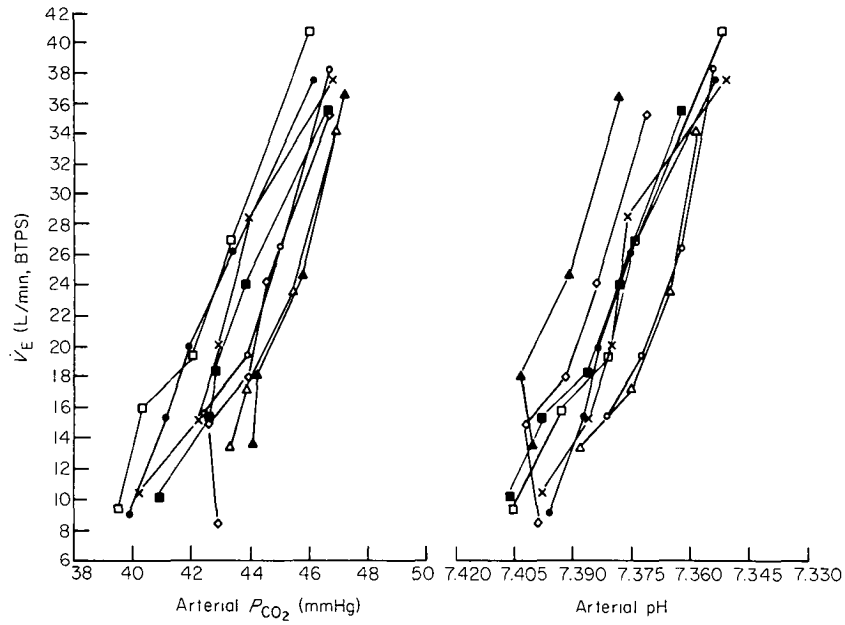

FIG. 5. Effect of a 30-day exposure to an ambient P_{CO_2} of 21 mmHg upon the relationship of ventilation to Pa_{CO_2} and pH in man. The 30-day exposure to CO_2 was preceded and followed by a 14-day period of air breathing. Measurements of ventilation, Pa_{CO_2}, and arterial pH were obtained at the times indicated in the figure. The data represent the average results of four subjects. A change in P_{CO_2} of 1 mmHg on the abscissa is equivalent to a pH change of 0.0075 units. (●) Control day 13; (×) start exposure; (○) 1 day exposure; (▲) 4 days exposure; (△) 14 days exposure; (◇) 30 days exposure; (■) 1 day recovery; (□) 4 days recovery.

the pH–ventilation response curve was shifted to the left by about 0.025 pH units. After 14 days of hypercapnia, the Pa_{CO_2}–ventilation response curve was still in the position of the 4-day curve, but the pH–ventilation response curve had shifted back to the right to become superimposed upon the 24-hr curve.

Following 30 days of hypercapnia, the Pa_{CO_2}–ventilation response curve was superimposed upon the 24-hr curve, whereas the pH–ventilation response curve was shifted to the left toward the 4-day curve. After 1 and 4 days of air breathing, the Pa_{CO_2} and pH–ventilation response curves shifted toward the control curves and became superimposed upon them. Comparing the ventilatory responses obtained at a constant Pa_{CO_2} shows that ventilation was reduced by about 8–10 L/min following acclimatization to hypercapnia.

Discussion

The data summarized in Figs. 4 and 5 indicate that the reduction of ventilatory response to CO_2 occurring during the first 24 hr of chronic hypercapnia cannot be attributed to a reduced stimulus level in respiratory chemoreceptors which are directly exposed to the $[H^+]$ of arterial blood. On the contrary, arterial pH measurements obtained after 24 hr of hypercapnia show that these chemoreceptors are exposed to $[H^+]$ at least equal to and probably

greater than the control level of acidity. Reduced ventilatory response to hypercapnia without a compensatory decrease in arterial [H^+] could be accounted for by a decreased stimulus level in the central chemoreceptors whose acid–base environment may be quickly influenced by changes in Pa_{CO_2}, but which are only slowly modified by alterations in arterial pH. The existence of such central chemoreceptor response has been demonstrated by Lambertsen *et al.* (16–18).

Comparison of the close agreement of the Pa_{CO_2}–ventilation response curves during CO_2 exposure with the much wider range of the arterial pH–ventilation response curves provides additional evidence that respiration during acclimatization to prolonged hypercapnia is predominantly controlled by central chemoreceptors which are not directly influenced by changes in arterial pH. The apparent lack of a change in the ventilatory response slope to increased Pa_{CO_2} or [H^+] does not support the concept that reactivity of the respiratory control centers is diminished during prolonged hypercapnia.

Elevations in Pa_{CO_2} were observed 16–20 hr after exposure to both an ambient P_{CO_2} of 30 mmHg and an ambient P_{CO_2} of 21 mmHg, and again after 16–20 hr of recovery in each instance. In all four cases, increased Pa_{CO_2} occurred when the subjects normally would have been sleeping. These data indicate that the decreased ventilatory response to CO_2 that occurs during sleep (4, 15) may also occur to some degree at the same time of day even when the subject is awake. Eger *et al.* (8) and Koepchen *et al.* (13) have also reported a diurnal variation in the ventilatory response to hypercapnia. Hormones—such as norepinephrine, epinephrine, ACTH, and cortisone—can all increase respiratory response to CO_2 administration (2, 7, 12, 13, 28). It is possible that diurnal variation in hormone blood levels (19, 27) may be partially responsible for the apparent diurnal variation in the ventilatory response to hypercapnia. The data summarized in Figs. 4 and 5 were obtained within a few hours after the subjects were awakened and should not therefore have been influenced by diurnal variation.

Conclusions

The results of the present studies of the rate of man's acclimatization to chronic hypercapnia agree with the findings of most previous investigators that maximal compensation for the initial decrease in arterial pH during exposure to hypercapnia requires as long as 3–5 days. However, the results of these experiments indicate that most of the reduction in ventilatory response to CO_2 occurs within the first 24 hr of exposure and, therefore, agree generally with the earlier observations of Chapin *et al.* (6). The rapid rate of respiratory acclimatization to chronic hypercapnia appears to be related to acclimatization of some central component of respiratory control that is not directly influenced by changes in arterial pH. Compensation for the initial decrease in arterial pH may cause a further reduction of ventilatory response to CO_2 that is smaller than the earlier reduction.

It should be emphasized that these preliminary conclusions are based upon data obtained from small numbers of subjects. There are obvious individual variations in both objective and subjective responses to CO_2 (21, 24), and the rate of acclimatization to hypercapnia may also vary among individuals. Furthermore, many of the changes observed in the present investigation are small in magnitude and difficult to measure by present-day techniques. More definitive information can be obtained by exposing larger numbers of subjects to higher levels of inspired P_{CO_2}, if such exposures are made safe by using suitable indices of CO_2 toxicity and if they can be subjectively tolerated.

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COMPARISON OF PHYSIOLOGICAL RESPONSES OF NORMAL MAN TO EXERCISE IN AIR AND IN ACUTE AND CHRONIC HYPERCAPNIA

R. D. Sinclair, J. M. Clark, and B. E. Welch

The results of studies on acute and chronic hypercapnia have shown that the healthy man at rest can tolerate an ambient P_{CO_2} of up to 30 mmHg without signs or severe symptoms of incapacitating physiological changes (4, 8, 13). However, this information does not completely answer the question of just what CO_2 exposures are tolerable for humans living in the artificial environment of habitats or vehicles used in underwater and space exploration. It must be assumed that individuals thus exposed will very likely be required to perform tasks calling for increased muscular work. Therefore, defining the interactions between the normal physiological responses to exercise and hypercapnia and the influence of these interactions on CO_2-tolerance limits in man is an important challenge in environmental research.

Exercise During Hypercapnia—The Problem

During acute hypercapnia man, even at rest, is in an environment unfavorable to metabolic CO_2 elimination (14), and he must therefore increase minute ventilation ($\dot{V}_E$) in response to elevated Pa_{CO_2}, and $[H^+]$. Thus, the basic physiological responses that adequately eliminate the CO_2 produced by the body during exercise in air are less effective when exercise is done in a high CO_2 atmosphere. Consequently, Pa_{CO_2} and $[H^+]$ increase progressively in proportion to elevations of inspired PI_{CO_2} (13). Although ventilation is not usually considered to be a limiting factor in man's capacity for doing heavy or maximal exercise in air (1, 20), exercise during hypercapnia places excessive demands upon ventilation that may in turn limit exercise capacity. Furthermore, the normally high incidence of premature ventricular contractions in man performing exercise in air (14) and cardiac arrhythmias while he breathes high concentrations of CO_2 (7–14% at ambient pressure) at rest (17) raise an important question: Is there a possible interaction of these combined stressors on rhythmicity of the heart?

Theoretically, during chronic exposure to CO_2 with its concomitant physiological adapta-

TABLE I

EXERCISE DURING EXPOSURE TO HYPERCAPNIA

Subjects	Ambient P_{CO_2} (mmHg)	Workload[a]	Duration (min)	Source
6	8	L	180	Krasnogor *et al.* (11)
7	21[b]	L	60	Glatte *et al.* (8)
4	21[b]	L–M–H	45	Sinclair *et al.* (this study)
8	8–30	M–H–Max	20–30	Menn *et al.* (14)
7 (dog)	15–60	M	15	Sinclair *et al.* (18)
4	16	Max	—	Finkelstein *et al.* (6)
2	21 (15–35)[b]	M	—	Schaefer (15)
32	7–35	L	10	Froeb (7)
11	35	H	2	Hickam *et al.* (9)
3	(7–45)	M–H	10	Asmussen and Nielsen (2)
3	(15–45)	M–H	12	Craig (5)

[a] Classified as low (L), moderate (M), heavy (H), and maximum (Max).
[b] Acute (hours) and chronic (days) exposure.

tions—e.g., increased body buffering of $[H^+]$, slightly abated $\dot{V}_E$ (3, 8), and readjustment of hormonal activity (16, 17, 21)—man might be somewhat better able to do exercise than he is during acute exposure to the same P_{CO_2}. This possibility has been the basis for studies of responses to exercise during prolonged hypercapnia.

Previous Studies Concerning Exercise During Hypercapnia

Table I summarizes a limited number of studies reported in the literature (2, 5–9, 11, 14, 15, 18) that provide data on exercise tolerance during exposure to CO_2, and shows the approximate degree of work performed, its duration, and the ambient P_{CO_2} during the exercise. The ambient P_{CO_2} values shown in parentheses indicate that CO_2-response curves were obtained during steady-state exercise; second footnote denotes the studies that were made during both acute and chronic exposure to CO_2 (21 mmHg in all cases). Man was the experimental subject in these studies; the single exception was a dog experiment, which was performed to extend the ambient P_{CO_2} to 60 mmHg—a P_{CO_2} level to which man has not been exposed during exercise. One can see from the data that heavy work has been done for relatively long periods in both 21 and 30 mmHg of ambient P_{CO_2}, and maximum exercise has been done in 16 and 21 mmHg. The table also shows that the highest ambient P_{CO_2} breathed by man during heavy exercise was 45 mmHg for 12 min.

The first five studies listed in the table were performed at the USAF School of Aerospace Medicine as a series of related experiments performed specifically to define the levels of CO_2 exposure that man can tolerate. These studies included measurements of ventilation, gas exchange, pH of the blood, and blood gases, in addition to cardiac monitoring.

The remaining studies listed in the table are from other laboratories and, with the exception of the work by Schaefer (15) and Finkelstein *et al.* (6), were not specifically designed to answer questions regarding tolerance levels for exposure to CO_2. Most of these studies concentrated on ventilation and gas exchange measurements, and omitted arterial acid–base and electrocardiographic data.

Graded Exercise in Acute and Chronic Exposure to Hypercapnia—21 mmHg P_{CO_2}

Methods

The present investigation involved four young male subjects in excellent physical condition, who performed three levels of work (low, moderate, and heavy). The work was done in air and during acute (1 hr) and chronic (15–20 days) exposure to an atmosphere containing 21 mmHg ambient P_{CO_2}. While in a supine position, the subjects performed 45 min of continuous steady state exercise on a bicycle ergometer. They breathed air or CO_2-enriched air directly from the environmental room using a modified Otis-McKerrow low-resistance valve. The expired air was directed to a 600-L Tissot spirometer. Two runs, 5 hr apart, were performed by the same subject on each experimental day in order to minimize the total number of arterial catheterizations. The data used to construct the curves in Figs. 1–4 represent measurements obtained

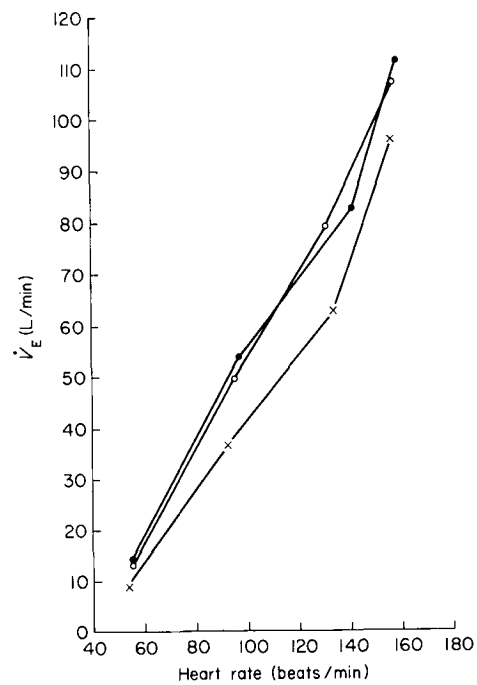

Fig. 1. Effect of graded exercise upon respiratory minute volume in air (×) and in acute (●) and chronic (○) exposure to hypercapnia (21 mmHg). $\dot{V}_E$ values are corrected to BTPS conditions.

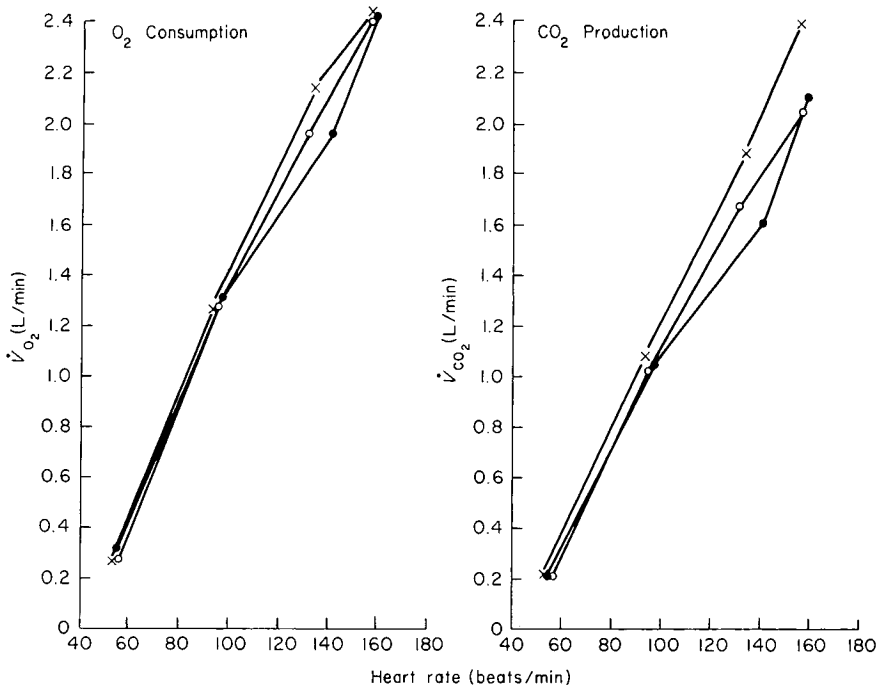

FIG. 2. Effect of graded exercise on respiratory gas exchange in air (×) and in acute (●) and chronic (○) hypercapnia. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ values were corrected to STPD conditions.

between the 12th and 15th minute of exercise. Analysis of variance was used to test the data, which are considered significant when $P < 0.05$.

RESULTS

In Figs. 1–4, the experimental data are plotted against the mean heart rate (HR) responses to exercise rather than against work loads. This method of plotting data is justified because of the highly significant linear correlation between exercise work load and cardiac rate (1, 20). The present study shows that this linear relationship also holds for exercise during acute and chronic CO_2 exposure. Each resting and exercise point on the graphs shown in Figs. 1–4 represents the mean of 12 and 4 individual measurements, respectively.

The effects of graded exercise on $\dot{V}_E$ both in air and CO_2-enriched air are illustrated in Fig. 1. The parallelism of the ventilation curves during exercise agrees with the results of work done by Asmussen and Nielsen (2) and Craig (5). These investigators found that the effect of CO_2 on hyperpnea during exercise, for a limited number of P_{CO_2} and work load combinations, approximates a simple addition of increments produced by the action of each stressor acting independently. However, the difference between $\dot{V}_E$ in air and in CO_2 at rest was approximately doubled at the low work load, and appeared to remain constant at higher work loads. The average $\dot{V}_E$ at similar work levels was essentially equal both in acute and chronic hyper-

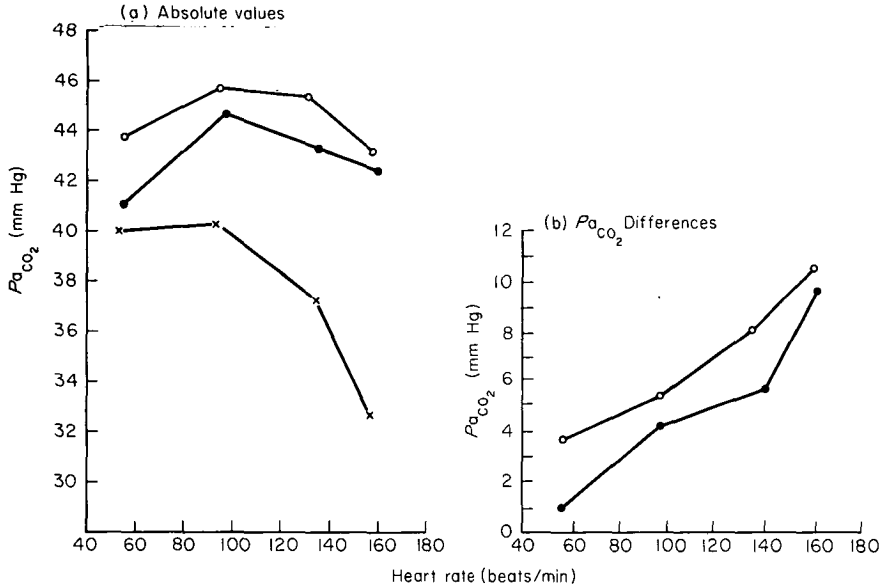

FIG. 3. Effect of graded exercise upon Pa_{CO_2} in air (×) and in acute (●) and chronic (○) hypercapnia. Blood gases were temperature corrected. Pa_{CO_2} *differences* refer to the difference between the absolute Pa_{CO_2} values in acute or chronic hypercapnia and the corresponding Pa_{CO_2} values during air breathing for rest and at each level of work.

capnia; the air curve was approximately 15 L/min lower at each work load. The difference among the three curves is considered highly significant ($P < 0.001$).

The changes in $\dot{V}_E$ with progressive exercise in each experimental condition resulted from changes in both respiratory rate and tidal volume, the former becoming relatively more important with increasing work load. Comparison of the respiratory patterns under similar work loads in both acute and chronic hypercapnia showed that tidal volume was higher and rate lower in chronic hypercapnia.

The finding that there was little difference between the average $\dot{V}_E$ at similar work loads in acute and chronic CO_2 conditions is in contrast to the finding of Schaefer (15). His data for one subject showed that the ventilatory response to exercise during acute hypercapnia was reduced when exercise was done after 8 days of exposure to 21 mmHg ambient P_{CO_2}. The individual data from the present study reveal that one of our four subjects showed a response similar to that described by Schaefer, but the ventilatory responses of the other three were not clearly different in acute and chronic hypercapnia.

The effects of graded exercise on gas exchange in air and in CO_2 are shown in Fig. 2. The oxygen uptake ($\dot{V}_{O_2}$) curves are essentially linear, and the mean values vary little from one another at the same work load in the different experimental conditions. There is no significant difference between the curves. Krasnogor *et al.* (11), Glatte *et al.* (8), and Menn *et al.* (14) likewise found no differences in $\dot{V}_{O_2}$ whether exercise was performed in air or CO_2-enriched air environments. In contrast, Finkelstein *et al.* (6) reported a 12% decrease in maximum $\dot{V}_{O_2}$ with exercise in an environment containing 16 mmHg inspired P_{CO_2} (PI_{CO_2}). However, they did not indicate whether the degree of exercise performed in CO_2 and in air was the same.

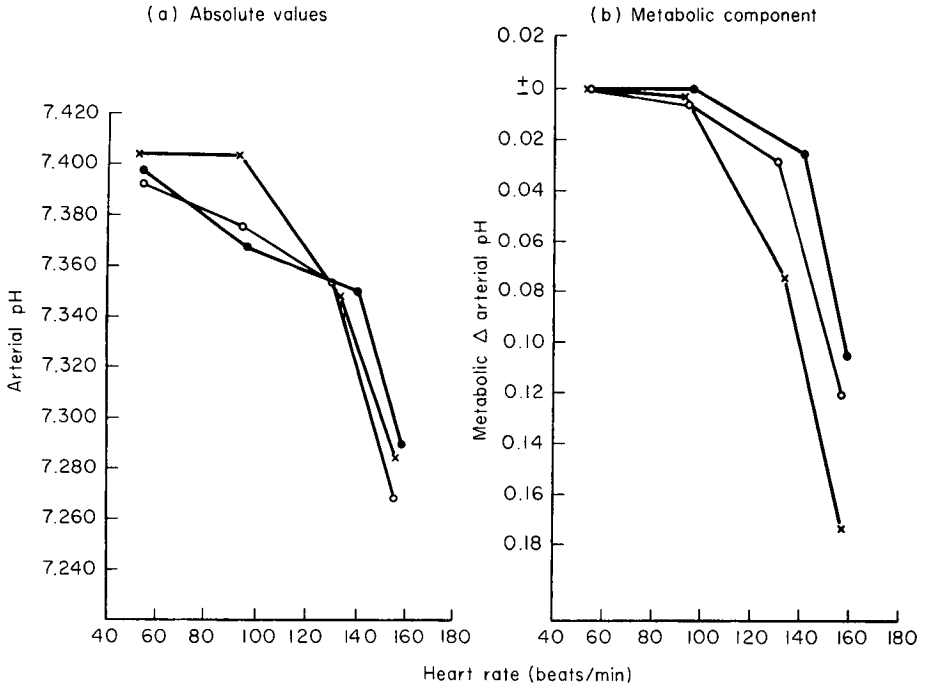

FIG. 4. Effect of graded exercise upon arterial pH in air (×) and in acute (●) and chronic (○) hypercapnia. The pH values were temperature corrected. The *metabolic component* was calculated by subtracting the respiratory component from the total change in pH. The relationship between change in P_{CO_2} and pH has a slope of −0.0075 pH units/1.0 mmHg increase in Pa_{CO_2} (12).

The mean values for $\dot{V}_{O_2}$ (L/min) and HR (beats/min) for the three experimental conditions at low, moderate, and heavy work loads were 1.281/95, 2.018/135, and 2.411/158, respectively. The average resting HR was 55 ± 6 beats/min, which reflects the excellent physical condition of the subjects and the absence of excitement prior to exercise.

Increases in CO_2 production ($\dot{V}_{CO_2}$) were also progressive as the work load under all three experimental conditions increased. The curves are significantly different ($P < 0.025$), with the $\dot{V}_{CO_2}$ curves for acute and chronic CO_2 exposure falling below the air curve. This finding agrees with those of Menn *et al.* (14) and Finkelstein *et al.* (6), who observed a CO_2 retention (decreased $\dot{V}_{CO_2}$) during exercise in hypercapnia that could not be explained by a decrease in metabolism.

Figure 3 shows a comparison of changes in Pa_{CO_2} during exercise in air and in acute and chronic hypercapnia. During air breathing, Pa_{CO_2} values showed essentially no change from the state of rest to the low work load, and then proceeded to decrease progressively with exercise to reach a low value of 32.5 mmHg at the highest work load (7.5 mmHg lower than the resting control value). During exercise at the low work load in acute and chronic hypercapnia, Pa_{CO_2} increased by 3.6 and 2.0 mmHg, respectively, and then decreased progressively to values at the highest work load that were near the mean resting control in both experimental conditions. With all work loads, the changes in Pa_{CO_2} from the resting value were smaller in prolonged than in acute hypercapnia.

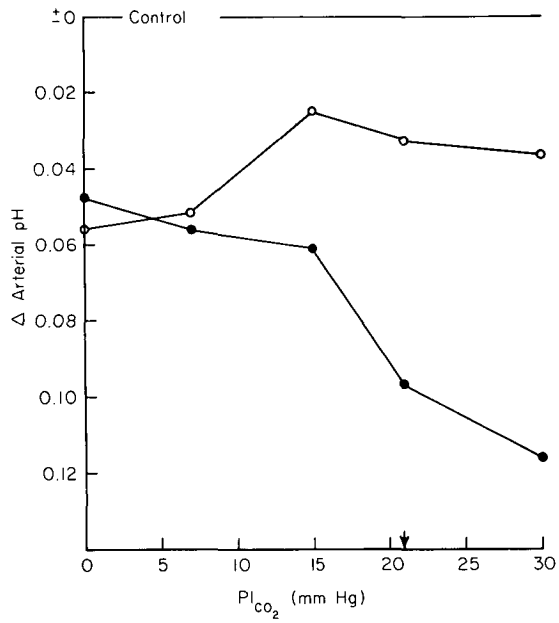

FIG. 5. Contribution of metabolic acidosis to changes in arterial pH with exercise in progressive hypercapnia (after Menn *et al.* [14]). Subjects exercised on a bicycle ergometer in upright position for 30-min periods in air and in four levels of ambient P_{CO_2} on different days. The metabolic acidosis component was calculated as described in legend for Fig. 4. The nearly identical heavy work loads in the Menn *et al.* and the present studies were performed during the same acute exposure to 21 mmHg P_{CO_2} (note arrow on abscissa). (○) Metabolic acidosis; (●) respiratory acidosis. PI_{CO_2} = 0, 8, 15, 21, 30 mmHg.

Although the Pa_{CO_2} changes were small during exercise in hypercapnia, the differences between the mean Pa_{CO_2} during acute or chronic CO_2 exposure and the corresponding Pa_{CO_2} values during air breathing increased progressively with higher work loads (Fig. 3). The differences in Pa_{CO_2} curves were highly significant ($P < 0.001$).

The changes in arterial pH during exercise in hypercapnia are shown in Fig. 4. The decreases in pH below resting control values were also progressive with increasing work loads, and were similar in magnitude at each work load under the three experimental conditions, despite the concomitant changes in Pa_{CO_2} shown in Fig. 3. The metabolic acidosis component of the pH changes was greatest during air breathing, smallest in acute hypercapnia, and intermediate in chronic hypercapnia—thus explaining the similarity in the pH values under the heavy work load for all three experimental conditions, despite quite dissimilar Pa_{CO_2} values.

Figure 5 is based on data drom the study of Menn *et al.* (14), and illustrates the contribution of metabolic acidosis to changes in arterial pH at the end of 30-min periods of heavy exercise in graded levels of hypercapnia. Each point on the graph represents the mean of seven individual measurements. The curves show that the metabolic component of the observed change in pH was considerably less with exercise in the higher levels of PI_{CO_2} than it was with exercise in air. In the Menn *et al.* study, the metabolic acidosis resulting from exercise performed during acute exposure to 21 mmHg P_{CO_2} was about 58% of that resulting from exercise in air. At a

comparably high level of exercise in the present study, the metabolic component of the change in arterial pH during acute hypercapnia was about 60% of that found during air breathing.

The only symptoms reported by our subjects during exercise in hypercapnia were occasional mild headaches and awareness of increased respiratory effort when the work load was heavy. The symptoms of air hunger and intercostal muscle pain reported by Menn *et al.* (14) in their subjects, during exercise at a heavy work load in 30 mmHg PI_{CO_2}, and at maximum exercise in 21 mmHg PI_{CO_2}, were not experienced by our subjects. Neither were the occasional premature ventricular contractions reported by Menn *et al.* (14) observed in our subjects.

Discussion and Conclusions

Analysis of the quantitative data in Figs. 1–4 indicates that some of the normal responses to graded exercise in air were significantly modified by the simultaneously imposed stress of exposure to an ambient P_{CO_2} of 21 mmHg. Of particular note was the retention of CO_2 as work increased, as indicated by a significant decrease in $\dot{V}_{CO_2}$ and increase in Pa_{CO_2}. These observations agree with the results of Menn *et al.* (14), which demonstrated progressive CO_2 retention both with submaximal and maximal exercise in increasing levels of hypercapnia. Finkelstein *et al.* (6) also reported decreased $\dot{V}_{CO_2}$ with maximum exercise during acute exposure to 16 mmHg PI_{CO_2}. Retention of CO_2 in exercise during exposure to hypercapnia indicates that alveolar ventilation does not increase sufficiently to compensate for its reduced effectiveness in CO_2 elimination.

The finding of reduced metabolic acidosis in response to heavy exercise in CO_2, as compared to that in air, may reflect decreased production of organic acids. This could be caused by a decrease in the requirement for energy obtained from anaerobic metabolism or by changes in enzyme activity. The former could be explained by improved perfusion of blood through the skeletal muscles during hypercapnia, which permits more rapid adjustment of the circulation to the demand for O_2 in the early exercise period. In support of an enzymic effect, Hughes *et al.* (10) showed that, during light exercise and voluntary hyperventilation in air (which lowered the Pa_{CO_2} to 27 mmHg), the blood lactate rose by 1.3 mmoles/L. However, no increase in lactate occurred if the Pa_{CO_2} was kept constant by the addition of CO_2 to the inspired air. Takeshita (19) likewise found that the rise in blood lactate owing to hypoxia at rest— which is in part the result of alkalemia due to hyperventilation—could be prevented by adding CO_2 to the inspired gas to keep the blood P_{CO_2} constant.

The present study demonstrates that an ambient atmosphere containing a CO_2 tension of 21 mmHg is well tolerated by a subject who is resting or engaging in strenuous steady state exercise. This general conclusion is applicable whether the CO_2 exposure is acute or chronic. In agreement with the previous reports, however, this study shows that exercise during exposure to increased ambient P_{CO_2} caused CO_2 retention and a concomitant elevation of Pa_{CO_2}. Similar interactions between the stresses of exercise and hypercapnia will become limiting at higher levels of inspired P_{CO_2}. Determination of these tolerance limits will require quantitative studies of larger groups of subjects performing graded exercise during exposure to progressively greater pressures of ambient CO_2.

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PART IX. CARBON DIOXIDE, EXERCISE, AND ACCLIMATIZATION TO HYPERCARBIA*

DISCUSSION

K. E. Schaefer, Chairman

Chairman Schaefer: Professor Chouteau appeared this morning to have reversed the usual procedure for pressure exposure. Usually we have stops during the decompression phase. He used stops during the compression phase and had a rather rapid linear decompression coming back from high pressure. Perhaps he can explain this.

Dr. Chouteau: As for the compression phase, our procedure was essentially intuitive. In the experiments when we compressed animals slowly but continuously, the animals had difficulty adapting. We have never had difficulty during the compression when it was interrupted. This may permit the animals to accomplish respiratory adaptation, perhaps by avoiding air trapping.

Chairman Schaefer: With the more rapid compression rate you probably have the condition of air trapping. Your experience would indicate that in order to keep animals alive at 3000 ft it is necessary to use a much slower compression rate than in previous animal experiments.

Dr. Chouteau, you consider that your goats developed an hypoxic condition when the inspired P_{O_2} was kept at normal atmospheric levels. Could you comment on whether CO_2 retention occurred?

Dr. Chouteau: I do not think we had CO_2 retention. Why? If we had, I do not believe that the slight rise of P_{O_2} from 0.05 atm would be sufficient to reverse CO_2 retention.

Dr. Summitt: Dr. Chouteau, you have referred to experiments in which you measured the arterial O_2 levels and some other experiments involving continuous electroencephalographic recordings. Could you tell us of these?

Dr. Chouteau: We have carried out experiments like those on goats with O_2–He, but in an equivalent density O_2–N_2 environment at lower pressure. We have also performed experiments with rabbits fitted with chronic electroencephalographic electrodes, and we have studied the effects of normoxic mixtures (O_2–Ar, O_2–N_2, O_2–He) at different pressures for each of the three inert gases. In these we have seen modifications of the EEG. In the first time it is replacement of α waves by θ waves, and then flatness of the tracings. When we raise P_{O_2} the tracings become normal.

In the second type of experiments we have put O_2 electrodes in about 20 rabbits. At 20 atm, after 10 min we see a fall of the arterial P_{O_2} of about 25 mmHg. When we work at 15 atm, we find nothing. We have also experimented with Ar and find the same phenomenon, but at lower pressures, between 10 and 15 atm.

Dr. Lahiri: With respect to the goats, these are ruminants and the amount of gases, particularly CO_2, produced in the rumen is considerable. In certain circumstances, like very high pressure, that could be important. I wonder what is the contribution of CO_2 from the rumen to the CO_2 you see in the animal's blood under these conditions.

Dr. Chouteau: I have no measurements of this. I know the ruminants have a high tolerance to CO_2. They have a high alkaline reserve. I do not see what influence this could have on resistance to pressure.

Dr. Glauser: I wish to offer a warning about SF_6, which does have narcotic effects and does make one intoxicated. The chemical producers of SF_6 do not know that it is being used in human beings. Any experimenter that uses it is responsible for the safety of the batch that he uses. This is just a general warning. The gas is not being produced for biological use.

* *Panelists:* D. C. Parker, J. M. Clark, J. Chouteau, R. D. Sinclair.

Dr. Parker: In addition to that, the commercially available gas has certain acid products in the cylinder and has to be filtered through an absorbent before it can be used, even in animals. It has been shown to produce pulmonary edema in animals if this is not done.

Dr. Glauser: There is sulfur tetrafluoride and the dimer, sulfur pentafluoride (S_2F_{10}), present in a great many of the batches, and that is why the investigator must take the responsibility for the safety.

Dr. Lever: In fact the toxic impurities present in supplies of gaseous fluoride compounds available commercially do differ enormously from sample to sample. Since we have been using fluoride compounds in Oxford, we have carried out detailed toxicity tests on the gases, and in long-term toxicity tests we have found no toxic effects whatsoever. Other investigators have found marked toxic effects to be produced by both these gases, but I would attribute these findings to toxic impurities that are present.

Dr. Parker: Some people who have breathed SF_6 seem to have a residual effect for several hours after it has been breathed. Our group has not noticed this. This may be due to the toxic impurities. We have analyzed our batch on a gas chromatograph and it seems to be very pure.

Dr. Glauser: The subjective reaction produced by the same batch of gas is extremely variable from person to person. Among our subjects, including myself, we did not experience any residual effect. On the other hand, the subjective response in terms of euphoria and depression was widely variable among the individuals.

Chairman Schaefer: This discussion indicates that if so many toxic effects exist, the fluorides are not the ideal gases for study of separate CO_2 effects and effects of CO_2 on inert gas narcosis.

We can now discuss the two studies on CO_2 acclimatization. The inclusion of the effect on central respiratory drives is a very important contribution in this area.

Dr. Kollias: Has anyone done maximal exercise studies during compression? If not, why? What are the reasons for not doing these? With the chronic hypercarbic experiments the subjects also worked submaximally. Could you state why you did not work them maximally? Were there any overt symptoms during the exercise that led you to terminate it at the low work levels?

Dr. Sinclair: There have been two experiments with exercise performed at the maximum level in hypercapnia. The first one was done by Finkelstein. He exercised several subjects in 16 mmHg to their maximum level. The second study was done by Menn *et al.* who exercised subjects maximally in air, and at 8 and 21 mmHg inspired P_{CO_2}.

With the Menn study the subjects performing maximum exercise in 21 mmHg P_{CO_2} performed quite well. They did report symptoms of intercostal muscle pain and more subjective distress from the ventilatory effort. Their maximal minute ventilation was the same in air, in 8 and in 21 mmHg P_{CO_2}. In other words, they did not increase their minute ventilation above the air value.

We did see a progressive decrease in CO_2 production with maximal exercise in both the 16 and the 21 mmHg P_{CO_2} experiments. We did not do blood gas studies in either one of these two experiments, so I cannot comment on that.

Dr. Lahiri: Dr. Clark showed that at the end of five days of exposure to CO_2 the CSF and also the arterial pH almost came back to normal. But the ventilation remained very high, more than double normal.

Dr. Clark: You are referring to the observations showing that at the end of 5 days of exposure arterial pH was essentially identical to the control measurement, CSF pH was about 0.01 pH units below normal and ventilation still remained high.

The measurements of arterial pH and CSF pH do not necessarily tell us a great deal about what is going on in cells in the CNS. Spinal fluid may partly reflect the acid–base environment of any cells that are immediately exposed to it, but it does not necessarily reflect the environment of cells deeper beneath the surface. Our data cannot explain what is going on in these deeper cells. Since we cannot find the basis for increased ventilation in arterial blood and we cannot find it in CSF, then the cause must be somewhere in these other cells that are deeper, and not responding directly to changes in either CSF or arterial blood.

Chairman Schaefer: Did you measure bicarbonate in the CSF, too?

Dr. Clark: Indirectly. We calculated the bicarbonate measurement from P_{CO_2} and pH.

Chairman Schaefer: What ideas do you have about the shift in CSF bicarbonate influencing the ventilatory response?

Dr. Clark: We got essentially parallel increases in the bicarbonate levels of arterial blood and CSF, and neither one of these was large enough to explain the drop in ventilation we found. Therefore I think that these indices provide rather poor bases for the magnitude of reduction that we got within 24 hr. Over the course of

5 days the bicarbonate increased enough for both arterial and CSF pH to come essentially back to normal, and this may have had some additional influence upon respiration. However, you will have to consider that the initial changes are somewhere in the CNS where they are is not accurately reflected by arterial acid–base changes or CSF acid–base changes.

Chairman Schaefer: You did not do any measurements at 3 days? Usually after 3 days exposure to CO_2 there is already a very strong tendency to return to normal, even at higher concentration in animals. As I understand your curve, you have 24 hr and then 5 days.

Dr. Clark: We did not make measurements at 3 days. We designed that experiment to look mainly at the first 24 hr of exposure because of the results of the previous experiment that seemed to indicate that nearly all changes were essentially complete by 24 hr. The technical work involved in doing measurements every day prohibited making daily measurements.

The fact that pH came back to normal by 5 days does not mean it took 5 days. It may have happened in 3 days. We don't know.

Dr. Sinclair: Regarding the comment concerning physical condition of the subjects, sometimes we do use this term loosely and do not define it. All the subjects in our exercise experiments at Brooks come right from Air Force basic training at Lackland Air Force Base. That training is a pretty good physical conditioning program in itself. When we receive these subjects they are given a thorough physical examination and then we keep them for about 6 weeks to 2 months on an extensive exercise program. In other words, if the high workload in our experiments was 190 or 200 W on a bicycle, we build up to that with the subjects. Then they do this at least twice a day for 45 min as an additional conditioning exercise.

We do not have the subjects running around the base, and so on, to increase their exercise capacity. But we do perform maximum O_2 uptake studies on the subjects prior to the start of the experiment. In the subject I reported on today the mean max $\dot{V}_{O_2}$ was about 3.7 L/min, which is good.

During the chronic exposures we do not let the subjects have days off. They continue to exercise on their days off at the maximum workload for the same duration of time that they perform the work in the actual experiment.

Dr. Clark: As one further comment, I have been impressed by the measurements which show that during exercise in exposures to extreme ambient pressures there seems to be a very prominent elevation of arterial P_{CO_2}. This may happen at rest as depth is increased. The data that Dr. Sinclair and I have shown with reference to the interactions of hypercapnia and exercise and hypercapnia and acclimatization will also apply to individuals at extreme ambient pressures even though they are breathing atmospheres not containing CO_2.

Chairman Schaefer: Have you any suggestions about the cause of the CO_2 retention during heavy exercise? Is that an altered elimination of CO_2 or a reduction of CO_2 production?

Dr. Sinclair: We do not feel that it is a result of decreased production of CO_2. Our evidence for this is the constancy of the O_2 uptake found in these studies. We consider that there is decreased elimination of CO_2 at the lung. There is a possible mechanism for this. If we consider that the normal alveolar inspired partial pressure gradient for CO_2 is 40 mmHg, with the ventilatory response that we get during exercise while breathing CO_2, this reduces that normal gradient.

The pressure gradient reflects a concentration gradient then between the pulmonary capillary blood in the alveolar CO_2, which means that the CO_2 coming from the pulmonary blood into the alveoli equilibrates sooner than it would with the normal 40 mmHg gradient. I am saying that CO_2 diffuses just as quickly, but it equilibrates with the alveolar CO_2 gas and, therefore, as the cycle continues, more and more CO_2 starts backing up.

Chairman Schaefer: In other words, you find the same thing in exercise as in a resting condition. I remember Shaw and Behnke's experiment in 1936 that showed that, with increase in CO_2 concentration during short exposure, the CO_2 elimination went down and the respiratory quotient dropped accordingly.

Related to the alveolar–arterial pressure gradient, do you have any evidence as far as the CO_2 production is concerned?

Dr. Sinclair: Only that the O_2 uptake level remained unchanged. We take this as evidence for no change in metabolism and an unchanged CO_2 production.

Part X. TEMPERATURE BALANCE IN SHALLOW AND DEEP EXPOSURES

HEAT EXCHANGE BETWEEN MAN AND THE WATER ENVIRONMENT

Albert B. Craig, Jr.

It is apparent that, for a given temperature, one loses or gains more heat when immersed in water than in air—or, said another way, cool water is "colder" than cool air, and conversely, warm water is "warmer" than warm air.

Despite the problems related to heat loss, man continues in his efforts to live and work in the hostile underwater environment. First, I should like to review some observations indicating that adaptations are possible for those who work in cold water. Second, I wish to discuss several problems concerning physiological responses to the cold stimulus and to point out their implications with respect to controlling the body temperature of the working diver.

Man has been working in water for a long time. The Ama of Japan and Korea have been gathering food from the underwater forests and fields for at least 2000 years (12). Figure 1 is a photograph of a Funado, or assisted diver. She is almost completely clothed, and in fact is wearing at least three layers of knitted garments. Just before diving she put on gloves and socks, and covered her head. She worked in 21.5° C water for about an hour during the morning diving shift. Figure 2 shows another diver about to leave the boat. She is a Cachido, who works alone in shallower water. In addition to several layers of clothing, she has carefully covered her head and neck, a procedure followed in an almost ritualistic manner. She worked in 25°C water for about 2 hr in the morning, followed by a similar work shift in the afternoon.

Between the morning and afternoon diving shifts, the Ama not only eats but rewarms herself. The Funado shown in Fig. 1 utilized the heat from a charcoal fire, an integral part of the boat. The stove is also used to warm tea, which serves as an additional source of heat. The Cachido's noon break is more sociable. Figure 3 shows a group of them gathered around a fire on the beach, where they have the added advantage of absorbing heat from the sand, which was very warm and which also reflected heat.

Korean Ama, whose diving activities are very similar to those of the Japanese Ama, have been extensively studied by Hong and his co-workers (10). In Korea some women work all year, and diving time is limited by tolerable body heat loss. During January, when the water temperature is 10°C, there is one shift a day, averaging 16 min. In August, when the water temperature is 27°C, there are two shifts a day lasting about 70 min each. Despite the different diving times, the rectal temperature at the end of a work period is about the same, i.e., 35°C.

FIG. 1. A Funado (assisted diver) dressed for diving, except for socks, gloves, and head covering.

Apparently, the major limiting factor in the Ama's ability to earn a living is the heat loss that she can tolerate.

Studies of the Ama have also revealed the interesting and dramatic adaptations she has made to cold stress. In the winter months, her basal metabolic rate (BMR) is 30% greater than that of nondiving women living in the same villages and eating the same type of diet. In the summer, the BMR's of these two groups do not differ from one another (10). Rennie *et al.* (14) have also shown that the diving women, who are unusually lean, tolerate without shivering much cooler water temperatures than nondivers of comparable fat thickness do. These results are reminiscent of the Australian aborigine whose core temperature decreases while he is sleeping at night yet in whom shivering is not evident (16).

Another group of people who have successfully performed in cold water are the adventuresome and perhaps masochistic swimmers who swim across the English Channel (13). They remain in 16–18°C water for 12–20 hr. In comparison with the Ama, the Channel swimmers have a greater layer of insulation in the form of subcutaneous fat. In addition, heat production resulting from their swimming is considerably more than that possible in breath-holding

FIG. 2. A Cachido (unassisted diver) about to enter the water for diving.

divers (8). Perhaps the long-distance swimmer is more closely related to the compressed-gas diver, who can work vigorously but who nevertheless gets cold. It has been reported that rectal temperatures of scuba divers working in 2°C water for about an hour decreases to 34°C, but we have little data on others diving in warmer water (9).

One study from Norway (17) indicates that, like the Ama, scuba divers show some acclimitization to cold stress. The subjects of that study maintained more normal rectal temperatures and showed a smaller increase in metabolic rate after working in 2–4°C water for 45 days than they did at the beginning of the test series.

Turning now from observations of working divers to planned experimental work, I should like to present several problems concerning physiological responses to cold stimulus. Qualitatively, immersion in water results in the same strains as those seen when the subject is in cold air. However, the responses are much more rapid and the course of events is greatly shortened. Vasoconstriction and decreased blood flow to the extremities occur quickly. Within 5 min of immersion, finger temperature decreases to a value that does not change during the remainder of the hour (6). In fact, the initial vasomotor responses are so rapid that there is an initial increase of the core temperature (3, 6, 11). The heat produced during this period is actually trapped in the central portion of the body.

FIG. 3. A group of Cachidoes during the noon break between the morning and afternoon diving shifts.

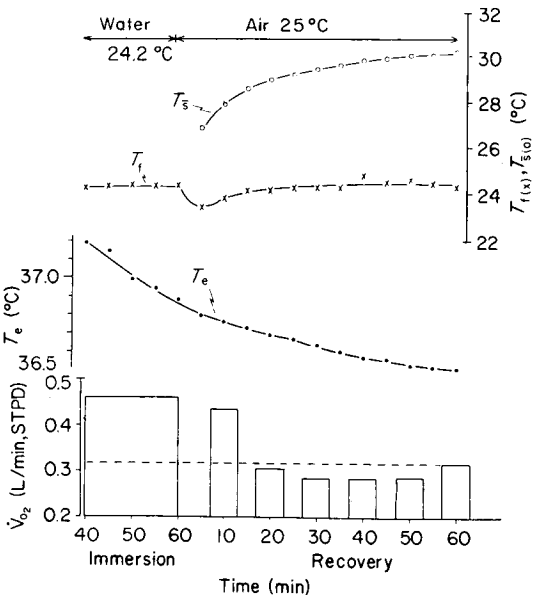

FIG. 4. Oxygen consumption and temperatures at the end of an hour of immersion in 24°C water and during recovery in 25°C air. Averages of five subjects. $T_{\bar{s}}$, mean skin temperature; T_f, finger temperature; T_e, middle ear temperature.

After the initial temperature increase there is a decrease in core temperature, the magnitude of which is related to water temperature. Shivering soon ensues, first in waves and then continuously and uncontrollably. The increased O_2 consumption reflects the magnitude of shivering.

The mechanism of shivering is of considerable importance to the diver. Benzinger (2) has suggested that metabolic response is dependent upon a decrease in the temperature of the hypothalamic thermoregulatory center, but that shivering does not occur unless there is also a cold stimulus from the periphery. A number of observations support this hypothesis. Subjects do not shiver immediately upon entering cold water. It takes time for the core temperature to decrease below the so-called "set point," and it is not surprising to find that shivering will start sooner in 24°C water than in 26 or 28°C water (6).

Another demonstration of this hypothesis is seen when the subject is removed from the water. Shivering stops when the skin is dried and begins to rewarm, a process which can be expected to reduce the peripheral stimulus. The average results of a series of cold-water exposures involving five subjects are shown in Fig. 4. Observations made during the last 20 min of a 1-hr immersion in 24°C water are shown on the left, and during the recovery phase, on the right. During immersion the subjects were shivering, and O_2 consumption increased above the control value (indicated by the dashed line). Oxygen consumption during the recovery period increased above control during the first measurement, after which it returned to control values. The subjects ceased shivering despite the continuously decreasing ear temperature. The mean skin temperature rose steadily during recovery. It is also of interest that finger temperature showed no significant increase suggesting that vasoconstriction in this area was still maximal even after an hour. Shivering evidently requires two conditions—a low central temperature and a peripheral stimulus. Removing either will relieve the shivering.

These observations are directly related to problems of deep diving and underwater habitat living. Consider a diver who works until the cold forces him to return to the shelter. He obviously will not be ready to dive again until he has rewarmed himself. If he were forced to

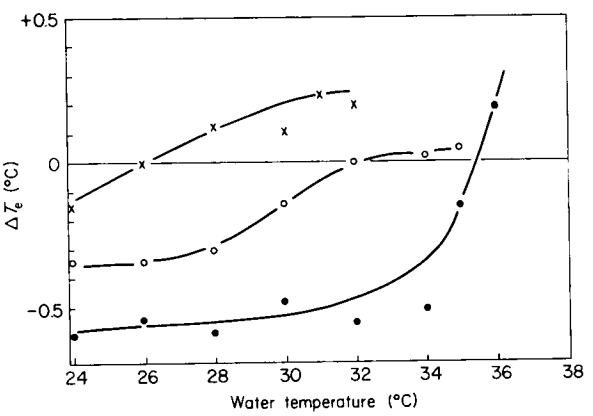

FIG. 5. Effect of exercise on middle ear temperature when subjects are immersed in water of different temperatures for 1 hour. Averages of 10 subjects. (●) Subjects resting; (○) subjects doing continuous work, doubling O_2 consumption; (×) subjects doing continuous work, tripling production. [From Craig and Dvorak (7), with permission of the publisher.]

return to the water before his core temperature has increased above the "set point", the peripheral stimulus will trigger shivering immediately. The Ama discovered this phenomenon without the assistance of physiologists. But what do we know about the modern diver? How long does it take him to recover from hypothermia? Could his effectiveness as a working diver be increased by providing an extra source of heat during rewarming?

There are many other questions one could ask about heat exchanges in this foreign, underwater environment. I should like to discuss two other experiments that are of interest to the working diver and to those concerned with protecting him against the cold.

Figure 5 illustrates the effect of two levels of exercise on core temperature during an hour-long immersion. As the abscissa of the graph indicates, these experiments were repeated in water of different temperatures (7). The solid dots on the graph show the change in the subjects' middle ear temperature while the subjects were at rest. The water temperature must be about 35°C to prevent a change. The open circles represent temperature changes occurring while the subjects exercised continuously at a load that doubled their O_2 consumption. The crosses depict another series of temperature changes occurring while the same subjects worked harder, thereby tripling their heat production. With the low work load, water of 32°C was adequate to prevent a temperature change, and with the higher work load they could maintain thermal balance in water of 26°C. One can look at this graph another way: If the water is 30°C, a person might feel cool at rest yet feel too warm doing even light work.

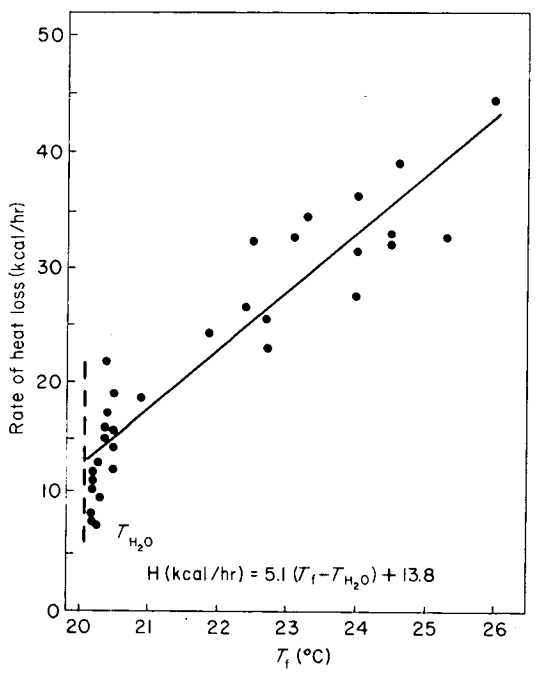

FIG. 6. Rate of heat loss from the hand and forearm immersed in a calorimeter filled with 20°C water as a function of finger temperature.

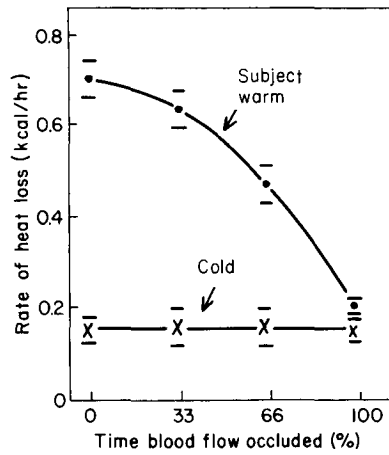

FIG.7. Rate of heat loss from the hand and forearm when a blood pressure cuff is inflated to 200 mmHg every 10 sec for a proportion of the time indicated. One subject studied in a series of six experiments in a cold room, and in another series when he was warm.

Most divers know that they might be quite comfortable under conditions of light work, but that when they are required to work harder, they suddenly feel unpleasantly warm. One method of cooling is to open the wet suit and let in some cold water. In effect, the diver is substituting manual control for automatic control. In water, one is dealing with a very narrow range of temperatures (5), and the microatmosphere of the protective wet suit is a poor buffer against body temperature change.

However, losing the automatic control system provided by sweating might not be so bad. Recent work in our laboratory indicates that vasomotor control of the peripheral parts of the body causes fantastic variations in heat loss. We have measured by direct calorimetry heat loss from the hand and forearm immersed in 20°C water. The results are shown in Fig. 6. The rate of heat loss from this extremity varied between 13 and 43 kcal/hr, and was directly and linearly related to the temperature of the finger. Extreme variations in heat loss were produced by cooling or warming the rest of the body. Worthy of note is that heat lost at a rate of 43 kcal/hr represents about half the heat production of the resting subject when only one extremity is exposed.

Last year Rennie and Marder (15) reported that heat loss from the forearm of subjects immersed in 30°C water was the same whether or not the blood flow to the extremity was completely occluded. Such results imply that under conditions of vascular occlusion the only heat lost from the forearm was produced locally. Blood flow in the forearm was also measured and found to be minimal, suggesting that countercurrent heat exchange was 100% efficient. These results are rather startling, and one is immediately tempted to write them off as artifacts resulting from the use of heat flow discs to measure heat loss.

We repeated this type of experiment (see Fig. 7) by immersing the subject's hand and forearm in the water calorimeter. Although it was not possible to have the rest of the subject's body immersed in water, we did have him warm in one series of experiments and cold in another. A pressure cuff inflated to 200 mmHg was used to occlude the flow of blood com-

pletely for 10 min. Intermittent occlusion using a cycle time of 10 sec, as indicated on the abscissa, was also tried for similar 10-min periods.

When the subject was warm, heat loss was inversely related to the time of occlusion. When the subject was cold, the occluding pressure did not change the rate of heat loss. These results are in agreement with those reported by Rennie and Marder (15). They demonstrate that the human body has an excellent method of regulating heat loss, one which is similar to that of aquatic mammals who can vary heat loss through their flippers. When one considers protective equipment designed to buffer the diver against the effects of cold water, taking advantage of these reflex mechanisms seems desirable.

Many of these experimental findings also raise questions about the usual evaluation of protective clothing. Beckman (1) has indicated that often of primary concern in the testing of wet suits is the maintenance of a desired skin temperature. One then attempts to relate core and skin temperature changes to heat loss. It is also worth remembering that the factors developed by Burton (3, 4) for use in indirect calorimetry were based on data obtained through using the traditional air calorimeter. These factors have not been proven; neither, in fact, have they been adequately tested for subjects immersed in water. But this type of investigation will no doubt be reported on in the future.

For the present I have tried simply to show that people can work successfully in a cold water environment. The physiologist is a newcomer on the underwater scene, and he has provided a number of interesting observations. These have implications for the diver who is pushing on to the border of the continental shelf—if not beyond.

ACKNOWLEDGMENTS

The experimental work from the author's laboratory was supported by funds from the National Heart Institute, Grant HE 09676. I wish also to acknowledge, with deep appreciation, the assistance of Maria Dvorak, who carried out the experiments; and of Gerald Harris, who helped design and construct the special equipment.

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THERMAL BALANCE AT DEPTH

J. S. P. Rawlins and J. F. Tauber

From a theoretical standpoint there is no great problem in achieving thermal balance for a diver at depth, and from the practical point of view satisfactory working dives, using a saturation technique, have already been carried out by commercial organizations at depths in excess of 600 FSW.

It seems remarkable, therefore, that in three recent major experimental dives, Hydra, Janus, and preparations for SeaLab III, cold was one of the greatest problems. In all these cases, cold limited the planned duration of the dive, and in SeaLab III it may well have been a major factor in the death of one of the divers.

Thermal regulation and the response to cold stress are highly complex physiological problems that have been studied by many workers over the years. But in order to achieve thermal balance in an undersea habitat or in the water, all that is required is to apply the basic laws of thermodynamics that have been developed over the last two centuries. As Raymond (3) points out, if thermal balance can be achieved, the physiological problems of cold stress do not arise.

At the Naval Medical Research Institute (NMRI) at Bethesda, we have been engaged in the development of heated suits for SeaLab III divers. To this end we have evolved an approach that should be applicable to any future requirement for keeping a diver warm.

Beckman *et al.* (1) investigated heat loss in men immersed in cold water and showed that it is impossible to maintain thermal balance at 40°F, even when the diver wears a wet suit of 1-in.-thick foamed neoprene. Since such a suit is in any case quite impractical, the solution then appeared to be the use of a thinner insulative garment and to supply supplementary heat.

The dry suit was rejected for SeaLab III use because of (*1*) its inadequate insulation in comparison with foamed neoprene; (*2*) the problems imposed by immersion and cold diuresis; and (*3*) the common experience that dry suits are in fact seldom dry, and that once undergarments are wet, a dry suit is virtually useless (2). Keeping the SeaLab divers warm, therefore, will be accomplished through wet suit insulation, and some form of heat replacement. (This same approach was used in both Hydra and Janus.)

During the past year, we have evaluated three types of heat replacement garments and have studied the diver under two circumstances: in the water and in a personnel transfer capsule (PTC). In both cases the ambient pressure was 600 FSW (19.2 atm abs), the temperature 40°F, and the breathing mixture 98% He.

Method

Our first step was to develop a simple mathematical model to predict the heat replacement a diver will need under specific conditions. This model is a steady state heat-balance equation, the diver being in thermal comfort with a stable deep-body temperature and a mean weighted skin temperature of 94°F. The heat replacement garment used was either an electric resistance wire suit or a closed-circuit tubing suit (originally designed for Apollo astronauts) with a hot water supply (4). These were worn beneath one or two layers of 3/16 in. closed-cell foamed neoprene.

The heat balance equation is:

Heat replacement + metabolic heat generated = respiratory heat loss + suit heat loss

Theoretical estimation of suit heat replacement for a resting diver when wearing 3/8-in. foamed neoprene insulation under the stated conditions—i.e., 600 FSW, 40°F sea temperature, and a 98% He–2% O_2 breathing mixture—is 1500 W, or for a 3/16 in. insulation, 3000 W. (1 W = 0.8598 kcal/hr.)

When the diver begins to work, metabolic heat generation increases, but this increase is more than offset by the effect of cold water flushing around the body underneath the wet suit. Hence, another 500–1000 W may be required to maintain heat balance.

These theoretical values have been repeatedly checked in the NMRI tank in 40°F water, with appropriate adjustments made for air breathing and the shallow (10-ft) depth. In our experimentation, heat balance was maintained in the subjects who were experienced divers of different ages and endomorphic indices. There was very small variation in heat replacement—800–900 W with 3/16-in. insulation and 400–450 W with 3/8-in. insulation. These

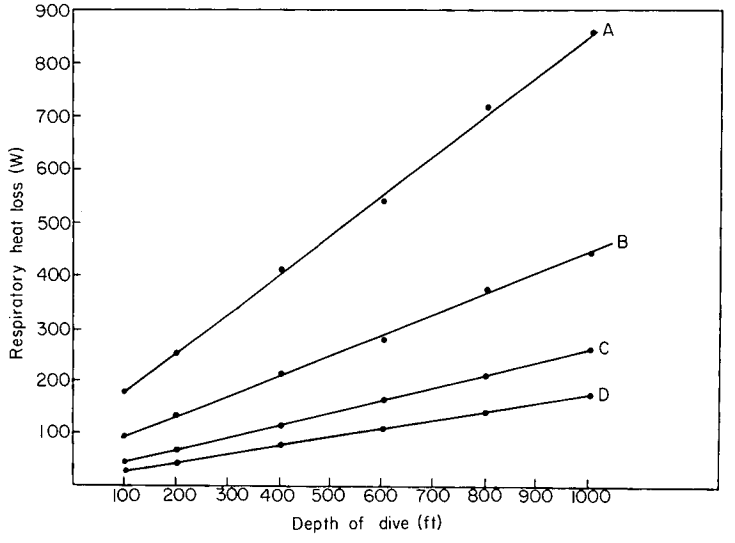

FIG. 1. Theoretical respiratory heat loss when subject breathes a 96% He–4% O_2 gas mixture at a temperature of 0°C through an open-circuit system. $\dot{V}_E$: (A) 50 L/min; (B) 25 L/min; (C) 15 L/min; (D) 10 L/min. [Adapted from Webb and Annis (5)].

results compare favorably with our prediction, giving us a measure of confidence in our suggested procedure for achieving thermal balance at depth.

Problem areas that have interested us include the respiratory heat loss at depth, which was investigated by Webb and Annis (5). Again, our own calculations compare favorably with their values, and we have extrapolated their curves for the SeaLab conditions (Fig. 1).

It is interesting to note that when a diver is at rest at 600 FSW and breathes He–O_2, his respiratory heat loss is approximately the same as the metabolic heat generation, or 125 W. Both these factors increase with work, so that at 600 FSW they tend to cancel each other in the heat balance equation.

At shallower depths, metabolic heat generation outstrips respiratory heat loss. The position is reversed at greater depths, so that for really deep dives some method of heating the inspired gas may be necessary (5).

Much work has been done on the effects of breathing cold air—as cold as −55°C—but we know of no studies on the effect of breathing cold He mixtures at great depths for prolonged periods. At high rates of ventilation with relatively dense gas mixtures of high heat capacity, there is a very large heat loss affecting a limited area of the body, i.e., the upper respiratory tract. The possibility of laryngeal damage, edema, or functional impairment should therefore be anticipated.

Suit Heat Loss

As already stated, the insulation selected for the SeaLab III divers is closed-cell foamed neoprene. Many samples were tested to establish what material provides the best thermal insulation, resistance to compression, reexpansion in a high-pressure He atmosphere, and resistance to tearing and abrasion consistent with good flexibility. However, the best foamed neoprene available is by no means an ideal material for divers at depth. Indeed, the greater heat replacement requirements at depth are largely due to the special properties of foamed neoprene when it is exposed to pressure and an atmosphere with a large percentage of He.

A material's insulating efficiency is dependent on its thickness and thermal conductivity. When a 3/16-in. wet suit is placed in a hyperbaric chamber and is slowly pressurized with He over 24 hr to 19.2 atm abs, the thickness decreases until about 4 atm abs, at which point it is 50–55% of its initial thickness. There is essentially no further change at greater depth (Fig. 2).

During compression He diffuses into the neoprene, and O_2 and N_2 diffuse out. After arrival at 600 FSW diffusion of He continues, as shown by the gradual reexpansion of the wet suit to about 65% of its initial thickness (Fig. 3).

The cells of the neoprene are now filled with He at 19.2 atm abs; should a diver wearing such a wet suit now enter the water (which of course has no dissolved He), the neoprene would rapidly outgas and lose thickness (Fig. 4). The pressure within the cells, however, is still 19.2 atm abs He, so that on return to the habitat there is no physical process to reexpand the neoprene, unless there is some sort of internal spring effect through which the neoprene cells tend to retain their volume and shape. Each subsequent dive will result in further loss of He so that a diver thus insulated is liable to end up with a very thin suit.

The thermal conductivity of a 3/16-in. foamed neoprene suit equilibrated in air at the surface is about 0.045 BTU/hr-ft-°F. When the suit is equilibrated in He under the same con-

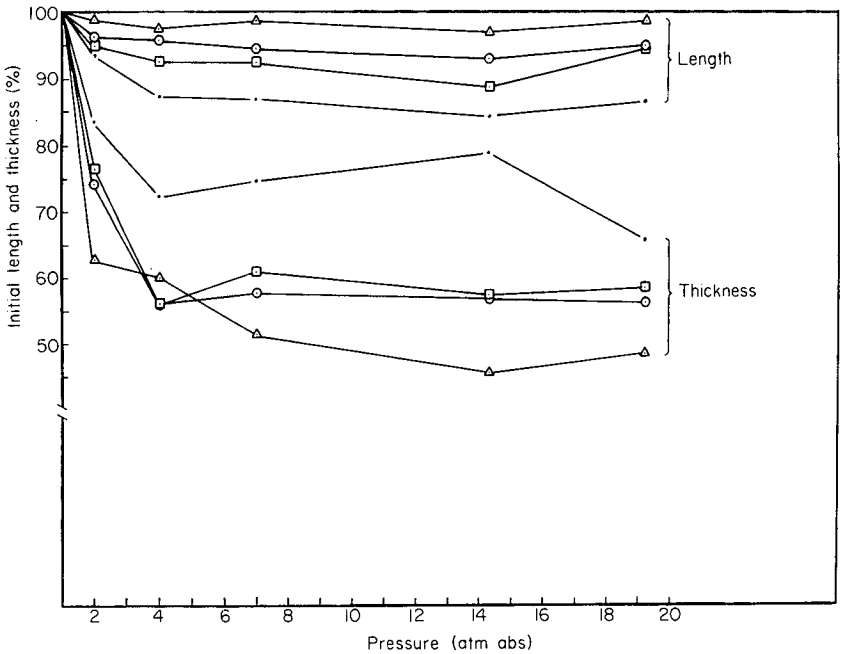

FIG. 2. Shrinkage and compression of neoprene wet suits during slow descent to 600 FSW (19.2 atm abs) in a He atmosphere. (•) $\frac{3}{8}$-in. suit; (⊙) $\frac{3}{16}$-in. inner suit; (⊡) $\frac{3}{16}$-in. outer suit; (△) $\frac{3}{16}$-in. single suit.

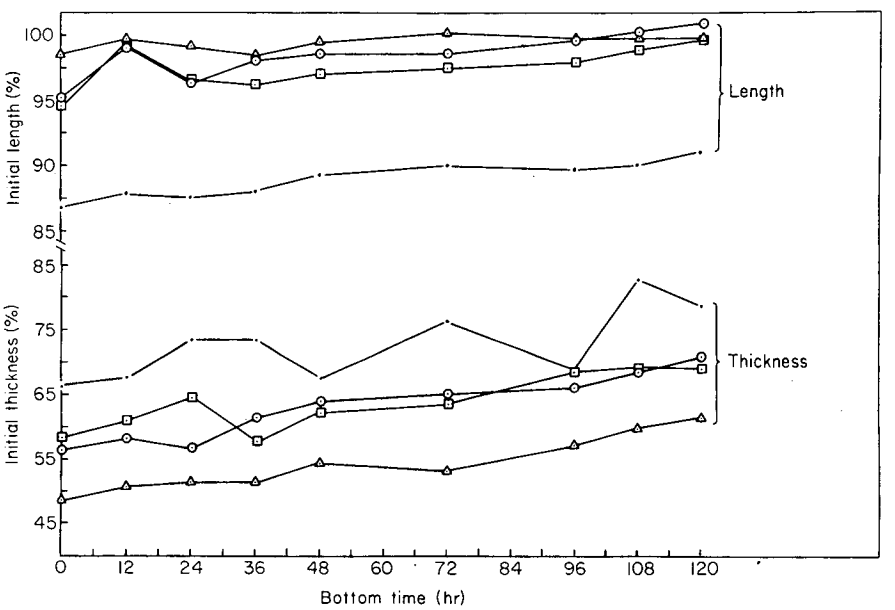

FIG. 3. Reexpansion of neoprene wet suits after a slow compression to 600 FSW (19.2 atm abs) in a He atmosphere. (•) $\frac{3}{8}$-in. suit; (⊙) $\frac{3}{16}$-in. inner suit; (⊡) $\frac{3}{16}$-in. outer suit; (△) $\frac{3}{16}$-in. single suit.

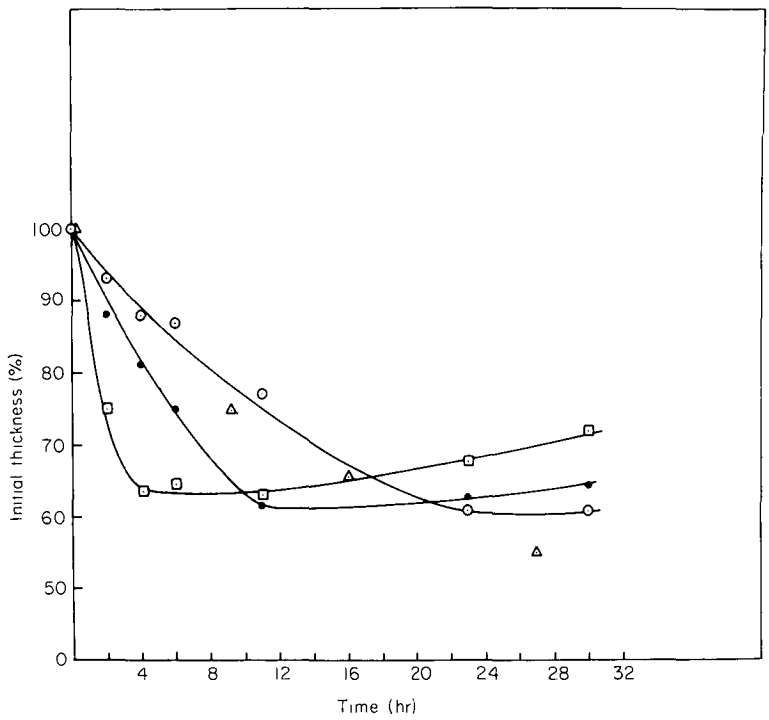

FIG. 4. Change in thickness of He-equilibrated neoprene wet suits during immersion in air-equilibrated water at 4°C. (⊙) $\frac{3}{8}$-in. neoprene; (•) $\frac{3}{16}$-in. neoprene; (⊡) $\frac{1}{16}$-in. neoprene; (△) $\frac{3}{16}$-in. neoprene (theoretical value).

ditions, its thermal conductivity is doubled, hence the insulation is halved. So at 600 FSW, after equilibration with He, the insulation is approximately one-fourth of its surface value, and will decrease with each dive.

Incompressible Suits

The obvious solution to the neoprene problem is the use of a suit made of incompressible material. We have examined various samples, some containing small tiles of rigid foam plastic, and one consisting of glass microspheres suspended in a mineral oil base. The former delaminated when the diver returned to surface from a simulated saturation dive in a He atmosphere. The glass microsphere material was an impressive technical achievement, but a suit made of it was unacceptably stiff and bulky and weighed over 40 lb. Before attempting further development of such a suit, it would be advisable to carry out thermal conductivity tests in air and in He at pressure. The effect of decompression on the He-saturated material should also be investigated.

Dry Suits

We feel now that the decision to reject dry suits was premature. Dry suits have the advantage of eliminating the flushing of cold water around the body; and if suit inflation is used, the insulative capacity at the surface is not too different from that at depth (provided the same inflating gas is used). Gases with low heat capacities, such as Freon and CO_2, are ideal. An advantage of CO_2 is that it can easily be absorbed chemically and therefore prevented from contaminating the breathing atmosphere of a PTC or underwater habitat.

With conventional underwear and air inflation of the dry suit, insulation at the surface is not quite as good as it is with 3/16-in. neoprene (Davies and Montgomery, personal communication, 1967), 1000 W being needed to maintain heat balance in our tank at 40°F. But there is little change with depth and the problem is simply one of supplying enough heat.

A better gas-trapping undergarment is needed, however. Around a 6 ft diver who is upright in the water, for instance, there is a 3 psi pressure gradient between the top of the head and the soles of the feet. Conventional insulative garments are therefore compressed around the lower legs while the insulating gas tends to inflate the upper part of the suit. What is required is a 3/8-in. material that is flexible yet resistant to compression, with small-diameter interconnecting cells or interstices. Fabricating such a material should not be beyond the capacity of the textile industry.

Thermal Balance of Divers in Personnel Transfer Capsule (PTC) or Submersible Decompression Chamber (SDC)

Saturation diving techniques demand that divers spend many hours in a submerged PTC or SDC. The steel shells of these chambers conduct heat more rapidly than water does, so that they must be insulated and heated to maintain the divers in thermal balance.

The SeaLab III system utilizes a PTC to transfer aquanauts from the deck decompression chamber (DDC) on the support vessel to and from the submerged habitat. A recent fatal incident involving the SeaLab system underlines the vital importance of maintaining the divers in thermal balance, not only in the water but also in the PTC or SDC.

In the incident referred to, the PTC was neither insulated nor heated. Furthermore, the insulation provided the aquanauts was a 3/8-in. foamed neoprene wet suit, but no supplemental heating system was made available.

The PTC had been on the deck of the support ship, attached to the DDC, and pressurized to 19.2 atm abs. The chamber atmosphere was 98% He. To maintain the aquanauts' thermal comfort in this environment—they were wearing wet suits with 1 clo* of insulation—an ambient temperature of 85°F was required. The PTC was equilibrated to the surrounding air temperature of 56°F. In their unheated suits, the aquanauts were conscious of the cold as soon as they were locked out of the warm DDC and into the PTC at 56°F.

The descent of the PTC to 600 FSW was slow, requiring 2½ hr, during which time it equilibrated with the 47°F thermocline temperature. The convective loss at this pressure was greatly accelerated by the gas movement produced by the CO_2 scrubbers and by the threefold increase in the density of the He atmosphere over that of air at 1 atm.

* clo is a unit of insulation described as 0.18°C/kcal-m²-hr.

Our calculations indicate that an aquanaut wearing a 3/8-in. foamed neoprene garment equilibrated with He at 19.2 atm abs would require approximately 1000 W of suit-heat replacement in a PTC under the circumstances described. Indeed, the rate of heat loss in the PTC in question was so high that when one aquanaut entered the 47°F water at 600 FSW after leaving the PTC, he stated that the immediate sensation was that of stepping into a hot bath.

The consequent fall in deep-body temperature caused shivering, increased metabolic rate, and accelerated CO_2 production with hyperventilation. These changes in turn may possibly have caused the acute dyspnea that the divers experienced when they donned their volume-limited Mk 9 breathing apparatus. They wrongly attributed their dyspnea to an inadequate gas flow, which they endeavored to adjust by operating the bypass. It should be added that in warm water this same apparatus was used without difficulty at the same depth in successful simulated working dives.

Rather than heating the aquanauts individually while they are in the PTC, the better solution, of course, is to insulate the whole PTC and then to heat it by some simple means—e.g., a hot-water heat exchanger supplied from the surface. We estimate that some 12,000 W would be required for this purpose. This value would be considerably less if 1/4-in. of efficient incompressible insulative material can be applied to the surface of the PTC.

Heat Sources

Finally, there may be an occasion when it is desirable to have a diver at depth who is independent of surface supply. Hence a self-contained source of heat is required.

The possibilities are legion. For electrically heated suits, a metal-gas battery with replaceable anodes offers an attractive solution. For a closed-circuit tubing suit, we have tested a radioisotope heat supply system. This system was inadequate, however, since most of the heat escaped into the surrounding water because the insulation was ineffective. Furthermore, γ-radiation and the neutron flux from the ^{238}Pu of the heating system limit the diver to about 10 hr exposure every 3 months. But there is no doubt that an effective radioisotope heater could be designed using, for example, ^{171}Tm (which is a β emitter), if the cost could be justified.

Battery-supplied heater-pump combinations have been designed and tested, and are satisfactory for limited operations. More sophisticated systems are currently under consideration, such as high-temperature heat-of-fusion systems, chemical dynamic systems employing catalytic burning of aviation fuel or metal foil, and a heat-pump system based on refrigeration principles but operating in the reverse mode. All these possibilities are matters for technical development.

Commercial organizations accomplish successful dives in cold water by programming a rapid descent for the divers in a PTC, in addition to supplying them with adequate heat once they are in the water by flooding their suits with hot water from the surface. The experimental dives described in this paper have been unsuccessful because the heat replacement required was underestimated, both in the PTC and in the water. In SeaLab III this led to a fall in body temperature, increased metabolic rate, increased CO_2 production, and, hence, acute dyspnea, which added to the distress of the divers.

For a diver to be fully efficient, there must be no fall in body temperature. The necessary heat replacement may be calculated on simple thermodynamic theory. If the known properties

of the available insulative materials are taken into account, current engineering expertise can provide the thermal balance required.

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PART X. TEMPERATURE BALANCE IN SHALLOW AND DEEP EXPOSURES*

DISCUSSION

J. D. Hardy, Chairman

Dr. Miller: One other way of heating a man would be to use a latent heat system. We have used this system to keep mice warm in a chamber. I don't know how it would work in practice. If you take saturated sodium sulfate solution, the latent heat as it changes to crystalline hydrate at 32°C is quite high; and you can maintain a tank of this stuff at 32°C for a very long time.

Dr. Rawlins: There are possibilities in latent heat diffusion systems; particularly for water which is not very cold. Suits have been proposed containing layers of material like sodium sulfate, and this works perfectly well. But you cannot have enough heat without a tremendous bulk if you are going to operate at great depth and in cold water. The preferred system for heat diffusion is to use an intense heat diffusion system—lithium hydroxide, lithium fluoride, eutectic mixtures—and to store heat at around 800°F. This can be used to boil water to drive a steam engine (and you can make a very small steam engine), and then some of the steam from that can be added to water circulated around the man.

The answer is that latent heat systems have great possibilities, but the technology is quite complex.

Dr. Hills: I think most of us are aware that the thermal conductivity of He is several times greater than that of air, but the rate of evaporation of water can go up many times in He. I was very impressed by an experiment I saw about two years ago in Brisbane, in which they had a large solar evaporator in which they had replaced air by He and had about 50 times the rate of evaporation and condensation. This is a very large factor and I wondered whether you had found similar heat losses.

I should also like to know whether induction heating has been tried, whether a diver could have a muff so that if his hands felt cold he could put them into the muff and would have induction deep heat.

Dr. Rawlins: I have never heard of it being tried. We think hot water is so good that we are prepared to settle for that most of the time.

Chairman Hardy: As a heat transfer fluid, there is nothing to beat water.

Dr. Webb: The respiratory heat loss data which Dr. Rawlins has shown is a rather alarming extrapolation. It is only fair to point out that it was for an open breathing system where all of the exhaled gas is essentially thrown away. For a recirculated system, if the apparatus can be kept from getting as cold as the water, then, of course, you conserve respiratory heat because you rebreathe much of the gas. Also, as you suggested, you can heat the inspired air by simply passing the expired air through a passive heat exchanger and letting the heat exchange just outside the mouth.

I also wanted to ask Dr. Craig if he has found specific limits for the tolerable loss of body heat content. There are tolerance limits in cold exposure which are often described in terms of the total number of kilocalories per man. A limit for survival might be in the neighborhood of 150–200 kcal. By this time you begin to get very ineffective indeed.

Dr. Craig: No, Dr. Webb. One of the problems is that the estimates of heat loss in terms of calories in water are basically derived from indirect calorimetry, using standard factors. But until we can do experiments in a calorimeter and capture the calories, literally measure them, we will not really know the heat loss quantitatively.

Dr. Webb: I think direct calorimetry, for which there are new techniques that are very interesting, will be the answer. It will help solve the problem of what to do in the repetitive diving situation, where instead of

* *Panelists:* A. B. Craig, J. S. P. Rawlins, H. T. Hammel.

just the decompression tables based on inert gas uptake and elimination in repetitive dives, we need similar information for repetitive dives in terms of heat content—the loss of body heat and how quickly it is replaced.

Chairman Hardy: It seems to me that the body temperature would be much more of an index rather than the number of calories. If the thin man loses a calorie, it is a lot more important to him than the loss would be to a fat man, since the mass of the two men is different and the number of calories depends upon the mass as well as temperature drop.

Dr. Beckman: I certainly agree with Dr. Hardy because the important thing that we found is not what the ambient temperature is, but the total caloric loss which the individual can tolerate.

Secondly, as one can easily see, if the skin temperature or the shell temperature drops to ambient water temperature, which may very well be at 10°C, you can lose a great deal of heat. However, if you can maintain the core temperature you do well. But this situation does not last.

I would agree with Dr. Craig that the information which is available on allowable heat loss, at least in water, was obtained by indirect calorimetry. However, in order to check this work, we did measure the heat loss on immersion subjects. We were able to do two experiments using Dr. Benzinger's gradient calorimeter and the inaccuracies of our indirect methods were not really great after all when we checked them in the gradient calorimeter.

I also want to ask Dr. Horvath about the amount of heat that one can lose, since he is an expert on heat loss in air.

Dr. Horvath: Most people have forgotten that the absolute temperature of a man is much more important than is the amount of heat he loses in terms of calories. A man who weighs only 50 kg and loses 100 cal has a real serious problem. His deep body temperature may be lowered to about 33°C. At such a low temperature this man will not be very functional. In the few experiments in which we have carried subjects down that low, they had a considerable amount of difficulty.

Another problem which almost everybody has ignored has to do with whether or not people become adapted to cold water. In SeaLab II we had pretty good evidence that there was an adaptive process occurring in the divers, and their ability to tolerate the cold was better after they had been going in and out of the cold water for 2 weeks than it was before they started. So there is a problem of adaptation which also has to be considered. The absolute heat loss, while it may be important, is only important in terms of what it does to the body temperature.

Chairman Hardy: Dr. Craig, it has been my impression that humans do not truly adapt to cold very much and if they do it is a kind of habituation rather than a true acclimatization. You observed a 30% increase in basal metabolic rate, which is not likely to do anything for the diver. However, I do think that both the behavioral type of adaptation, which one might call habituation, and the physiological adaptation may have to be taken into consideration.

Dr. Craig: I would like to turn your question over to Dr. Hong who is responsible for these observations.

Dr. Hong: About 10 years ago when we started working on Korean Ama, we noted that they dived in cold water. At that time we did not know how cold the water was, so I went down with a thermometer and measured it, and the temperature was about 10°C. We were surprised and shocked. We could not imagine how they could tolerate it.

The first thing we did was to measure their oral temperature at the end of a diving shift, which lasted about 10–15 min, although some dived about 30 min. The oral temperature at the end of the diving shift was 32°C, which is very low. Then we checked the rectal temperature and, as Dr. Craig said, at the end of the diving shift it was about 35°C.

Considering this reduction in cold temperature by 2°C within 15 min, we began to wonder about the extent of the cold distress they are getting. Then, of course, we began to wonder about the possible development of cold adaptation.

We know that whether metabolic adaptation occurs in human beings has been argued. Data obtained on Eskimos have been criticized on the basis of uncontrolled protein intake.

In demonstrating this metabolic adaptation, we did an experiment covering a period of about 3 years. We had 20 diving women and 20 nondiving women living in the same community, belonging to the same socio-economic class. We found out that their protein intake was about the same, although the carbohydrate intake was somewhat different. Ama ingest about 1000 kcal more than nondivers, possibly because they lose more heat through cold water.

When we studied their basal metabolism seasonally during the first year, we found that the basal metabo-

lism of Ama increases in winter about 30%, as compared to nondivers. And it decreases again in spring and summer. In the middle of the summer this difference between the Ama and other women disappears.

We have also studied thyroid functions and the data on thyroid functions are more complex, and we are still thinking about interpretation of the data. But this seasonal increase in basal metabolism such as we found in winter is highly reproducible; the interpretation could be different, but the facts are there.

Dr. Brauer: Sea lions do well in cold water with the type of insulating material they have. It occurs to me that it would be worthwhile to study some of the insulating materials that the diving mammals do well with. Certainly they are not bulkier than some of the insulated suits I have seen used.

Chairman Hardy: You mean some good old whale blubber?

Dr. Rawlins: I suspect such insulation is not entirely static, but is a dynamic insulation. I also suspect that if you wrap yourself in a tube of blubber you may not keep as warm as you might hope and you would not do as well as a seal would with the same amount.

The other thing is that one always has to bear in mind that the diver has to get in and out of the water as well as being in the water, and nongaseous insulation is heavy.

Chairman Hardy: Perhaps one should encourage the divers to be fatter than the normal individual.

Dr. Brauer: We have considered the effect of exposure of the entire body surface to cold. In clinical medicine the immersion of a hand in cold water produces effects, and cold exposure of the diver does involve local cooling. Also, under conditions where the body is enveloped either in a standard wet suit or in an air-insulated suit, shifting from air to He as a breathing medium often leaves the diver with the impression that he is very uncomfortably cold. From what I have heard, this comes on so quickly that I find it extremely difficult to believe that it is the result of heat loss. Can you tell me to what extent we should be concerned not with overall heat loss but with the type of information fed to the CNS about the heat balance of the individual? In that connection arises the question of many of us having experienced paradoxical thermal responses during protracted helium exposures in chamber environments, where hot and cold sensations occur at the same time, with shivering and profuse sweating.

Chairman Hardy: This question concerning the relative importance of central and superficial input in thermal sensing and regulation is still under extensive investigation and discussion today and is extremely complex.

Part XI. INFLUENCE OF INERT GASES AND PRESSURE UPON CENTRAL NERVOUS FUNCTIONS

QUANTITATION OF PERFORMANCE DECREMENTS IN NARCOTIZED MAN

James G. Dickson, Jr., C. J. Lambertsen, and John G. Cassils

Over many years there has accumulated a sizable body of both subjective and quantitative information describing effects of narcotic substances, including narcotic gases, upon various parameters of human performance (2, 6, 8, 10). Gases such as Ar, Ne, N_2, and N_2O have been shown to cause measurable impairment of function, ranging from interference with accomplishment of relatively simple sensory and motor tasks to disruption of more complex cognitive abilities (1, 3–5, 7, 9, 11, 13–16). In general, such studies have shown that for any single index of narcosis, the degree of performance decrement is related to the characteristics of the gas chosen and to its absolute pressure, or dose.

In the case of narcotic gases (as with other narcotic agents), precise information regarding the pattern of performance impairment under conditions progressing from light to deep narcosis is quite limited. In a previous paper from this laboratory (12) attention was called to this limitation and especially to the need for obtaining such data over the complete range of narcosis from zero effect to complete loss of purposeful function before it will be possible to predict quantitatively the tolerance of man to high pressures of various inert gases. It was further stated that the pattern of progressive impairment of a given human function, from the earliest detectable change to the abolition of function, as inert gas pressures increased could be expected to take the form of the classical S-shaped pharmacological dose–response curve. Since this curve is a mathematical entity, it would provide a basis for systematic analysis and comparison of inert gas effects (12).

To test this prediction, we gathered quantitative data on the performance of eight resting human subjects during inhalation of N_2O at several different concentrations. A deliberate attempt was made to select highly motivated, cooperative subjects so that any motivational changes occurring under narcosis might be minimized. The performance tests selected were of two general types. The first series consisted of tests of visual-reaction time in response to light stimuli. Both simple reaction times and performance in a four-choice reaction test were measured. Intentionally, techniques duplicated as closely as possible those used previously by Frankenhaeuser *et al.* (9). The second series was a group of written tests of cognitive ability adapted from standardized tests prepared by the Educational Testing Service, Princeton,

N.J. The cognitive tests and the functions tested are shown in the following tabulation:

Test	Function tested
Arithmetic	Multiplication and subtraction
Card rotation	Spatial orientation
Number comparison	Perceptive speed
Letter set	Inductive reasoning
Memory	Short-term memory

All five cognitive tests were administered during the same exposure period at each level of N_2O; to prevent repetition of identical tests by a subject, content was varied within each test format as the N_2O level changed.

Nitrous oxide in 21% O_2 was administered at atmospheric pressure in 20, 30, 40, and 50% concentrations. As a result of preliminary study, a maximum "dose" of 50% N_2O was chosen as being near the highest which could be tolerated by all subjects, allowing sufficient residual responsiveness to permit testing. At each gas dose the test procedure shown in the following tabulation was followed:

Elapsed time (min)	Procedure
0–10	Equilibration to new level of N_2O; practice on reaction test device
10–15	Visual reaction tests
15–28	Written cognitive tests

Control data for normal performance were collected while each subject breathed air, both before and after he breathed N_2O. The order in which the various N_2O doses were administered varied systematically from subject to subject. Prior to the experiment the subjects received sufficient training in carrying out the visual reaction tests to stabilize performance and were instructed how to perform the written tests. Prior to beginning the study they were repeatedly familiarized with the subjective sensations involved in N_2O breathing.

Effects of Nitrous Oxide on Visual Reaction Performance

The subjects' ability to perform the visual reaction tests decreased as the inspired N_2O concentration was increased (see Fig. 1). Both in the simple reaction (speed) and the choice reaction (time and accuracy) tests, progressive deterioration occurred as the N_2O dose was increased. The line defining accuracy was obtained from choice reaction test data by dividing the number of problems performed correctly by the number of problems presented. Although the degree of impairment was not sufficient to permit conclusions regarding the pattern of visual reaction response to be expected with still higher doses, the increasing impairment as the N_2O level rose is compatible with the S-shaped dose–response curve predicted for narcosis.

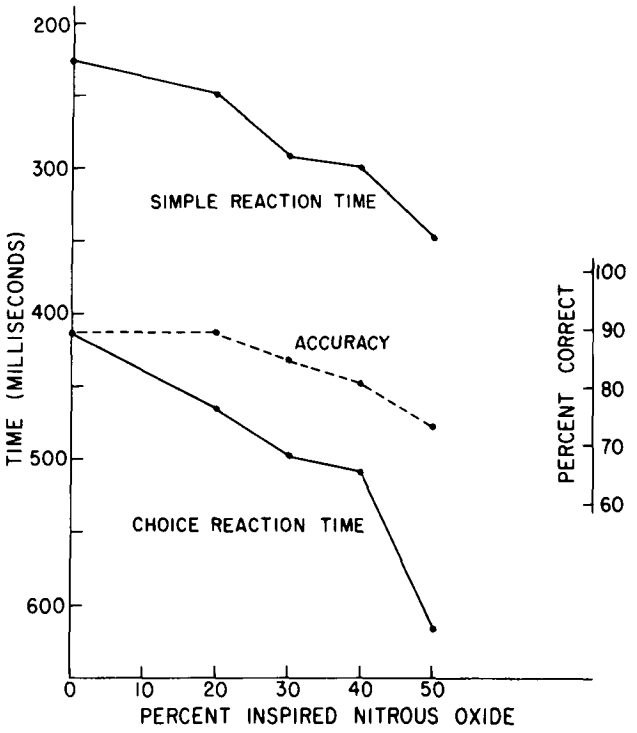

FIG. 1. Effect of breathing N_2O on performance of visual reaction tests. Mean values in eight subjects.

Effects on Cognitive Functions

With increasing pressures of N_2O, there was a progressive impairment in the performance of all the subjects in all of the cognitive tests. The impairment was minimal at low N_2O levels and severe at the maximum level. Some subjects, although conscious and responsive to questioning, were completely unable to perform independently a particular test at the highest N_2O level used. Figure 2 shows results obtained in one of the cognitive tests, the number comparison test; the results were generally representative of those of all five cognitive tests used.

Accuracy scores represent the number of problems answered correctly divided by the number of problems attempted during the time allowed at each N_2O level. Speed scores represent the number of problems attempted, and the combined scores represent the number of problems solved correctly. There was progressive decrement in accuracy, speed, and the combination of speed and accuracy as the inspired partial pressure of the narcotic gas increased.

For further analysis, the combined scores (number of problems answered correctly under test conditions as compared with control conditions) were plotted on probability coordinates and a linear regression drawn through the data. Figure 3 is a replot of these data and the derived regression line on rectangular coordinates. The regression line necessarily takes the form of an S-shaped curve when plotted in this manner. However, it is evident that the actual

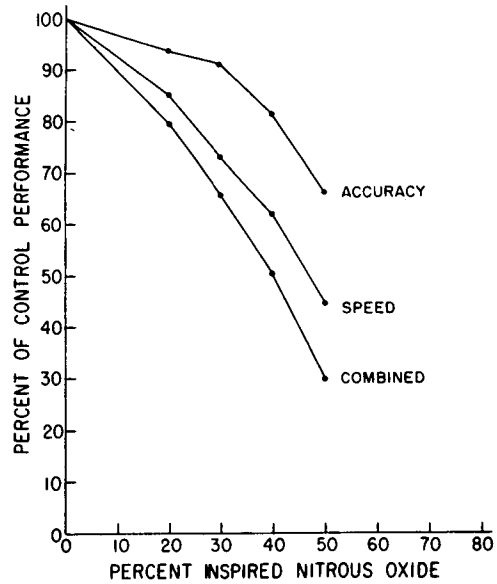

FIG. 2. Effect of breathing N_2O on perceptive speed (from number comparison test). Mean values in eight subjects.

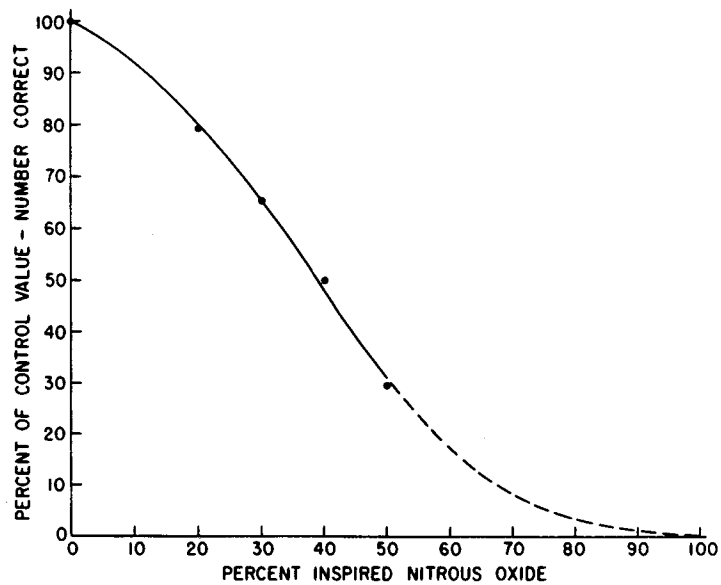

FIG. 3. Derived regression curve of effect of breathing N_2O on perceptive speed (number comparison test).

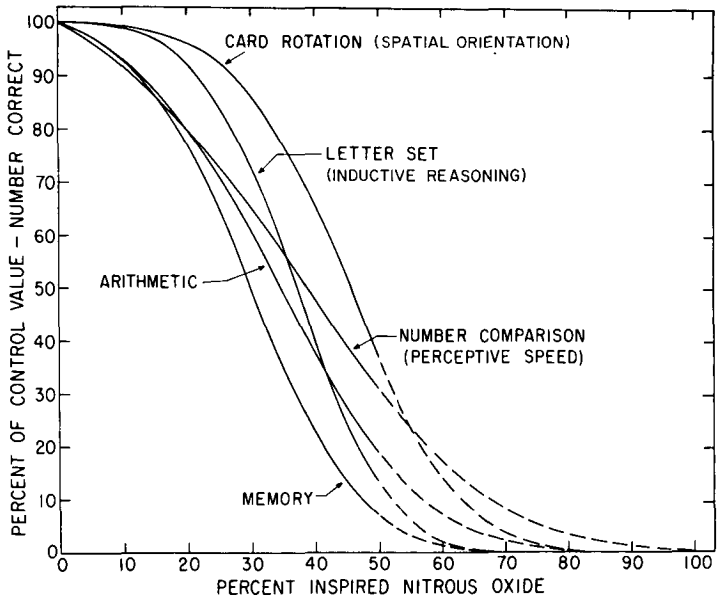

FIG. 4. Effect of breathing N_2O of increasing concentrations on performance of various written cognitive tests.

experimental points relate closely to the theroretically derived curve. An indication of the likelihood that an S-shaped relationship exists for all of the functions studied is that for the reaction time and for all five cognitive tests there is a remarkable degree of linearity when the data are plotted on probability coordinates.

Figure 4 shows the results of all five cognitive tests performed, the regression curves being derived as they were in the number comparison test. Through an inspection of these curves, we can get a clearer picture of the effects of N_2O on the cognitive functions studied. First, each function showed progressive impairment as PI_{N_2O} was increased. The pattern of the narcotic impairment in each case, over the range of impairment studied, is also quite consistent with the predicted S-shaped pharmacological dose–response curve. Changes induced by N_2O in these cognitive functions, even though they are neurophysiologically complex, appear to occur in a manner similar to the pattern of other and simpler drug-induced biological responses. Finally, differences in narcotic effects upon the several different functions can be seen from the curves. Such differences can take two general forms. One is of position reflecting a degree of narcosis necessary to produce a demonstrable effect. This is in some biological systems considered to represent a "threshold" for effect, but this is probably not true in the more complex systems studied here. The differences in slope could be considered as reflecting sensitivity of the neurological mechanisms involved. For example, the curves for short-term memory and for spatial orientation, while parallel, are displaced from one another positionally and indicate chiefly a difference in dose required to produce significant effects. Decrement in memory is readily apparent at the 20% N_2O level, while a much higher dose is required for a comparable loss in spatial orientation. On the other hand, a comparison of the curves representing short-term memory and perceptive speed (number comparison) reveals a significant difference in slope. Although equivalent decrements at low doses of N_2O indicate that these

functions are roughly equally affected by low doses of the narcotic gas, the steeper slope for loss of capacity for short-term memory clearly shows that memory is more sensitive to increasing doses of N_2O than is perceptive speed. This finding with respect to memory impairment is consistent with those of other investigators (13, 14, 16).

Application of Dose–Response Data to Human Performance at Increased Ambient Pressure

These data concerning effects of N_2O at sea level pressure were obtained as a step toward quantitative prediction of human performance while breathing other gases at increased ambient pressure. It is probable that under increasing pressures of another inert gas (such as N_2), the decremental pattern of a particular function will follow an S-shaped curve similar to that produced by increasing pressures of N_2O. It is even possible that, when N_2 (or other inert gas) is breathed at increased pressure, the relationships among the performance curves for various functions will be similar to those found during N_2O breathing at atmospheric pressure. Such findings would be compatible with the existence of common mechanisms for the production of narcosis by the several inert gases. It is now necessary to determine whether the dose–effect relationships found for N_2O can in fact be empirically linked to performance decrement produced by increased partial pressures of N_2, Ar, Ne, and, eventually, He. By accomplishing such a cross correlation of dose and effect for the several gases at critical effect levels, it should be possible to derive the needed predictive capability for a wide range of pressures and mixtures of inert gases.

A further advantage of the quantitative dose–response curves for N_2O is in delineation of the effects of factors which can modify inert gas narcosis (such as CO_2, hypothermia, drugs, and exercise). These too should be approached experimentally as far as possible through collection of dose–response information across the full range from light to deep narcosis. The definition of the nature and degree of performance decrement produced by inert gas narcosis, alone and in conjunction with other factors, will become increasingly important as man pushes toward the limits of his mental and physical performance in the sea.

Acknowledgment

This work was supported in part by grants (HE-08184 and HE-08899) from the National Institutes of Health and by a contract (NONR 551) with the Office of Naval Research.

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PSYCHOLOGICAL, PHYSIOLOGICAL, AND BIOPHYSICAL STUDIES OF NARCOSIS

Peter B. Bennett

The problem of inert gas narcosis, like the majority of other saturation diving problems, requires a multidisciplinary approach to measure effectively and understand the mechanisms involved and to develop a means for its prevention. With the advent of more sophisticated diving techniques, major concern in the matter of man's limitations at depth has shifted from decompression sickness to other factors—e.g., hydrostatic pressure, the narcotic action of inert gases, respiratory embarrassment, and more recently observed phenomena, such as He tremors and convulsions (8). All these problems can adversely affect man's capability at depth—and often they all exist at the same time.

This paper will discuss briefly various investigations that indicate connections between seemingly unrelated problems in saturation diving and the benefits to be gained from a multidisciplinary approach to solving them.

Psychological and Physiological Testing for Narcosis

One of the greatest difficulties encountered in deep diving studies is effective measurement of the performance impairment caused by such conditions as inert gas narcosis. In the past, reliance has been placed on psychological evaluation, yet there has been little conformity among the tests or techniques. The inherent problems of most psychological tests (e.g., training effects and differing motivation among subjects), combined with the possible synergistic action of such variables as O_2, CO_2, and pressure, make comparison of results extremely difficult. The solution to many of these problems appears to be a suitable *physiological* test for narcosis that is less sensitive to subject manipulation than are existing ones (23), together with carefully controlled experiments to establish the degree of synergistic involvement of such factors as pressure, O_2, and CO_2.

Previous attempts to find a suitable physiological test based on such indicators as changes in the EEG (9) or the fusion frequency of flicker (7) were not entirely successful. However, the steady evolution of our understanding of the electrical activity of the brain now suggests other methods—in particular, the use of potentials evoked in the brain by a sharp sound or flash of light. As an example of the use of this method, measurements of the amplitude of the

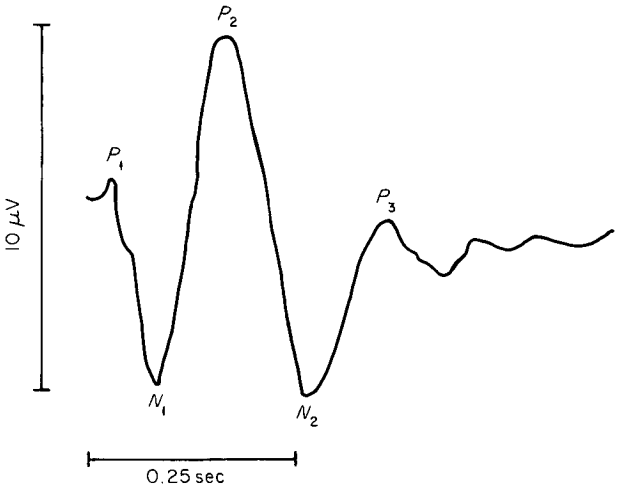

FIG. 1. Average of typical human AER after 60 clicks. There is an initial positive potential (P_1) at 50 msec, followed by a large negative wave (N_1) at 90 msec; P_2 occurs at 160 msec, and sometimes N_2 at 270 msec (6). (By permission of the publishers.)

auditory evoked response (AER) in cats exposed to high pressures of inert gases showed a significant depression (3). Although the amplitude of the AER in man is only some 10 μV in a spontaneous activity of 50 μV, it can be measured by modern averaging computers.

In a recent series of experiments (6) electrodes were attached to the vertex of the subject's head and to the right or left occipital and sphenoidal areas. Sharp sounds or clicks, triggered by an averaging computer, were transmitted through binaural earphones at the rate of 1/sec

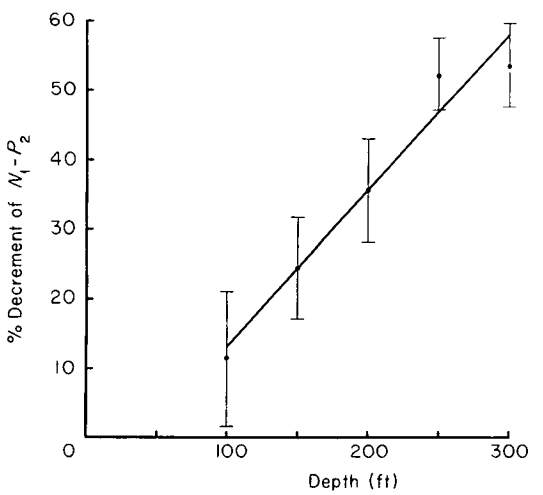

FIG. 2. The mean percentage change in the amplitude of the N_1–P_2 wave of the AER of five divers exposed to increased depths while breathing air (6). $y = 0.23x - 9.4$; $r = 0.98$. (By permission of the publishers.)

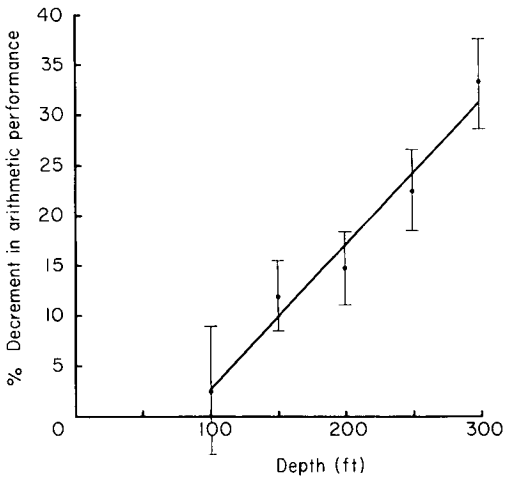

FIG. 3. The mean percentage decrement in the number of multiplication problems correctly performed in a test by five divers exposed to increased depths while breathing compressed air. Results of changes in the AER and the number of multiplication problems attempted at the same time are shown in Figs. 2 and 4, respectively (6). $y = 0.15x - 12.1$; $r = 0.99$. (By permission of the publishers.)

for periods of either 1 or 5 min, and the averaged response was displayed on an X–Y plotter. A typical result is shown in Fig. 1.

Figure 2 shows the effects of exposure to increasing depths on the N_1–P_2 amplitude. At depths greater than 150 FSW (5.5 atm abs), there is a significant decrement in the amplitude of N_1–P_2, which correlates well with increasing depth.

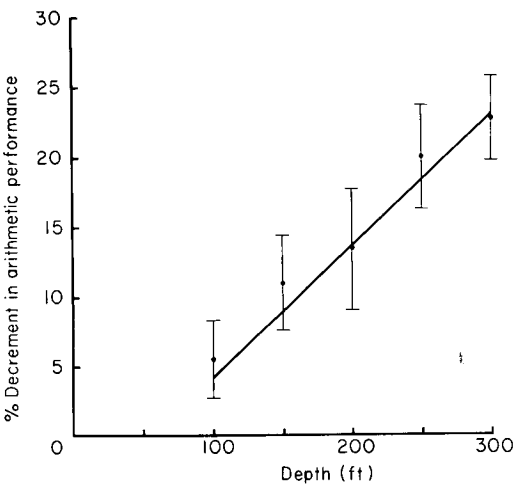

FIG. 4. The mean percentage decrement in the number of multiplication problems attempted in a test performed by five divers exposed to increased depths while breathing compressed air. Results of changes in the AER and in the number of multiplication problems correctly performed at the same time are shown in Figs. 2 and 3, respectively (6). $y = 0.087x - 2.8$; $r = 0.99$. (By permission of the publishers.)

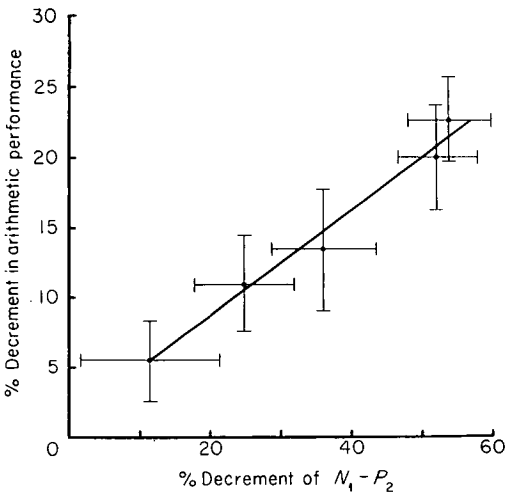

FIG. 5. Correlation between the percentage decrement in number of multiplication problems attempted and the percentage decrement in the N_1–P_2 wave amplitude of the auditory evoked response in five divers exposed to increased pressures of compressed air as shown in Figs. 2 to 4 (6). $y = 0.38x + 1.1$; $r = 0.99$. (By permission of the publishers.)

The mean changes in the AER of five divers breathing compressed air at 50 FSW (2.5 atm abs) intervals to 300 FSW (10 atm abs), as indicated by measurement of the amplitude of the N_1–P_2 wave, were compared with the divers' ability to perform a test of two-digit by one-digit multiplication (6). The N_1–P_2 decrement was accompanied by a decrease both in the number of multiplication problems attempted and the number done correctly (Figs. 3 and 4). The correlation between decrements in the number of multiplication problems attempted and the N_1–P_2 wave is 0.99 (Fig. 5); the correlation is 0.95 when the number of correct multiplications and the decrement in amplitude of the AER are compared. This test is therefore as effective as the much-used arithmetic test in quantifying inert gas narcosis, and it has the major advantage of objectivity, which is lacking in most psychological tests.

Some of the AER amplitude variation among individuals indicated by the standard errors of the mean in this study might have been reduced by controlling diving frequency, for it was found that men who dived daily prior to the test showed only a 40% reduction in amplitude in comparison with the novices' 60%. Production of the click by tone noise at 200–350 msec and 90 dB, rather than the 60 dB used in the present study, also markedly reduces individual amplitude variation.

The possible synergistic effects on the narcosis of pressure (17) or O_2 (16, 19) were studied through use of the AER and multiplication test with five divers who breathed air or He–O_2 at 300 FSW and O_2 at 33 FSW. Unexpectedly, the He–O_2 mixture produced a 29% mean reduction in the AER without a concomitant reduction in arithmetic efficiency. Furthermore, pure O_2 at 33 FSW (2 atm abs) caused a similar reduction (27.8%) in the AER, again without a reduction in arithmetic efficiency (Table I).

Ten divers, breathing a N_2–He–O_2 mixture, were exposed to 300-FSW (10 atm abs) pressure. Helium at 1.8 atm abs (leaving only 0.2 atm abs O_2) was used in place of pure O_2 at 2

TABLE I

MEAN PERCENTAGE CHANGE IN N_1–P_2 WAVE OF AER AND MULTIPLICATION EFFICIENCY DURING FIRST 5 MIN AT DEPTH (6)[a]

	Air at 300 FSW 2.0 atm abs O_2 8.0 atm abs N_2	O_2–He at 300 FSW 2.0 atm abs O_2 8.0 atm abs He	O_2 at 33 FSW 2.0 atm abs O_2	Mixture at 300 FSW 0.2 atm abs O_2 1.8 atm abs He 8.0 atm abs N_2
Spike height N_1–P_2	−55.5 ± 10.5	−29.4 ± 4.6	−27.8 ± 8.0	−54.1 ± 4.3
Arithmetic correct	−31.2 ± 10.95	+ 6.3 ± 7.1	+ 1.0 ± 5.7	−30.0 ± 5.3
Arithmetic attempted	−28.0 ± 11.8	+ 7.5 ± 4.6	+ 1.3 ± 5.7	−23.9 ± 4.7

[a] By permission of the publishers.

atm abs to determine if removing most of the O_2 would result in a smaller decrease in the AER and less narcosis. However, the result was no different from what it had been when the subjects breathed compressed air; the mean AER decrement was 54.1% and there were equivalent decrements in arithmetic ability (Table I). On the basis of these experiments, one can conclude that O_2 does not seem to be synergistic, and the fundamental role of N_2 in narcosis is supported. The role of CO_2 as a narcotic will be established shortly on a similar basis.

The results of other experiments (1, 5) involving measurements at surface, 33 and 66 FSW (1, 2, and 3 atm abs) indicate that O_2 at various hyperbaric pressures also will depress the AER, as will altitude hypoxia (Fig. 6). These and other results suggest the possibility that both high and low O_2 pressures can produce conduction deficiencies in the brain, thus acting as narcotics. However, such deficiencies are insufficient to cause mental impairment before some other factor such as enzyme inhibition becomes involved and convulsions result. For example, unpublished work by J. D. Wood and me showed that although the concentration of the inhibitory nerve transmitter γ-aminobutyric acid falls in the rat brain before the animal convulses, no such change occurs with inert gas narcosis.

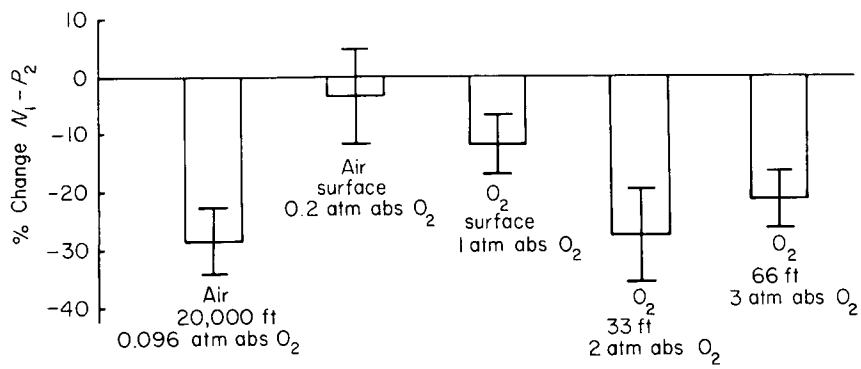

FIG. 6. Mean percentage change in N_1–P_2 wave of AER and multiplication efficiency during first 5 min exposure to increased O_2 pressures and altitude (1).

Biophysical Mechanisms of Narcosis

Speculation continues regarding the mechanism of narcosis, but biophysical explanations related to the polarizability and volume of the inert gas molecule appear to be the most probable (4, 15, 26, 33, 34). The majority of the physical theories relate to two phenomena, either separately or together—interference with the release of the chemical transmitters necessary for transmission of the nerve impulse across the synaptic gap and a block of the ion exchange across the cell membrane.

Another hypothesis is based on the formation of clathrates (29), or "iceberg" formation (25), and much controversy revolves around whether the fundamental site of narcosis is in the lipid or aqueous phase. However, Miller *et al.* (24), working with F compounds, found no evidence to support the aqueous phase as the site of action.

Clements and Wilson (14) have suggested that the difference between hydrate and lipid theories is irrelevant, as the narcotic agent forms lipid–protein–water–narcotic complexes in cellular membranes. Their work on the affinity of narcotics such as N_2O for interfacial films or model membranes of stearic acid or lipoprotein led them to conclude that "inert gases, sufficient to bring about a standard effect in a biological system, act on a lipoprotein–water interface to cause a standard decrease of 0.39 dyne/cm in the interfacial tension." This decrease in tension will result in a tendency to expand the model membrane, replace water at the interface, or change the dielectric constant at the interface. Such effects, Clements and Wilson suggest, might result in changes in permeability or alter the critical structural relationships in the enzymes that support phosphorylation and electron transport in mitochondrial membranes. The results of this work also support the hypothesis that lipids play a fundamental causative role in inert gas narcosis.

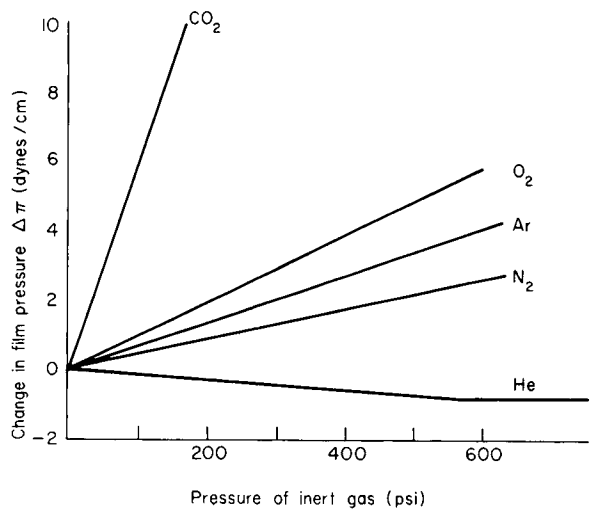

FIG. 7. Penetration of an egg phospholipid monolayer or model membrane system by inert gases, O_2, and CO_2 at increased pressures. All the gases except He adsorb to the membrane (11). (By permission of the publishers.)

TABLE II

MEAN CHANGES IN IONS IN CEREBROSPINAL FLUID OF FIVE CATS AFTER 1-HR EXPOSURE TO VARIOUS BREATHING MIXTURES AT 330 FSW FOLLOWING AIR BREATHING (10)[a]

	Values after 1 hr of breathing							
	Air at atm pressure		20% O_2–80% He		20% O_2–80% N_2		20% O_2–80% Ar	
Ions	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
Sodium	+ 7.6	± 4.4	+ 4.8	± 2.1	− 14.2	± 3.1[b]	− 11.2	± 3.4[b]
Potassium	− 0.1	± 0.22	− 0.1	± 0.25	− 0.54	± 0.18	+ 0.14	± 0.29
Chloride	+ 0.6	± 1.4	+ 0.6	± 0.7	− 9.6	± 3.0[c]	− 6.0	± 2.7[d]

[a] By permission of the publishers.
[b] $P > 0.01$.
[c] $P = 0.05 - 0.02$.
[d] $P = 0.05$.

On the basis of the Meyer-Overton theory—i.e., that narcosis commences when any chemically indifferent substance has attained a certain molar concentration in the lipids of the cell (22, 27)—an increased ion permeability can be expected to result, whereas the hydrate theories predict a decreased ion permeability. In further support of the lipid theory are the ion permeability experiments of Bangham *et al.* (2), who adapted a model membrane system developed by Clements and Wilson (14) to study the effects of *n* alcohols, chloroform, and ether on membrane permeability. They concluded that "narcosis represents a transient reversible increase in membrane permeability to cations, higher concentrations resulting in a degree of permeability which the energy-driven pumps in biological membranes cannot keep up with."

More recent work (11) has established that at increased pressures, O_2, CO_2, and inert gases also will adsorb to lipid membranes. Monolayer pressures or surface tensions were obtained with a platinized needle attached to the transducer of a microforce balance. The needle was dipped in a polythene trough containing an aqueous solution on which was spread a phospholipid monolayer. As the gas pressure increased, N_2, Ar, O_2, and CO_2 produced a linear increase in film pressure (Fig. 7); but He, even under pressures as high as 1600 psi (and Ne in a similar experiment) failed to penetrate the membrane. The most significant adsorption was by CO_2, followed by O_2, Ar, and N_2; the degree of penetration agreed with the predictions of Clements and Wilson (14).

In conjunction with these experiments measurements were also made, using the technique of Bangham *et al.* (2) of the effects of high pressures of Ar, N_2, and He on the permeability of $^{22}Na^+$ from liquid crystals, but the results were inconclusive (11).

However, measurements of the concentration of sodium, potassium, and chloride ions in the extracellular or cerebrospinal fluid of cats exposed to 330 FSW (11 atm abs) may provide support for the permeability theory (10) (see Table II). There was a significant fall in the

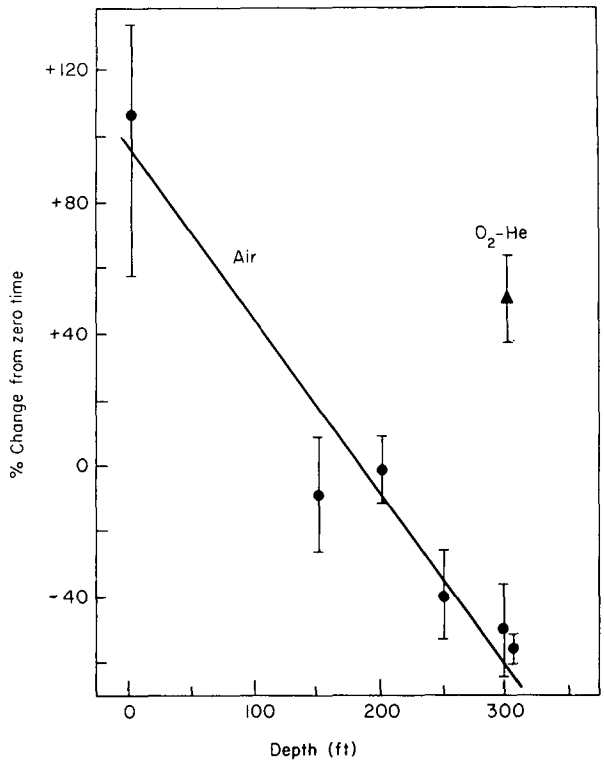

FIG. 8. Mean percentage change in excretion of calcium ions in the urine of divers breathing air at various pressures and an O_2–He mixture at 300-FSW (10 atm abs) pressure. Comparison of values obtained at experimental and atmospheric pressures revealed a significant decrement with compressed air but none with He–O_2; O_2 at 2 atm abs (33 FSW) also produced no change (30). $y = 96 - 0.53x$; $r = -0.968$; $P < 5\%$. (By permission of the publishers.)

sodium and chloride ion concentration in animals exposed to Ar–O_2 and N_2–O_2 mixtures, which was accompanied by narcosis, as determined by the decrement in AER. Potassium was unaffected, and neither the control animals exposed to air at 1 atm abs nor those animals exposed to the He–O_2 mixture under pressure showed significant changes in sodium or chloride levels.

Similar changes have been reported at high O_2 pressures by Kaplan and Stein (20); and Radomski and I (30) have found electrolyte changes in the urine of divers during exposures to 150, 200, 250, and 300 FSW for up to 1 hr. Significant decreases were found in Na and Ca excretion, which correlated with increasing P_{N_2} and were reversible upon decompression (Figs. 8 and 9). At comparable partial pressures, He and O_2 had no effect on these electrolytes. It is of interest that similar electrolyte shifts have been reported during general anesthesia and in alcoholic intoxication (28).

Although these changes were related to P_{N_2}, they are also a function of increasing depth and might well be due to other factors, such as an acidosis (21, 31), or, more probably, to a combination of increased N_2 and CO_2 tensions.

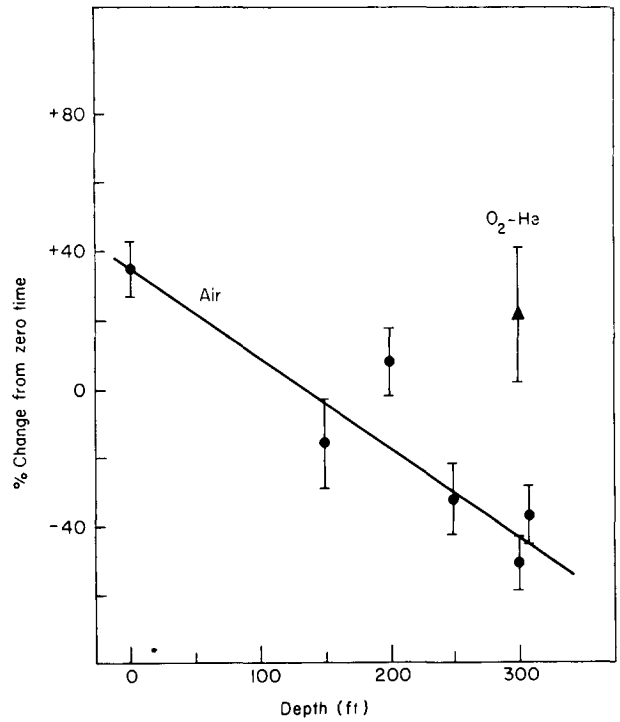

FIG. 9. Mean percentage change in excretion of sodium ions in the urine of divers breathing air at various pressures and an O_2–He mixture at 300-FSW (10 atm abs) pressure. Comparison of values obtained at experimental and atmospheric pressures revealed a significant decrement with compressed air but none with He–O_2; O_2 at 2 atm abs (33 FSW) also produced no change (30). $y = 36 - 0.26x$; $r = -0.904$; $P < 5\%$. (By permission of the publishers.)

Helium Diving

That He did not penetrate the model membrane, even at depths as great as 3500 FSW (107 atm abs) is significant, for it suggests that He is not a narcotic, and that He tremors and the decrement in performance found in some deep He dives are caused by other factors, such as CO_2 retention.

It is now clear that He tremors can be virtually eliminated by ensuring a slow rate of compression, low P_{O_2}, and comfortable temperature, although recent animal studies suggest that the tremors may recur at greater depths (18). Brauer (12) has reported that monkeys breathing He–O_2 convulsed at depths 35% deeper than the one at which tremors began. However, in recent deep dives in France with the COMEX organization (12), which involved human subjects, Brauer reported He tremors, which were accompanied by dizziness and nausea during compression. There were, in addition, changes in the EEG and periods of somnolence during 4 min at some 1190 FSW (37 atm abs), all of which were of sufficient severity to necessitate immediate decompression. Yet O_2 in the breathing mixture was kept low, and compression was conducted at a very slow rate. On the basis of these experiments, Brauer has postulated that there may be a heretofore unrecognized physiological barrier to deep diving.

TABLE III

MEAN RESULTS OF THREE SUBJECTS' EFFICIENCY IN SERIES OF PERFORMANCE TESTS CARRIED OUT DURING 1969 SWISS–BRITISH 3-DAY SATURATION DIVE (13)[a]

Tests			1 atm abs (Control)	31 atm abs 0 hr	1 hr	2 hr	3 hr	24 hr	48 hr	72 hr	36 atm abs (Excursion)
Ball-bearing test		$\bar{x}$	12	6	10	11	12	16	18	17	19
		SD	4	5	6	4	5	6	3	4	4
		n	10	7	5	7	5	6	3	6	12
Drawn points		$\bar{x}$	69	66	63	63	62	61	67	69	63
		SD	12	9	9	7	13	6	6	8	9
		n	12	9	7	9	5	6	3	6	12
Marked points		$\bar{x}$	38	32	32	34	35	39	36	41	38
		SD	4	4	4	3	5	2	3	2	3
	errors	%	2	6	3	0	3	6	5	10	9
		n	12	9	7	9	5	6	3	6	12
Deciphered letters		$\bar{x}$	39	33	30	32	33	38	35	41	37
		SD	6	5	6	4	6	8	4	5	3
	errors	%	0	7	7	2	0	0	0	2	0
		n	12	9	7	9	5	6	3	6	12
Arithmetic test %		$\bar{x}$	100		144		146	166		188	161
		SD	0		20		31	36		27	43
	errors	%	3		9		9	4		6	9
		n	3		3		3	6		6	9

[a] By permission of the publishers.

The 3-day Swiss–British dive conducted in February 1969 (13) to 1000 FSW (31 atm abs), in which there were three underwater excursions to 1150 FSW (36 atm abs) for a total of 5 hr, revealed no evidence of such a barrier. The breathing mixture was 1.5% O_2–3% N_2–95.5% He, and rate of compression was 1000 FSW /hr, with a decompression time of 88 hr.

With one exception, there was no significant impairment in the performance of such tasks as the ball-bearing test, and the arithmetic, visual analogies, and motor-coordination tests. The exception occurred during the first hour, upon reaching 1000 FSW, when there was a significant 50% decrement in the ball-bearing test (Table III). This and minor depressions in some other performances were possible indicators of He tremors. But after the first hour at depth, there was no major decrement in performance.

The EEG remained virtually normal, as did the AER. The men, wearing Draeger semiclosed breathing equipment (FG 111), were able to skin dive and to swim underwater against an ergometer at 1150 FSW (36 atm abs). Other work performed was the lifting of a 50-lb weight 20 times in 2 min.

The morale of the men was good; their leisure time was given special attention through the provision of games, construction kits, movies, reading matter, and good food. They experi-

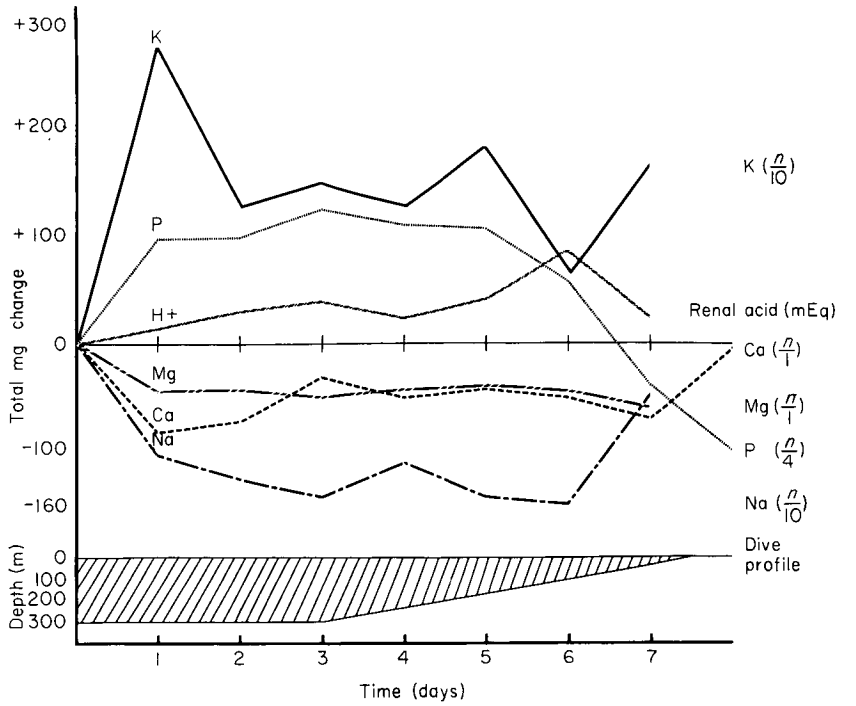

FIG. 10. Mean total mg change in electrolytes in 24-hr urine samples of three divers exposed to 1.5% O_2–3% N_2–95.5% He at 31 atm abs (300 m) for 3 days, with a 1-hr excursion at 36 atm abs (350 m) on day 1, and a 2-hr excursion (same depth) on days 2 and 3 (13).

enced some skin infection problems and ear irritation because of the relatively high humidity (78%) of their environment. These conditions were treated with tetracyclines. Among other problems experienced by the men were loss of taste and smell, and the usual difficulty in communication because of He speech distortion.

One of the most significant changes was in the 24-hr urine biochemistry. There were falls in calcium, sodium, and magnesium ions that recovered during decompression; phosphorus levels first rose and then fell. These changes were accompanied by an increased net excretion of acid (Fig. 10).

Now phosphaturia is associated more with respiratory acidosis than with metabolic acidosis. These changes are similar to the changes in electrolytes measured in compressed air dives to 300 FSW (10 atm abs); in such dives, respiratory embarrassment can be expected although N_2 narcosis may be an associated factor. However, Schaefer and Carey (32) have reported no elevated alveolar or blood CO_2 during a similar 1000 FSW dive, but did note an increased urine CO_2 output.

The relevance of these findings to the problems encountered during the dive reported by Brauer (12) and to the lack of them during the Swiss–British exposure (13) remains to be determined; but on the basis of the latter work, the limits of deep diving appear yet to be reached.

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NEUROPSYCHOLOGICAL EFFECTS OF EXPOSURE TO COMPRESSED AIR

P. M. Criscuoli and G. Albano

In previous papers (1–4) we proposed that the term *neuropsychic syndrome of compressed air* (NSCA) be used to describe the group of biophysical and neurochemical signs and symptoms of the CNS caused by breathing certain gases under high pressure. Our research began with an analysis of the individual roles played in this syndrome by such gases as N_2, O_2, and CO_2. That narcosis can be caused by inert gases under high pressure has long been recognized (7, 15). Nevertheless, we believe that some symptoms manifested during compressed air exposure, such as psychomotor excitability, are not part of the N_2 narcosis syndrome.

To study the effects of N_2 narcosis thoroughly, we measured the EEG changes in white rats compressed slowly (to avoid hypercapnia) to 21 atm abs with pure N_2 added to ambient air (5). Figure 1 demonstrates the typical EEG patterns in N_2 narcosis with progressive synaptic blockage of the mesodiencephalic system (brain stem reticular formation) (5, 11).

The biological effects of HPO (13) and the EEG patterns of O_2 convulsions (16, 17) are widely known. We have shown (Fig. 2) that the addition of He to the breathing mixture of animals subjected to HPO does not modify substantially the EEG pattern in O_2 toxicity. (The breathing mixture consisted of O_2 at 4.4 atm and He at 16.6 atm.) The EEG patterns 10–15 min after the beginning of pressure exposure show hypersynchronized spindle bursts dispersed by mild slow-wave polymorphous activity, tending to desynchronization. Although the animal's behavior did not change grossly at this point from its normal behavior, the wave pattern suggests a preepileptic phase, which lasted for some time.

Some 50–60 min later, muscular tremors developed, followed by twitching and, finally, tonic–clonic convulsions (second tracing, Fig. 2). The EEG is now similar to that of a typical grand-mal epileptic seizure (epileptic phase).

In another experiment (Fig. 3) in which air was administered at 21 atm abs, both phases of EEG changes occurred, to our surprise, but with modification from those observed in the O_2 and O_2–He experiment. In the preepileptic phase (3–5 min following compression), the spikes appeared more frequently and persistently. The dominant rhythm was θ polymorphous activity that never tended toward desynchronization. This phase lasted for 5–20 min and was followed by an intermediate phase, during which there was a slow dominant rhythm, which was continuous, with occasional superimposed spikes.

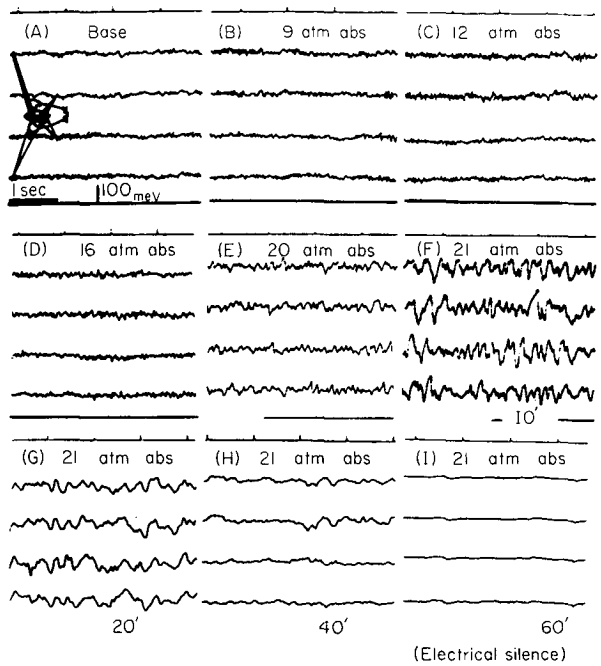


FIG. 1. EEG records of a white rat compressed with pure N_2 added to ambient air. [From Albano *et al.* (5).]

The epileptic phase (40–45 min) was characterized by hypersynchronous modulations with persistent, polymorphous spikes, during which we observed brief and repeated hyperkinetic activity in the animal. From these observations, we concluded that the epileptic phase is uniquely characteristic (to say nothing of dangerous) in the NSCA syndrome, and consists of an O_2-induced seizure (paroxysmal EEG activity) that can be eased by an early impairment of inhibitory synapses caused by N_2 narcosis (10), as this impairment lowers the convulsion threshold.

Since the dynamic physiopathological effect of the NSCA has been demonstrated, it is not valid to speak of the synergistic effect of HPO with respect to N_2 narcosis (12); instead, the N_2 narcosis may reduce the neurotoxic discharge. To test these concepts, we studied the effect of high N_2 pressure in another condition of neuronic hyperpolarization, such as that which occurs when adrenalectomized rats (6) are exposed to high P_{N_2}.

In these earlier experiments we administered a P_{O_2} of 1 atm. We observed that at 21 atm abs, the paroxysmal EEG record (Fig. 4) was similar to that observed with HPO, confirming the effect of N_2 on the inhibitory synapses and on the convulsion threshold.

NSCA Syndrome in Man

In order to give a more detailed outline of the effects of compressed air on man, we conducted a series of experiments at pressures of 2.5, 10, and 12 atm abs. The EEG changes observed at these O_2 and N_2 pressures (Fig. 5) were less pronounced than they were in the animal

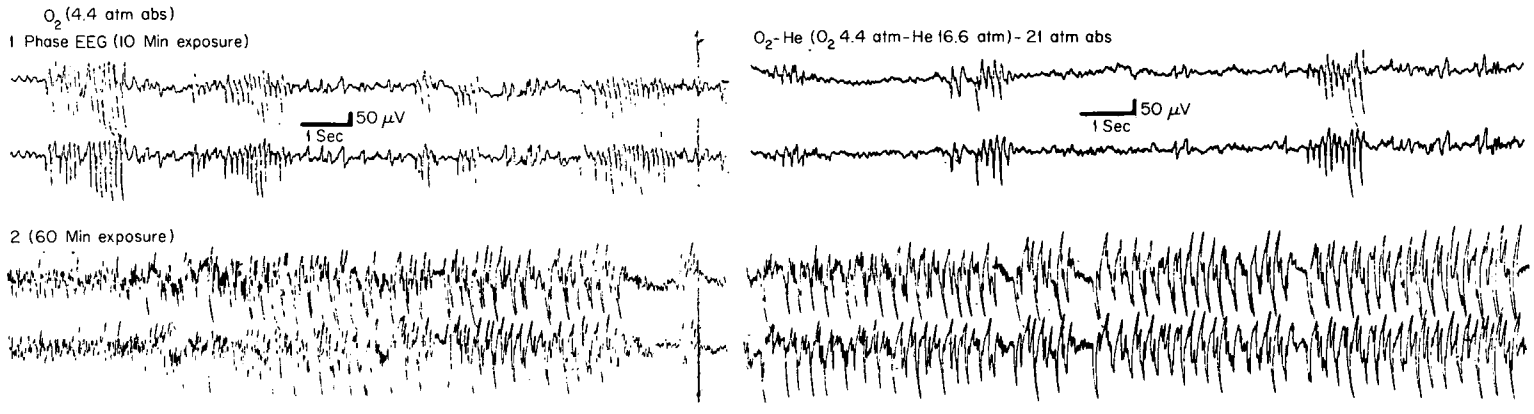


FIG. 2. EEG records of a white rat. Comparison between pure O_2 and O_2–He toxicity.

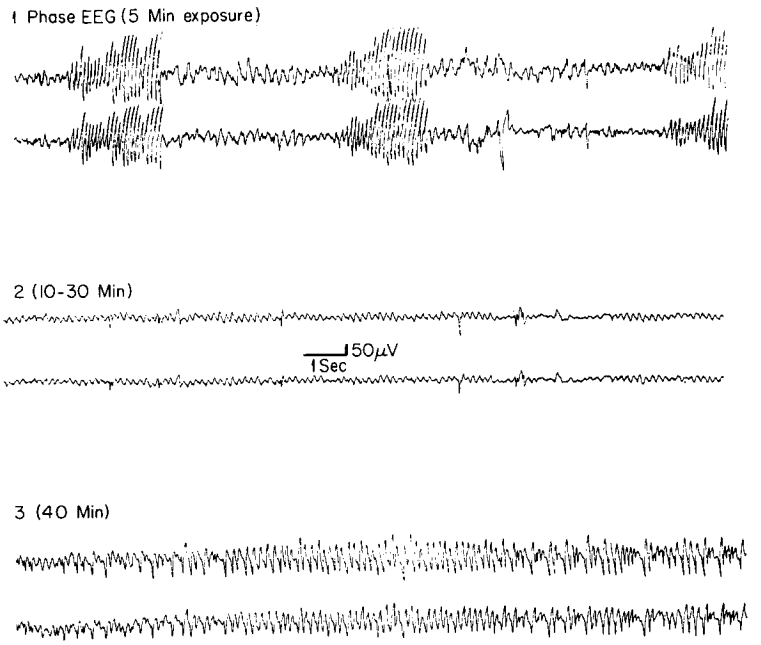


FIG. 3. EEG records of a white rat breathing compressed air at 21 atm abs (P_{O_2}, 4.4 atm; P_{N_2}, 16.6 atm).

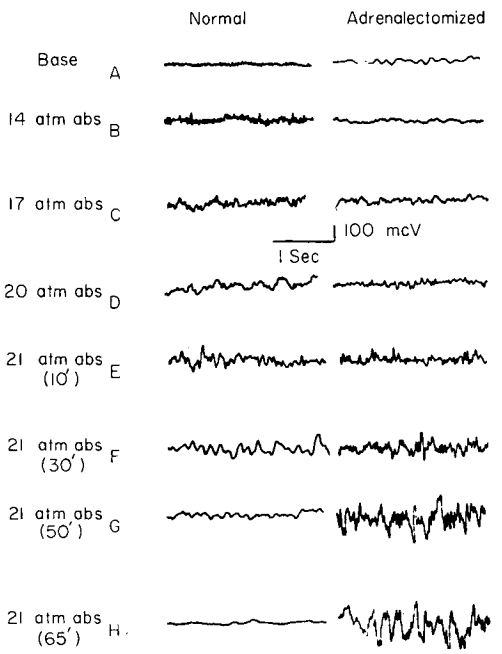


FIG. 4. EEG records of normal and adrenalectomized rats submitted to high N_2 pressure. [From Albano *et al.* (6).]

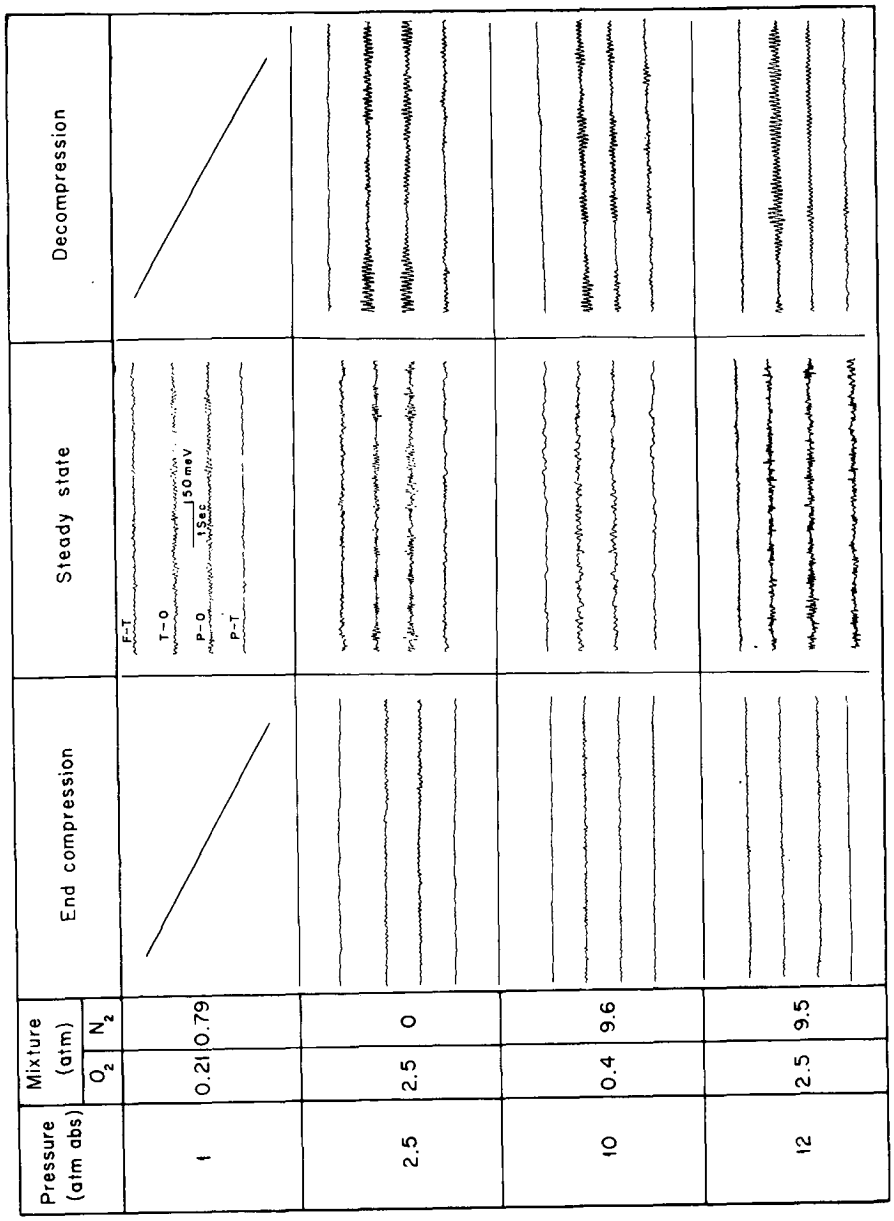


FIG. 5. EEG records in man under various experimental conditions.

experimentation; yet an accurate comparison of the EEG records in the steady state of three different experimental conditions (pure O_2 at 2.5 atm abs, 4% O_2 with N_2 at 10 atm abs, air at 12 atm abs) demonstrated a mild slowing of electrical frequencies. A prenarcosis electrical pattern was observed when an O_2–N_2 mixture was breathed at 10 atm abs; and signs of cortical hyperexcitability (high amplitude of dominant rhythm, with occasional superimposed, low amplitude spikes) developed during administration of O_2 at 2.5 atm abs. When air was administered at 12 atm abs, we observed mixed records.

The EEG records taken during compression (performed at the rate of 3 atm/min) and upon arrival at maximum pressure were desynchronized in all subjects. This was attributed to excitation of the reticular activating system owing to hypercapnia occurring during compression (4). The excitation was attenuated within 5–7 min after maximum pressure was reached. During decompression a progressive return to more normal EEG activity occurred in all subjects, but with synchronous, subcontinuous, high amplitude α waves, which were indicative of hypocapnia caused by decompression.

Psychological tests were used to help evaluate the subjects' responses, to indicate any defects in memory, calculation, and judgment, and to assess precisely their ability to handle practical situations calling for performance and manual dexterity. Of the different types of psychometric tests administered, some deserve particular attention (Fig. 6).

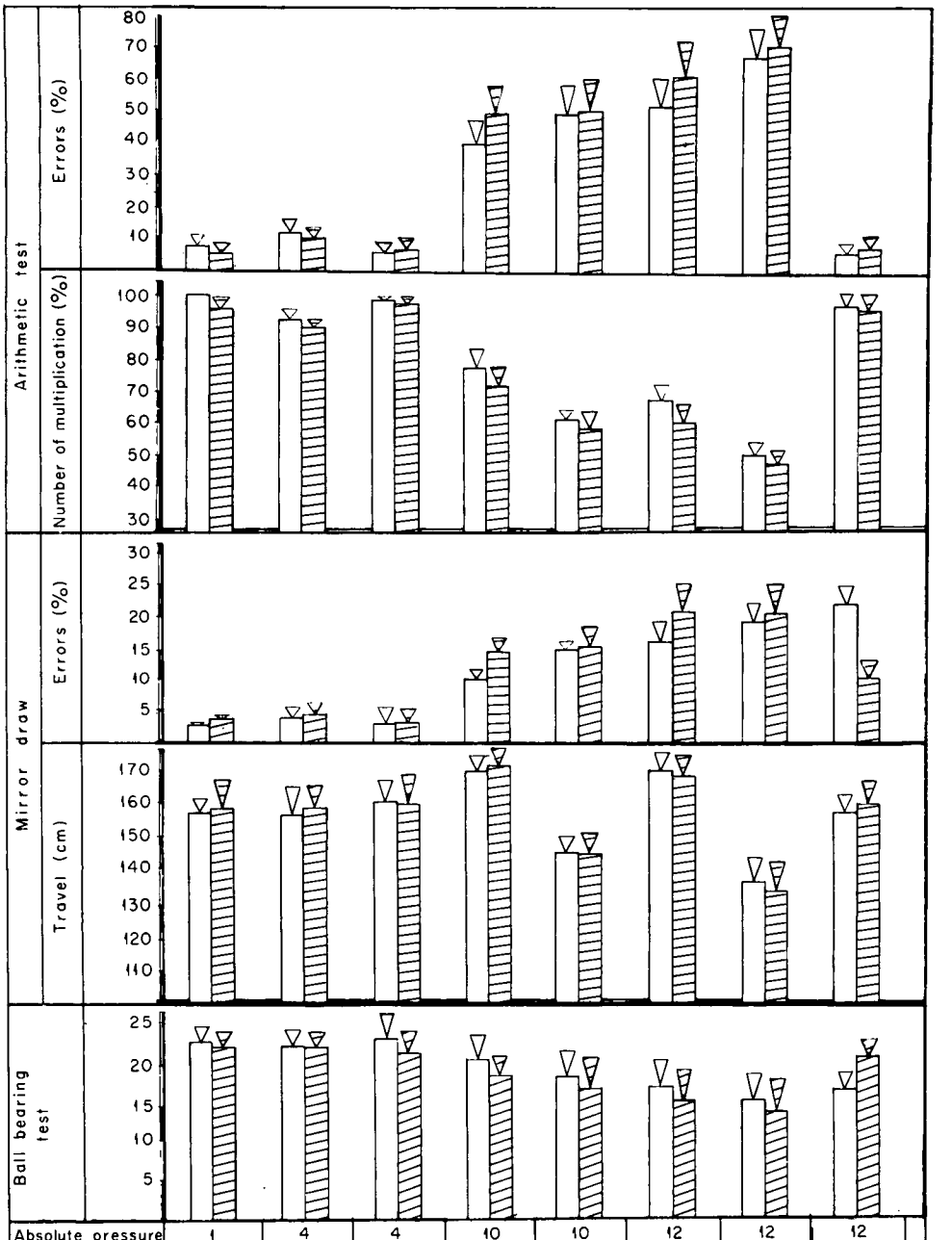
The Arithmetic Test and the Mirror Drawing Test, described in earlier work (2), have pointed out remarkable variations both in the number of tasks executed and in the error percentages tabulated under different experimental conditions. The error percentages appear to be dependent upon the P_{N_2} (threshold: 3.6 atm) and—in the hyperoxic mixtures only—upon the extent of the individual's activity. Indeed, it is recognized that the magnitude of HPO determines the degree to which ventilatory response is impeded during exercise (14), and that CO_2 retention synergizes N_2 narcosis (9). On the other hand, we could establish no valid relationship between the density of the breathing mixture and the error percentages.

The P_{O_2} correlates well with the subjects' performance on the Mirror Drawing Test. Indeed, at both 10 and 12 atm abs with hyperoxic mixtures, the distance traced per unit time was greater than the distance covered when the subjects breathed air.

A He tremor was detected in the subjects during the first phase of exposure at 12 atm abs in the Ball Bearing Test (8) and in the Mirror Drawing Test. Trembling decreased rapidly as the exposure continued, and almost disappeared after the subjects exercised (about 25 min).

A corresponding difference in psychological behavior was noted to be dependent upon the particular breathing mixture used. Exposure to high N_2 pressures, with normal P_{O_2}, caused a progressive impairment in the subjects of the vigilance state, ideation, fixation memory, practical activity, and affective self-control. Logical thought and attenuated associative processes were clouded, as well as the capacity for and the consistency of critical synthesis. The use of sensory functions for practical purposes was also impaired. Furthermore, the subjects exhibited reactive dysphoria, which tended to break down under external stimulation.

The exposure to compressed air at 12 atm abs produced a different symptomatology. The qualitative change in the conscious state was distinguished by euphoria and was accompanied by irritability and impulsiveness. Frequently, there was perceptual dysfunction, particularly in the senses of touch, sight, and hearing. Distal motor hyperkinesis and subliminal tremor impaired working ability. Execution of physical tasks remained defective during the test period, and the habitual motor acts were often performed with perseveration. The subjects displayed childish behavior and were obsessed by some particular detail of their tasks.



Absolute pressure	1	4	4	10	10	12	12	12
O_2	0.21	0.4	2.4	2.1	0.4	2.5	0.5	0.5
N_2	0.79	3.6	1.6	7.9	9.6	9.5	11.5	—
He	—	—	—	—	—	—	—	11.5
Density related to atmospheric air	1	3.92	4.19	10	9.73	12	11.68	2.14

FIG. 6. Results of some psychometric tests performed while the subjects were at rest (white bars) and after exercise (crosshatched bars) (350 kpm/min for 10 min) under various experimental conditions.

Finally, all our experiments tend to underline the fact that the neuropsychological effects of exposure to compressed air appear to be like a polymorphous syndrome. The symptoms of N_2 narcosis are more apparent at low air pressures (up to 12–15 atm abs) than are other effects of the air. Under greater pressures, the neurotoxic effects of exposure to the O_2 in air—e.g., the neuronic hyperexcitability manifested in behavior, EEG records, and the psychomotor tests—are already detectable at 10 atm abs and can terminate in an epileptiform seizure of O_2 toxicity. This syndrome is predictable, and can be eased by a lowering of the convulsive threshold by the blockade of inhibitory synapses through N_2 narcosis. Carbon dioxide retention resulting from alveolar hypoventilation during work in compressed air causes the entire constellation of symptoms to worsen.

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HUMAN PERFORMANCE AT GREAT DEPTHS

R. J. Biersner

Undersea technology has progressed at such a rate that the human organism may soon be the only limiting factor in diving systems. Narcosis, O_2 toxicity, tremors, and breathing resistance are just a few of the dangers that threaten personnel involved in advanced diving operations, and these dangers will continue to pose new problems as greater depths are reached. In studying these problems, performance has become an important index of the physiological state and usefulness of the diver. Performance indices may also be helpful in selecting personnel for hazardous diving conditions.

Behavioral techniques have been used to evaluate diving performance for many years, and they still serve as reliable and practical indicators of O_2 toxicity and inert gas narcosis. Perhaps the most extensive study to date of human performance in underwater environments was completed during SeaLab II (6), in which the subjects breathed an O_2–N_2–He mixture. Although the results of these studies showed that man could function adequately under the conditions of that experiment, problems did arise that previously had not been considered important in diving. It was found, for example, that some kinds of performance can be impaired by the fears and anxieties associated with a hostile environment. Divers who reported heightened feelings of fear and anxiety were rated lower on assigned projects, and they participated less in group activities than those not so fearful and anxious. The degree of psychological stress was found to differ according to education, place of birth, and birth order. Little has been done to further define these variables, or to devise tests that could be used to select personnel for such hazardous conditions.

The results of other deep He dives reveal a progressive deterioration in performance when pressure depth approached 1300 FSW (3, 7). Accurate interpretation of these results is difficult because the studies are not always comparable; there are often no control subjects in such studies, and often only a small number of experimental subjects is involved. It is generally agreed, however, that motor coordination shows the greatest decline under high pressures, whereas cognitive and intellectual functions undergo only small transitory changes. It is unclear, however, whether the impaired motor performance is related to hypercapnia, compression rate, temperature, or psychological stress, or to some combination of these factors. Moreover, the extent of this impairment cannot be fully appreciated until the performance of several different motor tasks has been used to evaluate it.

Cognitive performance during deep He dives is even more variable and uncertain, prompting one investigator (7) to suggest that marked individual differences may reflect psychological adjustment to the particular diving hazard, and that these differences may be useful in screening divers for hyperbaric duty.

Not much is known about human performance at great depths, and the few observations available are often rendered unreliable by such nebulous factors as fear and anxiety. It is only reasonable to assume that the risks and dangers involved in experimental diving will be reflected in performance and they must therefore be appropriately controlled. As diving becomes increasingly hazardous, moreover, the need to reduce or eliminate psychological stress for the sake of task efficiency will become more critical.

Little has been done to study the psychological stress of diving or to assess various mechanisms that might be used in combating it. A better understanding of such stress would be highly useful in the selection of personnel for hazardous diving duty. The problem is to find a measure of stress that not only is objective and valid, but also differentiates among individuals. Such an approach, incidentally, has been used successfully to study psychological stress in parachutists (4). These studies show that experienced and skillful jumpers apparently become conditioned to the autonomic effects of danger, which allows for voluntary inhibition of arousal before it become excessive, and which provides a useful method for coping with threat. As a result, jumpers confidently perform many difficult maneuvers during free fall, which is the period of maximum threat. The psychological stress of deep saturation diving is probably more severe (and is certainly more prolonged) than that of parachuting. The means used to adapt to it should be of concern not only to divers but also to those persons involved in the surface control of the diving program. An attempt was therefore made in the present study to define the pattern of psychological stress during a deep saturation dive.

Psychological stress may be generated by many factors during a hazardous experimental dive, one of which is increased concern about the environment. Several studies (8) have revealed that certain perceptual tasks are particularly sensitive to psychological stress. The performance of these tasks, which usually require the subject to locate a simple figure within a complex design, has been found to correlate highly with environmental dependence. Using one of these perceptual tasks, the author demonstrated (unpublished report, 1968) that U.S. Navy divers are significantly more independent of their environment than are a comparable group of nondiving naval enlisted men. A modified version of the same task was used during the present deep saturation dive to study perceptual changes that might have occurred because of increased dependence on the environment.

Two other performance measures—manual tracking and a memory test—were used to study motor performance and narcosis. The greatest deterioration in motor coordination is known to come about as the result of tremors, whereas impairment of memory is one of the best measures of narcosis (1). Motor performance and memory were considered to be the most important factors studied during the present experimentation, because significant impairments in either would impose serious limitations on future deep diving.

Method

This investigation was undertaken as part of a deep saturation dive made by the Navy at Duke University during December, 1968. The diving procedures used have been amply des-

cribed by others in this volume (cf. Salzano *et al.*). The experimental group consisted of five divers: two civilian diving technicians and three professional divers. There were also five divers in a control group: one civilian diving technician and four professional Navy divers. Control and experimental divers were matched as closely as possible for age, diving experience, and occupation.

Performance was studied through the use of an automated measurement system, which included a magnetic keyboard activated by the subject diver, a set of tracking controls, and a rear projection screen for presenting visual test material. A master control console was operated by the experimenter to program the tasks and display the results. The four performance tests included measures of psychological stress, environmental dependence, memory, and motor coordination.

The performance tests were administered during a shallow training dive at 320 FSW held 1 week before the experimental dive, and at 900, 1000, 500, and 50 FSW during the saturation dive and subsequent decompression. A final testing session was held 2 days after decompression. Each of the performance tests was also administered to the control group six times, at time intervals approximating those of the experimental group, in order to control for possible changes in performance resulting from practice rather than the experimental conditions.

Psychological stress was evaluated using the Stroop Color Conflict Test (SCCT), which consisted of two control slides and a test slide. One of the control slides, containing words describing colors, was used to establish a baseline for word identification. The other control slide consisted of blocks of colors which provided a baseline for color identification. The test slide contained words that were printed in colors different from those described in the control slide and required the subject to identify the color and ignore the word. A performance score was derived by averaging the time taken to complete the two control cards, and then subtracting this figure from the time taken to finish the test card. This test procedure is often used to induce psychological stress (2), and it was considered possible that the additional stress of a hazardous environment might magnify any effects.

Environmental dependence was measured by use of the Hidden Patterns Test (HPT) (5). This test required the subject diver to determine whether a simple standard shape was present in each of 200 complex patterns. The score was based on the number of errors made, as well as the length of time taken to complete the 20 slides on which the complex patterns were printed.

Memory was tested using a simple word association test designed by the author that involved learning 12 pairs of word and number combinations projected on the screen inside the chamber. The same 12 words were used during each testing session, but were arranged differently each time. The two-digit numbers associated with each word were varied at random for each test session. Three minutes were allowed for learning the associations, followed 60 sec later by a test slide containing only the rearranged words. The score consisted of the number of associations correctly recalled.

Motor coordination was evaluated by means of a simple tracking task. Viewing an oscilloscope located at one of the ports, the diver used a single manual control stick to follow a target dot moving in a circular path around the screen. A trial lasted 60 sec, and five consecutive trials were made during each test session. The final score was the average voltage error obtained for the horizontal and vertical display axes during the five trials.

Results

The results revealed a general decline in performance as pressure increased. However, there were noticeable differences in performance among the four tasks, suggesting that the impairment was not the result of any single factor. Although the saturation divers did not vary significantly from control divers in performance on any of the initial tasks (Figs. 1 and 2), performance at 900 FSW during compression was markedly different. Figure 1 demonstrates that significant changes occurred in the SCCT ($p < 0.05$), and Fig. 2 shows similar results in manual tracking ($p < 0.025$). Performance of the experimental subjects on the HPT and the memory task did not differ significantly from that of the control group.

After 3 days on the bottom, the performance of the experimental subjects had improved to such an extent that only their manual tracking remained statistically different from that of the control group ($p < 0.01$). Performance continued to improve during the ascent, and was essentially normal during the final stage of decompression. The results of the tests taken by the divers at surface pressure 2 days after completion of the dive did not vary significantly from those of the controls.

Although performance was noticeably impaired at 1000 FSW, such deterioration is not

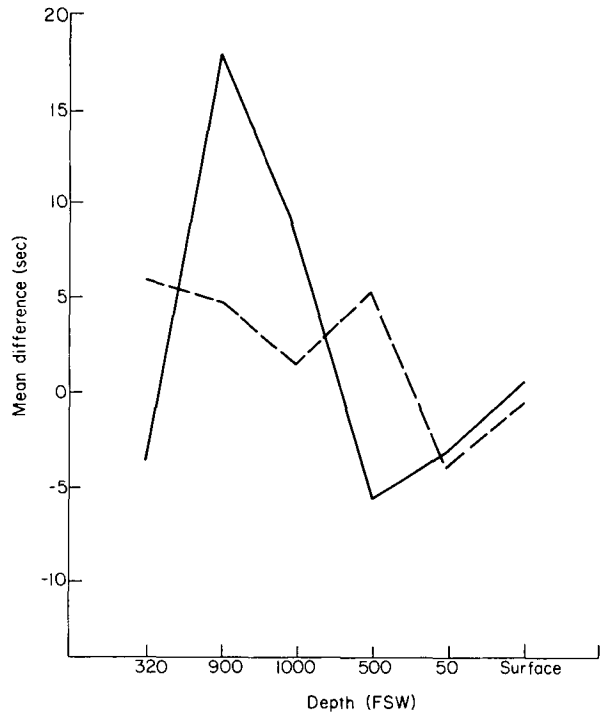

FIG. 1. Results of the Stroop Color Conflict Test (SCCT) (for psychological stress) during the six test sessions. (——) Saturation divers; (— —) control divers.

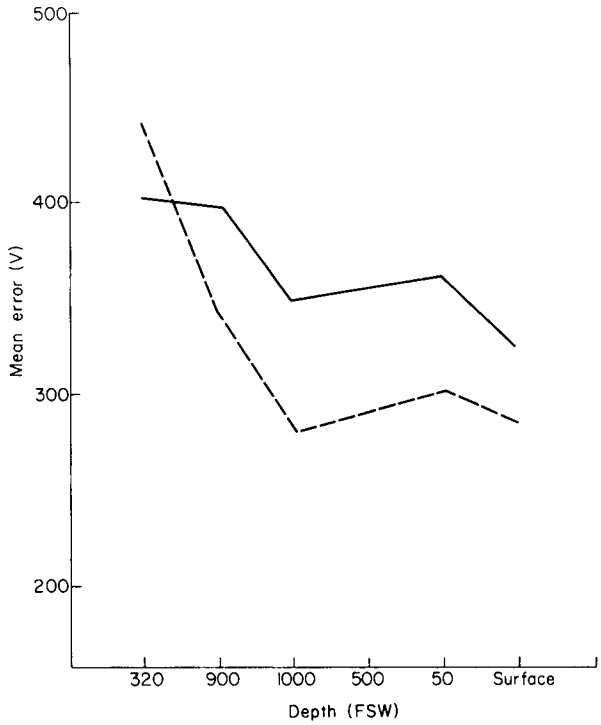

FIG. 2. Mean voltage error on the tracking task performed by saturation and control divers (motor coordination). (——) Saturation divers; (— —) control divers.

considered irreversible. Normal memory performance at this depth suggests an absence of narcosis, a contention that is substantiated by improvements in the SCCT. However, performance trends on the SCCT indicate that the divers experienced marked psychological stress and increased concern about their environment during descent, but that they began to adjust to these distractions while on the bottom. Only their motor performance was significantly impaired on the bottom, and remained so until after their return to the surface (Fig. 2).

Several factors might account for the motor decrement, such as compression, temperature, humidity, and fatigue. The results suggest a combination of these factors; but since the decrement persisted throughout the lengthy decompression while performance of the other tasks improved, the indication is that fatigue was probably not significantly involved. The divers did experience joint pains during compression and while on the bottom, the pain decreasing noticeably during the early stages of decompression. Moreover, the divers found that control of temperature and humidity was inadequate, and they complained of periodic shivering. The persistence of the motor impairment, which stabilized after 3 days on the bottom and continued until the end of decompression, suggests that environmental factors, especially temperature and humidity, were primarily involved. The marked partial recovery that occurred after the divers reached the bottom (Fig. 2) indicates that compression effects, if present, were transitory.

Discussion

Performance in this study indicates that (*1*) psychological stress and environmental dependence increase during the initial phase of a hazardous dive, but tend to decline noticeably during the period of maximum threat; (*2*) motor performance shows prolonged impairment under the conditions of this study; and (*3*) learning and memory capacities apparently remain normal at 1000 FSW. This last finding is perhaps the most important. Impairments in cognitive performance, especially immediate memory, are probably the most reliable signs of narcosis. These results indicate that He is not detectably narcotic at 1000 FSW, and that divers can perform at this depth without threat of He narcosis.

The foregoing conclusions are supported by the results of two other studies completed during this dive. A study by L. W. Thompson (personal communication, 1969), who used a complex reaction time task, found that mean reaction time increased only slightly at 1000 FSW. This impairment was not statistically significant and could have been the result of other factors, such as psychological stress. The results of EEG and evoked potential studies by W. P. Wilson (personal communication, 1969) were also normal under all conditions.

The effects of compression and environmental factors on performance at depth are serious, however, and require further investigation. The results of the present investigation suggest that important changes need to be made in environmental control during deep saturation dives to improve not only the subjective comfort of the divers, but also their efficiency in performing their assigned tasks. Unless effective efforts are made to control the environment more adequately, a diver's ability to perform difficult motor tasks at depth will remain significantly impaired.

The results of the tests of psychological stress and environmental dependence were similar to those of the parachutist studies mentioned earlier. These studies (4) have correlated performance with several physiological indices of stress. Recordings of skin conductance, heart rate, and respiration rate, obtained from parachutists of various experience and skill, have been correlated with actual performance and instructor ratings. Experienced parachutists show a steady but modest rise in physiological activity that declines rapidly before the jump is made. Novice parachutists, however, show a marked increase in activity that continues until after the jump is made. The same is true for skilled and unskilled jumpers in that the physiological activity of those judged as skillful is significantly reduced. The experienced and skillful divers tested during this study showed a similar pattern of performance—a decrement during compression and an improvement while they were on the bottom. Moreover, there were noticeable differences in performance among individuals, suggesting that these tests may be useful in the selection of personnel for hazardous diving conditions.

It is apparent from the present investigation that divers can perform normally at a simulated depth of 1000 FSW. Given the skills and necessary training to overcome threatening environmental conditions, along with better control of the environment itself, divers may soon live comfortably at this and greater depths.

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EXPERIMENTAL STUDIES ON THE HIGH PRESSURE HYPEREXCITABILITY SYNDROME IN VARIOUS MAMMALIAN SPECIES

R. W. Brauer, R. O. Way, M. R. Jordan, and D. E. Parrish

In 1965 we began a series of investigations to establish the relative narcotic potency of H_2, He, and N_2, a comparison that is of considerable theoretical interest. Early experiments using rhesus monkeys revealed that under pressures at which early narcotic manifestations of either He or H_2 might be expected, the reaction was not narcosis but, rather, severe convulsive seizures. There was a suggestion that such seizures might be less frequent on H_2 than on He, and that they might occur at slightly higher pressures (3). Subsequently, a similar response pattern was observed to occur in mice at somewhat higher pressures than in the monkeys. Furthermore, H_2 produced narcosis rather than convulsions in a high proportion (between 80 and 90%) of the strain of mice we used (7).

Since convulsions preclude meaningful quantitative evaluation of the relative narcotic potency of He and H_2 at high pressure, an alternative technique was developed based on the concept of linear additivity of the narcotic effects of inert gases in binary mixtures. This technique led us to use a series of He–N_2 and H_2–N_2 mixtures to determine their relative narcotic potency by mathematical analysis of the resulting curves (Fig. 1) (6). Helium was thus shown to exert a negligible narcotic effect, whereas H_2 was shown to be about one-fourth as narcotic as N_2 is. A correlation of these results with other available data is shown in Fig. 2, demonstrating that narcotic potency is somehow related to molecular interaction, possibly of a type involving bonding based on the induction of dipole moments in the inert gas molecule [cf. Featherstone and Muehlbaecher (10)].

The syndrome of tremors and convulsions observed in monkeys and mice will be referred to hereinafter as the *high pressure hyperexcitability syndrome* (HPH syndrome). In earlier experiments, we have observed the same general pattern of response in a number of other species of animals (Table I). Susceptibility to this syndrome appears to be greatest in the primates and to bear some rough relation to the extent to which the CNS has evolved. We have studied the syndrome more fully, to date, in the mouse and squirrel monkey than in other animals, and the following paragraphs contain a resumé of our findings.

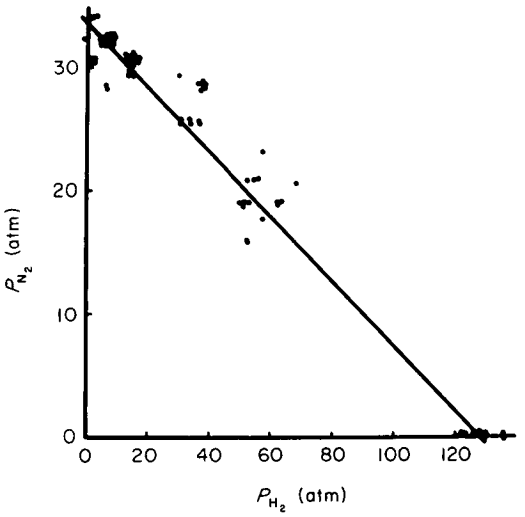

FIG. 1. Relation between the P_{N_2} and P_{H_2} at the point where loss of righting reflexes occurs in CD–1 mice compressed with mixtures containing these gases in various proportions, the P_{O_2} being held constant at 0.5 atm.

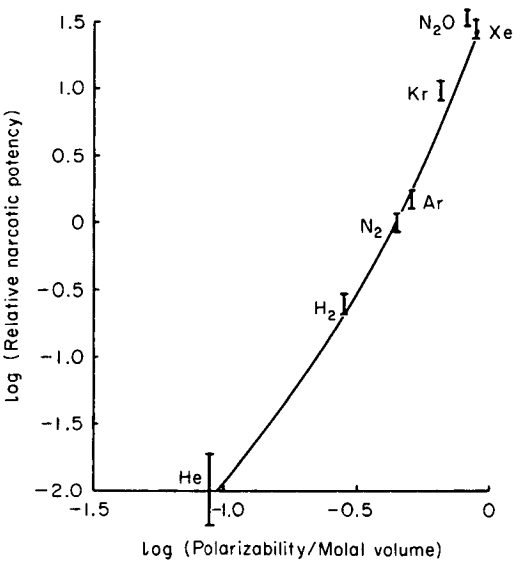

FIG. 2. The relative narcotic potency of He, H_2, and N_2O obtained by methods used in Fig. 1, together with data of other workers using other inert gases.

TABLE I

CONVULSION PRESSURE IN SEVERAL SPECIES
($He–O_2$, 22.8 atm/hr)

Species	Pressure at first convulsion (atm)
Birds	
Serinus canarius	101
Marsupials	
Didelphis virginiana	81
Rodents	
Mus musculus spp.	80–110
CD–1, 5 days old	63
CD–1, adult	98
Epimys rattus Norv. (S-D)	110
Oryclolagus cuniculus	99
Carnivores	
Procyon lotor	82
Primates	
Saimiri sciureus	61
Simia rhesus	57
Homo sapiens	22

Observations in Mice

We tried from the beginning to exclude from consideration, as far as it was possible, any trivial response to artifacts that inadequate environmental control might cause. The systems we used allowed precise control of P_{O_2} at any desired level. The systems also assured negligible CO_2 tensions throughout the experiments, and permitted adequate control of chamber temperature to $\pm$ 1°C. In all the experiments described hereafter, chamber temperature was kept between 30 and 33°C, which appears to be near the middle of the comfort range for mice in He or H_2 environments. In monkeys, this temperature range allows maintenance of constant euthermia, even in immobilized animals. To test the possibility that, in the relatively dense atmosphere encountered at the high pressures required to produce the HPH syndrome, hypoxia due to respiratory causes is not a factor, compressions were conducted using gas mixtures containing several different O_2 concentrations (4).

The convulsion threshold pressure was found to be independent of P_{O_2} in the range of 0.4 atm to approximately 2 atm (Fig. 3). At 2.4 atm, a significant depression of the convulsion threshold pressure was observed, suggesting synergism between the convulsant effects of O_2 and high pressure. Indeed, 2.4 atm of O_2 caused convulsions in our CD-1 mice only after 200 min of exposure, whereas 60 atm of He caused convulsions within 5 min. Synergism between the HPH syndrome and convulsant agents appears to be the rule rather than the exception.

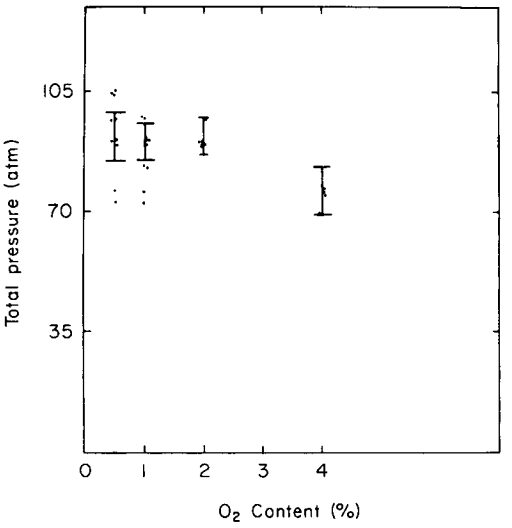

FIG. 3. Convulsion threshold pressures in CD-1 mice breathing He–O_2 mixtures of various O_2 content.

A further example of this type of effect is the depression of electroshock convulsion thresholds as a function of atmospheric pressures (Fig. 4).

The high gas density at which the HPH syndrome occurs may possibly interfere not only with O_2 transfer in the lung but also with CO_2 elimination. However, administration of an amine buffer in biologically effective doses fails to alter the mean convulsion threshold pres-

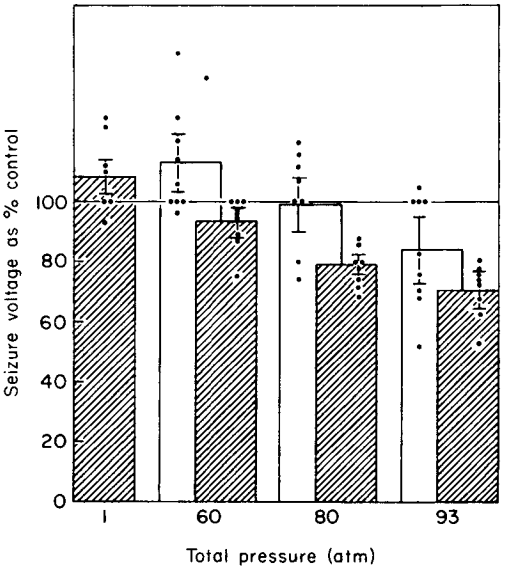

FIG. 4. Modification of electroshock convulsion threshold in CD-1 mice. (P_{O_2} is 0.5–0.6 atm throughout.) (▨) He–O_2; (□) H_2–O_2.

TABLE II

SUSCEPTIBILITY OF MICE TO THREE CONVULSANT CONDITIONS

	$He–O_2$ EP_{50} atm		O_2 T_{50} min		Audiogenic % not convulsing
BALB/CJ	89	BALB/CJ	10	BALB/CJ	35
DBA/2J	103	DBA/2J	17	DBA/2J	0
C57BL/6J	106	C57BL/6J	43	C57BL/6J	75
129/J	116	129/J	35	129/J	17.5
CD-1	99	CD-1	20		
A/J	82	A/J	15		

sure in mice, rendering this interpretation improbable (7). The relationship between gas density and altered respiratory mechanics in the HPH syndrome will be reviewed hereinafter in the light of data comparing the effects of different gas mixtures.

Mice of six inbred strains were compared with respect to their susceptibility to high pressure convulsions, hyperoxic convulsions, and audiogenic seizures [cf. Fuller and Sjunsen (11)]. The results, shown in Table II, suggest that these three responses are not related to one another, and, in particular, that susceptibilities to audiogenic seizures and the HPH syndrome do not correlate. Worth noting in Table II are the wide differences among the mouse strains in the animals' susceptibility to the HPH syndrome. These differences probably explain why this phenomenon did not emerge in earlier experimentation with mice compressed between 130 and 150 atm (15, 16).

Generalized intention tremors, and possibly some degree of spontaneous myoclonic seizure, occur in humans subjected to high pressures of $He–O_2$ (1), but the actual depth at which these effects are first noted depends greatly upon the compression rate. To ascertain to what extent in mice these He tremors might be related to the HPH syndrome, the animals' convulsion thresholds in a $He–O_2$ environment at P_{O_2} of 0.50 atm were compared at compression rates of 40 atm/hr and 5.1 atm/hr. The mean convulsion threshold at the faster compression rate was 98 atm, while at the slower rate it was 133 atm. The onset of visible tremors was postponed from about 50 atm (at the faster rate) to near 80 atm (at the slower rate). Considering our observation that severe tremors also occur when H_2 is used in the breathing mixture and that convulsions are only seen at very high pressures (or not at all), our tentative conclusion is that the underlying cause of convulsive seizures may be different from the one causing the HPH syndrome when He is contained in the breathing mixture.

In the mice as in the rhesus monkeys, H_2 produced fewer convulsions, and the seizures occurred at a higher mean pressure than when $He–O_2$ mixtures were used. These differences might be attributed either to the physical properties of the two gas mixtures (and hence to the smaller H_2 resistance) or to the higher narcotic potency of H_2. To clarify this matter, we determined the convulsion threshold of mice compressed with graded binary inert gas mixtures—$He–H_2$, $He–N_2$, and $He–N_2O$. In each case, an increased concentration in the mixtures of the narcotically active components resulted in an elevation of the mean convulsion threshold (Fig. 5). During compression, when the narcotic component reached a certain concentration at 130

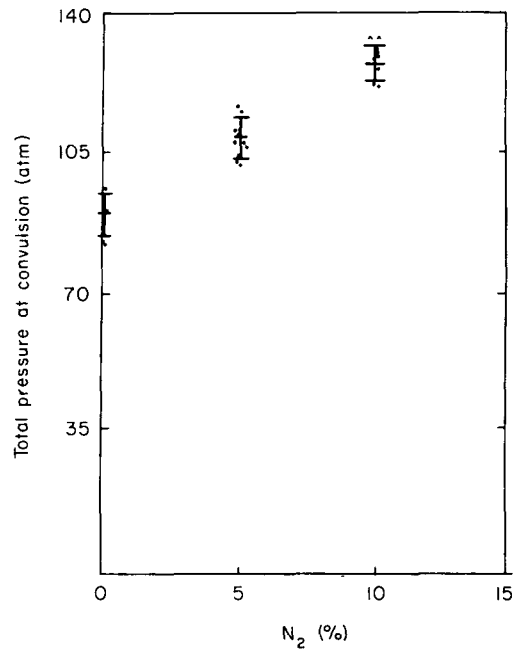

FIG. 5. Modification of convulsion thresholds in $He–O_2$ atm as function of the amount of N_2 added to the breathing mixture. Similar relations have been observed with H_2 or with N_2O admixture. Convulsions are no longer observed when 12% or more of N_2 is added so that the maximum pressure at which convulsions are observed before the onset of narcosis is approximately 130 atm. The gas concentration which produces 50% of this maximum convulsion threshold elevation is used for the calculation of relative anticonvulsant potency of the gases shown in Table III.

atm and no convulsions occurred, the majority of the animals showed loss of righting reflexes. Higher pressures then failed to induce convulsions.

Increasing the concentration of H_2, N_2, or N_2O in the $He–O_2$ mixture to a pressure midway between the pressure at which convulsions typically occurred in the $He–O_2$ mixture and the pressure at which convulsion frequency had dropped below 10% was used to calculate the

TABLE III

RELATIVE BIOLOGICAL EFFECTIVENESS OF METABOLICALLY INERT GASES

	Index of comparison (CD-1 mice ♂)	
	Loss of righting reflex	Elevation of convulsion pressure (He–0.5 atm O_2)
H_2	0.27	0.25
N_2	1.00	1.00
N_2O	28	35

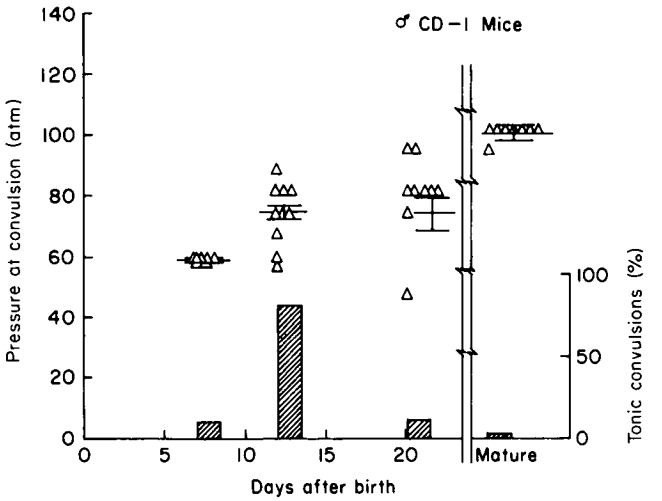

FIG. 6. Modification of convulsion thresholds with age in mice compressed with He–O_2 mixtures (P_{O_2} = 0.4–0.5 atm). The vertical bars refer only to the tonic seizure pattern.

convulsive potency of the three gases. Table III shows the results of such calculations, using loss of righting reflexes and convulsions as the indicators of narcotic potency. Since the two sets of values agree, one may conclude that indeed the narcotic potency of a specific gas is involved in the elevation of convulsion thresholds.

One may further conclude that modification of respiratory resistance—either downward by substituting H_2 for He, or upward by partial substitution of N_2 for He—fails to affect the convulsion threshold, except insofar as the gas acts as a CNS depressant. This latter conclusion agrees with the observations of other investigators, who suggest that nonvolatile CNS depressants also can counteract certain manifestations of HPH in mice and newts (17.) High pressure convulsions in the gill-breathing newt, furthermore, suggest that such responses are caused by hydrostatic pressure, as such, or by hydrostatic pressure changes; and that the convulsions should not be attributed simply to the gaseous environment in which pressure is exerted. These observations are not restricted to the newt—we have observed similar responses in mice breathing oxygenated fluorocarbon liquid, and our findings have been confirmed and extended by others (14).

A final series of tests shows that susceptibility to HPH convulsions is markedly greater in very young mice than in adults (Fig. 6), and that susceptibility decreases after the first 3 weeks of life. There is a curious change in the pattern of the convulsions. In the adult, the seizures are characteristically clonic; a tonic phase is rarely observed, if at all. A similar pattern occurs in very young mice. In numerous tests, however, 13- to 15-day-old CD-1 mice demonstrated a strong tendency toward tonic hyperextension seizures. A similar pattern was observed in adult mice only when the inert gas mixture contained 0.15% N_2O in He. These observations suggest that the seizures may originate not in the dendritic surface layer of the cortex (which is poorly developed in the young animal) but, rather, in some of the older, deeper layers of the brain. The significance of these curious changes in convulsive patterns with age remains to be determined by future investigations.

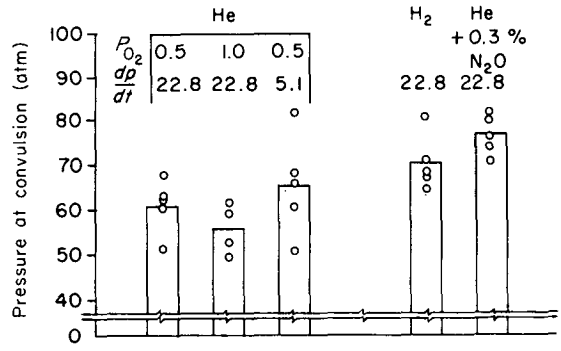

FIG. 7. Onset of convulsions in squirrel monkeys in various high pressure atmospheres as a function of P_{O_2} and compression rate.

Observations in Squirrel Monkeys

Elevating the O_2 pressure from 0.5 to 1.0 atm lowered the convulsion threshold of the squirrel monkey very slightly, if at all (Fig. 7). Slowing the compression rate from 22.8 to 5 atm/hr produced a slight but probably significant increase in convulsion thresholds, quite similar to the responses observed in mice (5). Substituting He for H_2 raised the mean convulsion thresholds in the squirrel monkeys as it did in mice. Addition of 0.3% N_2O to He produced a similar effect. In general, the responses of the squirrel monkey and the mouse closely

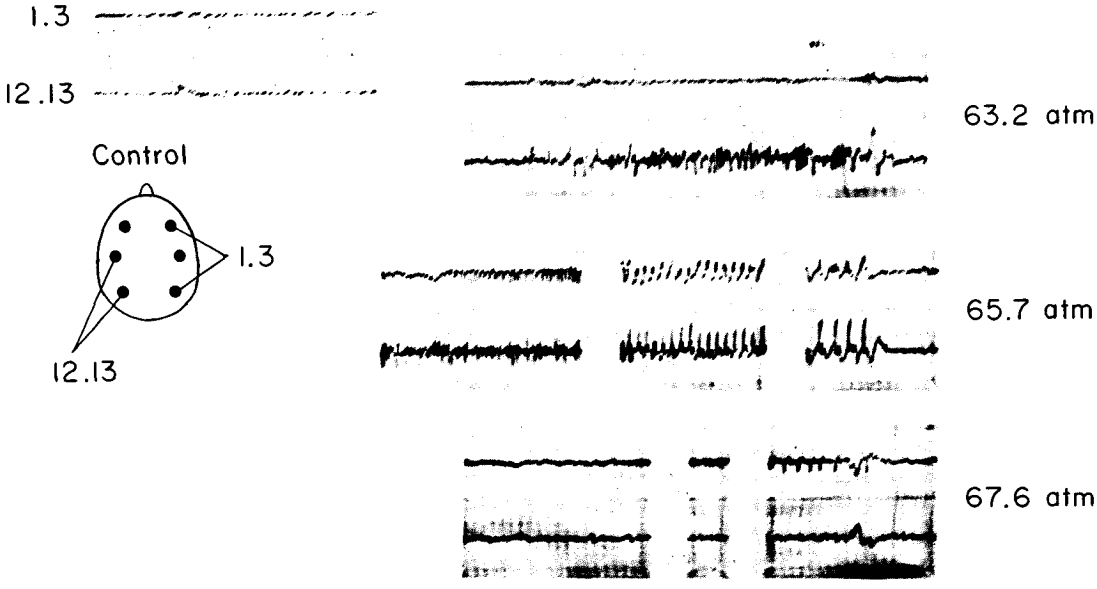

FIG. 8. EEG showing spontaneous seizures in a squirrel monkey compressed with He–O_2 at a P_{O_2} of 0.45 atm and a compression rate of 22.8 atm/hr.

corresponded, except that the monkey's convulsive seizures occurred at a lower pressure. There appears to be a marked difference between the lower mammalian species and the primates, judging from the results shown in Table I.

We studied the EEG changes associated with the monkeys' convulsions by implanting extradural electrodes. After at least a week's recovery, the monkeys were observed either in an unrestrained state or on a specially constructed restraining couch, the latter allowing for more detailed measurements. Compression, regardless of rate, has been associated with the eventual development of spiking and electrical seizure activity in all the animals (Fig. 8). At 63.2 atm, a localized seizure occurred; at 65.7 atm, a seizure started in the left temporo-occipital region and quickly became generalized, terminating in a postictal depression. The tracing at 67.6 atm illustrates that from this point forward, some measure of seizure activity was almost continuously present at one point or another in the brain.

Characteristically, tremors preceded any marked EEG change. The first clearly recognizable change was focal spiking, which at the outset was unaccompanied by any recognizable myoclonus. As the experiment progressed, focal spiking became more marked and electrical activity from epileptogenic foci sometimes appeared. Brief myoclonic seizures occurred along with the focal spiking. This suggests that a distinction may be made between the fine tremors associated with voluntary movements that have no concomitant in the EEG (which may be similar to He tremors in man), and the coarse tremors localized in a circumscribed muscle group that are not obviously associated with voluntary movements. As pressures were raised,

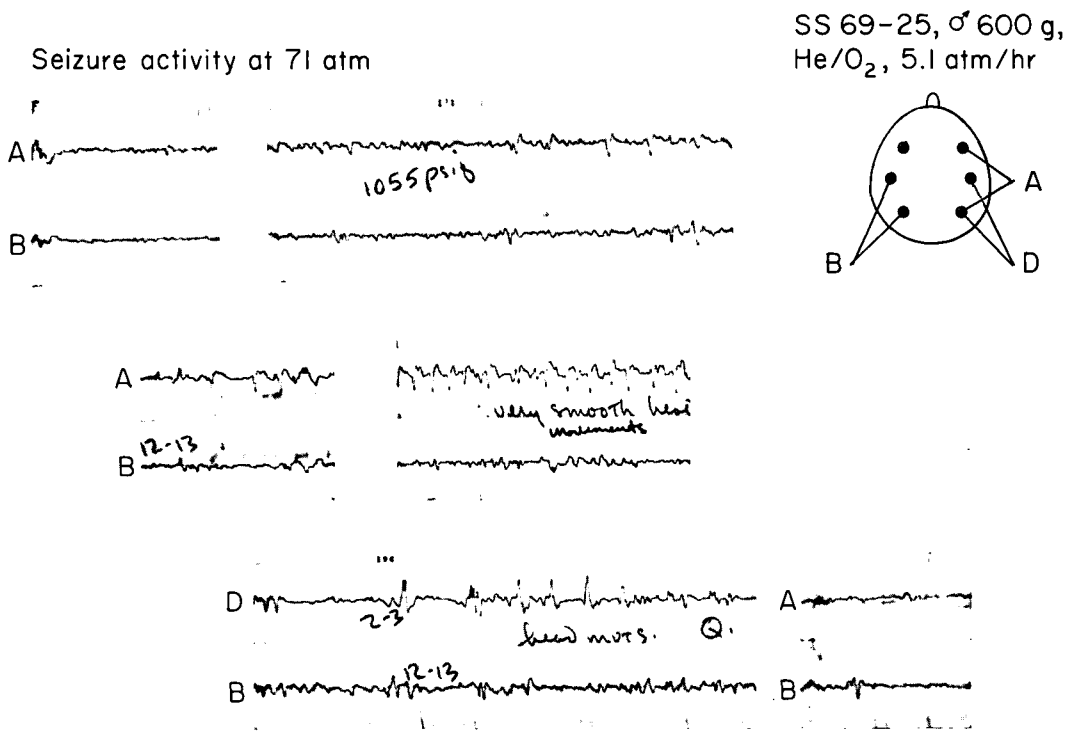

FIG. 9. EEG's of seizures associated with very limited motor activity resembling a petit mal seizure.

focal seizure activity became prolonged and more generalized. Several types of generalized seizures occurred occasionally and spontaneously at this point. The electrical seizure pattern either appeared simultaneously throughout the cortex, or by the spreading of a focal discharge (Figs. 8 and 9).

The EKG's taken through this period revealed no marked changes in heart rate or pattern, even just before generalized motor seizures began. Recovery after all but the most severe grand mal seizures was prompt. Generalized seizures were usually followed by postictal electrical pauses lasting 20–60 sec and by gradual return to a normal EEG. Once a generalized seizure began and compression continued, EEG seizure activity never subsided completely. Further occurrences of generalized seizures appeared to be dependent in part upon the degree of damage done during the first severe attack. A generalized seizure was sometimes followed by a 30-min period of relative quiescence marked by scattered focal spiking on the EEG, then by increasing hyperexcitability, and then perhaps by a buildup to another generalized seizure. If pressure was maintained at the level at which the first convulsion took place, convulsive

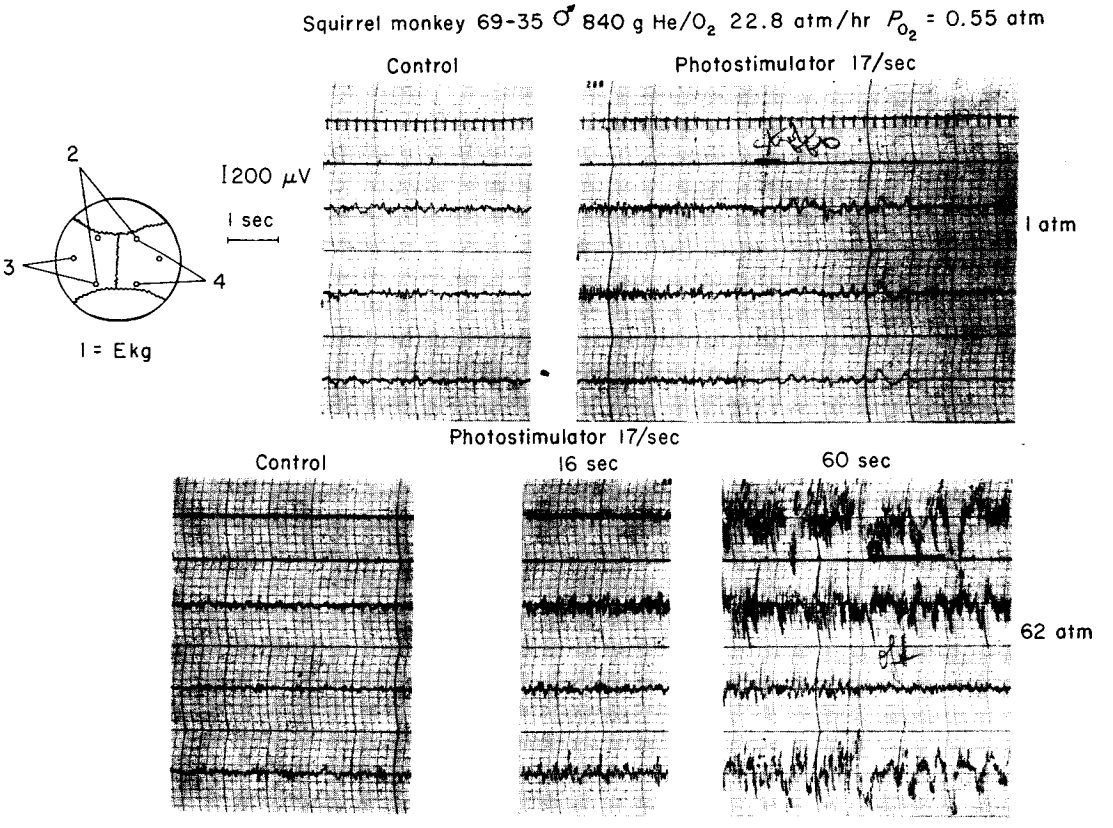

FIG. 10. Modification of the response to repetitive photostimulation as a result of compression. In the upper diagram the characteristic self-limiting response in the normal animal; in the lower tracing, marked recruitment, spiking, and eventual development of a grand mal seizure at 62 atm.

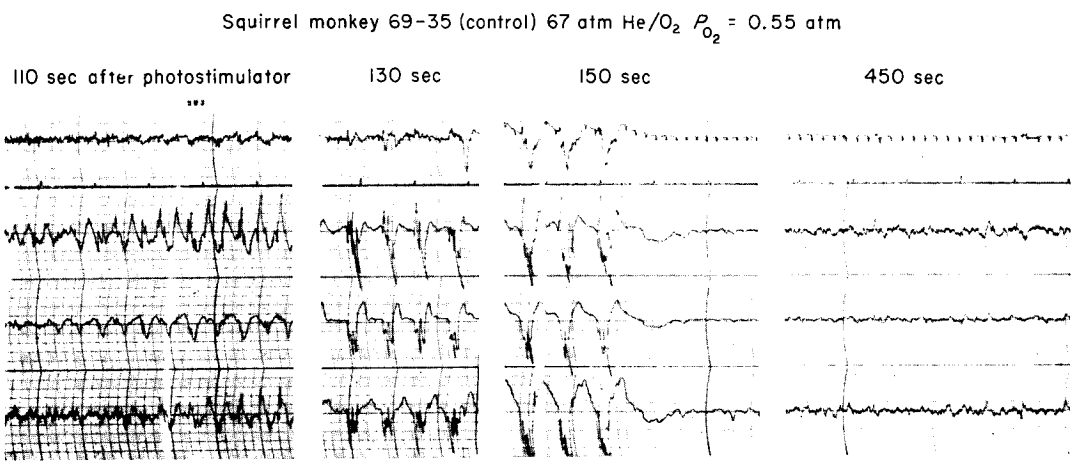

FIG. 11. Two minutes after the end of photostimulation shown in Fig. 10 there is a secondary buildup to a generalized petit mal seizure followed by a postictal period, and resumption of spiking activity 5 min later.

activity tended to subside after a few attacks; but seizures recurred as long as 12 hr after the first attack, and were accompanied by hyperexcitability a large part of the time.

While this paper was in preparation, we learned of two experiments conducted by Dr. X. Fructus and Dr. R. Naquet of Marseilles, France. In each experiment, a baboon (Papio papio) was compressed in an He–O_2 environment very slowly (approximately 3 atm/hr). The animals were held for 12 hr, one at a pressure of 60 atm and the other, 100 atm. The latter animal showed characteristic epileptiform EEG changes and began motor seizures at 92 atm. There then followed the pattern of quiescence and resumption of convulsive activity observed in our experimentation with squirrel monkeys. This animal died during decompression, and the preliminary analysis of the EEG and EKG suggests that death was caused by CNS damage that was not necessarily associated with the decompression sequence. The second animal was reported to have shown "alarming CNS changes" at depth, and it also died during decompression. A more complete analysis of these data is not yet available.

Experiments by Killam, Killam, and Naquet (13) suggest that generalized seizures can be induced in a given animal by repetitive photostimulation at pressures significantly below those that could reasonably be expected to provoke a spontaneous convulsion (Figs. 10 and 11). Above 50 atm, photostimulation produces an abnormal EEG pattern of numerous high voltage, high frequency discharges. If photostimulation is discontinued at this point, a few seconds of persistent self-sustained spiking activity may follow. At slightly higher pressures, recruitment and buildup of response amplitude become pronounced, and myoclonus occurs with each spike. In 30–40 sec this sequence tends to build up to a self-sustaining seizure. After the initial seizure, a brief period of near quiescence may ensue, followed, without further stimulus, by gradual recovery and then by a buildup to a second seizure, which now may be petit mal rather than grand mal.

EEG changes in squirrel monkeys exposed to high pressure He–O_2 environments appear in many respects to resemble those of idiopathic epilepsy. At the pressures we chose, significant changes may have occurred in the cortex. These changes may have triggered both the synchronous repetitive discharges seen in the EEG at various foci and the spread of seizure

activity. Future investigations must determine if the predominant site of the HPH syndrome is in the outer layers of the cortex, or if the responses reflect paroxysmal activity in deeper structures, such as the thalamus or hippocampus.

We have speculated very little up to this point about the biophysical mechanisms that might induce the HPH syndrome. Some suggestions regarding this aspect of the problem might be made here. There is a strong possibility, we feel, that the observed changes in CNS excitability are attributable either to the effect of hydrostatic pressure, as such, upon the membranes or upon the energy metabolism of CNS neurons; or to the magnitude of hydrostatic pressure used. Hydrostatic pressures from 50 to 150 atm are now recognized as being high enough to produce significant biological changes. Thus the Ussing frog skin preparation responds to hydrostatic pressures of 50 atm by a marked increase in membrane potential. This potential appears to be associated with increased ability of sodium ions to permeate one part of the composite membrane (8). If such changes were to occur in the CNS, they obviously could cause depolarization of neuronal membranes, thereby increasing excitability and facilitating impulse spread.

Changes have been noted in polymerization of certain fibrous proteins at 50–150 atm (12). The O_2–hemoglobin dissociation curve is displaced to the right at similar pressures (18), suggesting a possible, significant change in protein/small molecule interaction in this pressure range. It is far too early to single out any one of these phenomena as having particular significance in the HPH syndrome, but these examples suggest that they must have some importance. Future investigation into these relationships should be highly rewarding.

A second point is that different species possibly respond to high pressures in ways that differ qualitatively rather than quantitatively. Recent findings (9) suggest that in sheep CNS depression rather than the HPH syndrome may be the characteristic response to high pressures. The P_{O_2} of about 0.5 atm in our present experiments is above the values producing the symptoms described in Professor Chouteau's paper published in this volume. Other effects must also be taken into account—e.g., different compression rates, and the amount of rumen gases that might possibly be absorbed at these high pressures. Speculation must of course be replaced by experimentation.

Concerning the relationship between our findings in animals and the physiology of man under deep-diving conditions, several observations should be mentioned. In a series of experimental chamber dives* to pressures exceeding that of 1000 FSW, EEG changes were observed in each of the four human subjects studied (2). The changes consisted of Θ wave activity, especially in the occipitotemporal region; they appeared in the more susceptible subjects from approximately 30 atm onward. The phenomenon appeared to be reproducible, the relative susceptibilities of the different individuals seeming to bear a constant relation to one another throughout the series of dives. The EEG changes were concomitant with such behavioral changes as decreased attention, drowsiness (not unlike the pattern described as *microsleep*), and, at the highest pressures—equivalent to 1150–1190 FSW—some degree of confusion and motor disturbance. These last manifestations seemed to exceed those usually associated with He tremors, and were observed in all subjects when pressure reached 750 FSW and greater. Voluntary hyperventilation (2 min) at 28 and 35 atm failed to alter the EEG.

* Jointly performed in 1968 by the French National Research Council, the Compagnie Maritime d'Expertise of Marseilles, and the Wrightsville Marine Bio-Medical Laboratory.

These experiments suggest that, with the compression schedule used, man may begin to show evidence of the high pressure neurological syndrome at about 36 bar. No attempt was made to set a record in the experiments, of course. Rather, we had hoped to produce in the human subjects controllable symptoms relating to the sequence of CNS changes known to occur in animals under high pressures. The series was terminated sooner than we intended and before the anticipated severe symptoms developed, because one subject displayed an EEG change that could have meant a possible destructive hypoxic or ischemic condition in the CNS if exposure had been continued.

Before such experiments are resumed, it is essential that additional information be assembled. Data are needed regarding not only changes in blood gas composition of primates that are sufficiently compressed to evoke neurological changes, but also the degree of reversibility of such changes. To this end, decompression procedures have been developed that allowed us to recover safely six monkeys subjected to various time exposures at pressures up to 75 atm in He and H_2. These animals appeared grossly normal once they recovered from the fatigue of their 2- to 3-day exposure. They are being allowed recovery periods lasting 1–4 months before they are recompressed to establish new convulsion thresholds.

While it is too early for us to formulate firm conclusions, other studies with mice suggest that previously exposed and decompressed animals may prove much more sensitive to the HPH syndrome on their second exposure than on their first. If this is so, the heightened sensitivity implies residual CNS damage, and may provide a standard against which safe compression procedures can be planned in the future.

ACKNOWLEDGMENTS

This paper was supported by research grant NIH 5-RO1-GM154440-02 and by research grants from the United Medical Research Foundation, Inc., and the Z. Smith Reynolds Foundation. We wish to acknowledge the help of Dr. Heinz Schreiner, Director of Research, Ocean Systems, Inc., and of the Linde Corp. (Division of the Union Carbide Corp.) for making available part of the helium and helium–oxygen mixtures used in this work. The authors also wish to thank Dr. John B. Storer, formerly of the Jackson Memorial Laboratory, Miami, Fla., for his advice regarding the mice strains used in our experiment, and for making the animals available to us. The authors express their warm thanks to Col. Michael M. Conrad for making available the original design of a restraining couch used by the United States Air Force.

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ELECTRICAL ACTIVITY IN THE CENTRAL NERVOUS SYSTEM IN EXTREME NARCOSIS

P. V. Van Tassel, C. J. Knight, and C. J. Lambertsen

A search for a sensitive, objective, valid, and reliable method of studying the EEG has been made over the period of four decades since Hans Berger (1), in 1929, first published his findings on the electrical phenomena occurring in the brain of man.

Initially, this effort was expended in attempts to correlate brain wave patterns with behavioral characteristics. Additional attention was devoted early in the history of encephalography to quantitative analysis of brain waves; probably the first reasonable approach to a quantitative study was measurement of the height of the recorded waves (5). Kornmüller (4) in 1937 presented such a system of amplitude measurements from positive to negative extremes. Although this early attempt at quantification yielded some interesting facts, such peak-to-peak measurements proved to have little or no predictive value (3).

Study of amplitude measurements has progressed to methods of measuring the area under the recorded wave, which represents integration of the voltage represented by the wave. The electrical output is further integrated over intervals of time, the resulting average voltages giving a series of numbers describing the mean electrical activity over the selected period. This entire voltage integration procedure can now be accomplished electronically. With the electronic integrator, as with all circuits for analysis of harmonic functions, the incoming EEG signals must first be rectified, so that the average value will not be zero (3).

By 1948, Drohocki (2) had designed an automatic integrator for electroencephalography giving an output in the form of pulses which could be treated as numbers and submitted to statistical analytical procedures.

Murphree (5), in association with Goldstein, Pfeiffer, and others (6–8), further extended the method by using an integrator which was solid state in design, thus overcoming the recognized disadvantages of the Drohocki integrator (i.e., high noise level, limited stability and limited dynamic range).

Analysis of electrical energy at various frequencies is an extension of the voltage integrative technique, and involves the use of filters employed to divide the EEG frequency spectrum into narrow, adjacent frequency bandwidths. However, the possibility of overlaps or gaps between contiguous filter bands must always be taken into account. In this system the output is also

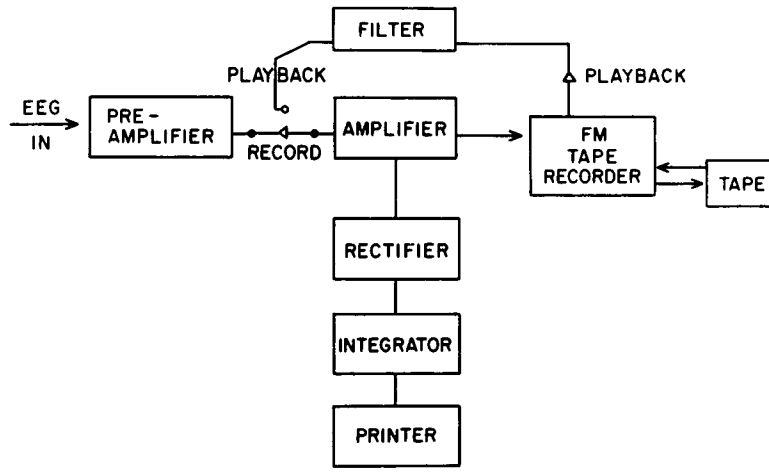

FIG. 1. Block diagram of recording and processing apparatus.

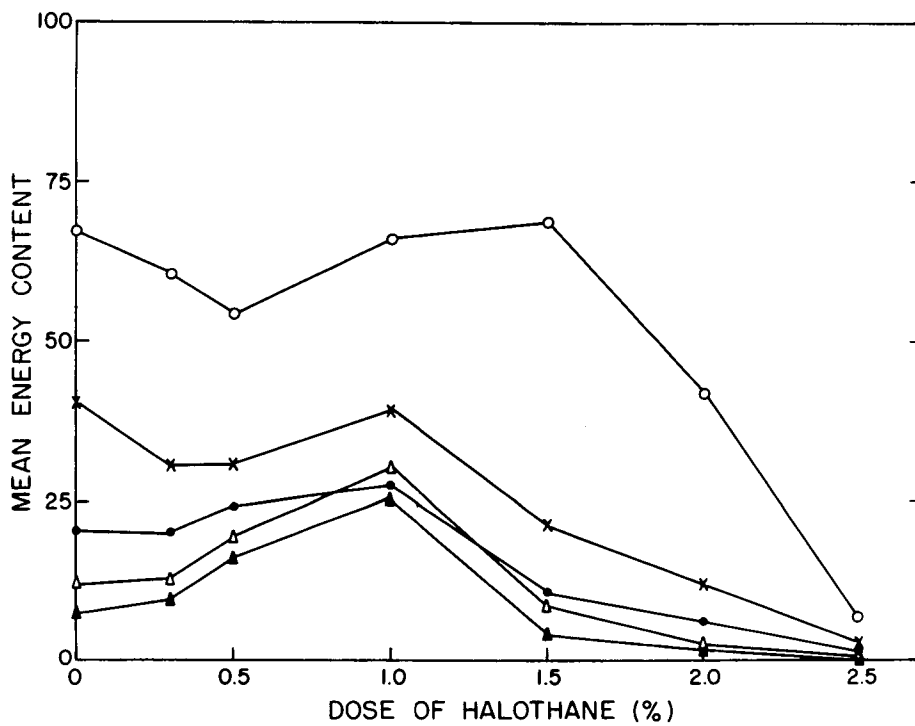

FIG. 2. Quantitative EEG effect of halothane in five subjects. Mean energy content of filtered EEG's showing peak levels in area of deep anesthesia (1.0% halothane). (○) 5 cycle; (×) 10 cycle; (●) 15 cycle; (△) 20 cycle; (▲) 25 cycle.

integrated at intervals, the mean voltages providing a series of numbers describing the electrical activity within the bandwidth being analyzed (5).

Following publication of reports of the use of this EEG quantification technique in studies of drug effects in animals and man, this laboratory became interested in evaluating the technique as a quantitative method for determining the narcotic effect of various inert gas mixtures in animals and man at normal and at high ambient pressures.

In the initial studies, N_2O was used in various concentrations at sea level pressure in an attempt to produce narcotic effects in rhesus monkeys. Concentrations up to 80% N_2O in O_2 neither put the monkey to sleep nor produced significant changes in the mean energy content as measured by integration of the EEG.

Experimentation with N_2O was not pursued at pressures greater than atmospheric since it was decided to employ a narcotic that would assure ability to cover the full range of CNS depression.

Halothane was selected as the narcotic, anesthetic agent that would be effective enough, yet still relatively safe and convenient to use. It was further decided to facilitate the overall evaluation of EEG quantitation by avoiding an excitement stage in the monkey, and concentrating upon EEG recordings from the stage of deep sleep to essentially complete abolition of electrical activity.

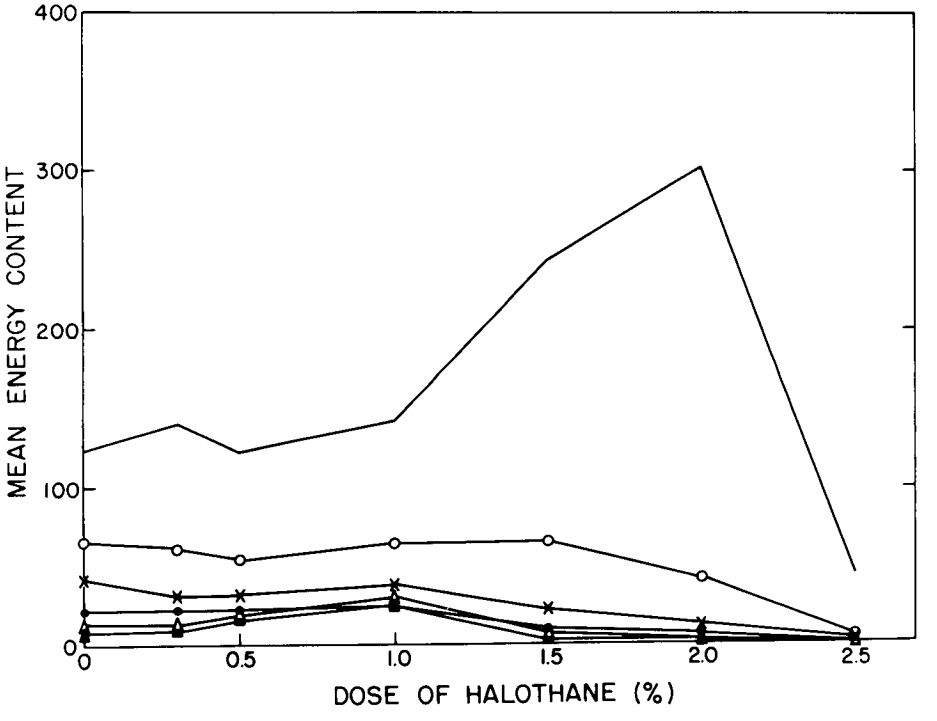

FIG. 3. Quantitative EEG effect of halothane in 5 subjects. Mean energy content of unfiltered EEG's compared to mean energy content at various filter levels. (——) All frequency; (○) 5 cycle; (×) 10 cycle; (●) 15 cycle; (△) 20 cycle; (▲) 25 cycle.

Recordings were made from electrodes implanted in the skull of the monkey. A seven-pin electrical socket was permanently attached to the skull by wires secured by screws penetrating into but not through the skull. The screws, wires and base of the socket were then embedded in dental plastic, the scalp replaced over the operative site with only the socket protruding through a slit in the scalp to the surface, thus providing a ready connection to the recording apparatus. Healing around the socket was rapid and complete.

From the several pairs of leads, a single channel recording was made on magnetic tape from the left frontal to left occipital areas. Figure 1 shows a block diagram of the recording and processing apparatus.

During each experiment respiration was supported by a small animal respirator. This was adjusted to maintain end-expiratory CO_2 at physiological levels, thus avoiding affecting the EEG. Halothane concentrations (end-expiratory) were monitored with an UV analyzer that had been previously calibrated by gas chromatography. In addition, blood pressure was measured through an indwelling femoral catheter, and deep-body temperature was monitored both by a tympanic thermometer and rectally.

The magnetic tapes from the last series of experiments revealed, in the frequency bands examined, an energy peak in the area of deep anesthesia that falls off only as the EEG approaches a flat tracing in the state of essentially complete CNS dysfunction (Fig. 2).

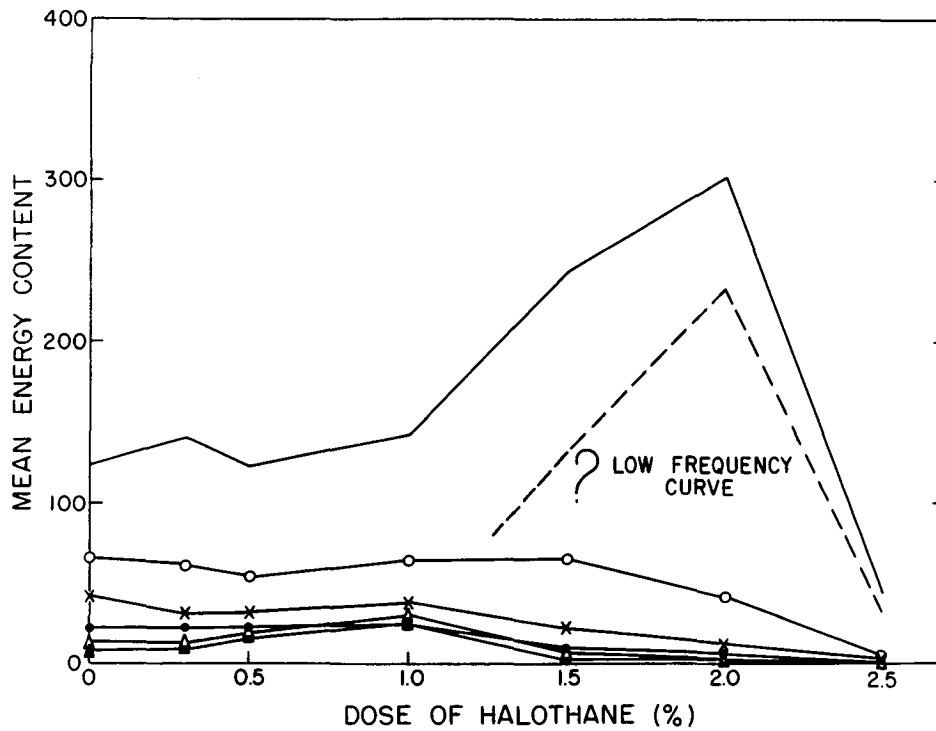

FIG. 4. Quantitative EEG effect of halothane in 5 subjects. Mean energy content of unfiltered and filtered EEG's showing hypothetic curve of mean energy content unrecorded by this filtering technique. (——) All frequency; (○) 5 cycle; (×) 10 cycle; (●) 15 cycle; (△) 20 cycle; (▲) 25 cycle.

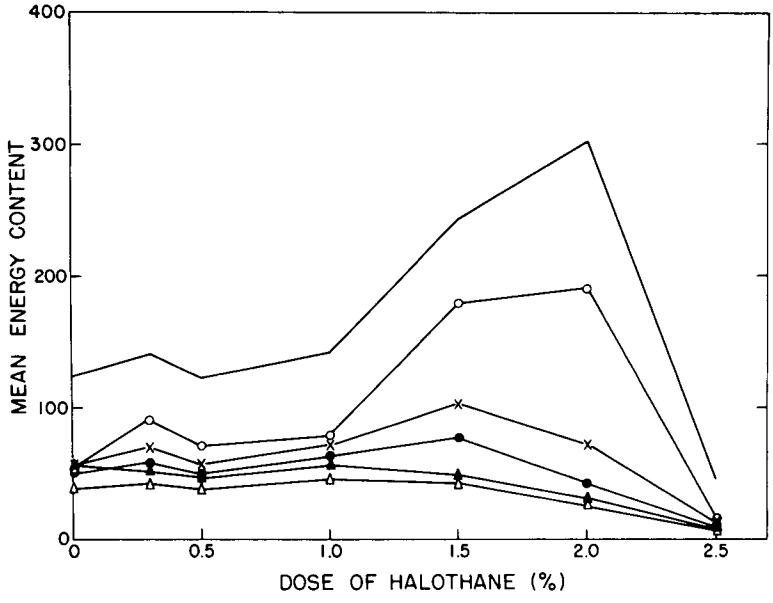

FIG. 5. Quantitative EEG effect of halothane in 5 subjects. Mean energy content of unfiltered EEG's compared to mean energy content at various low frequency filter levels. (——) All frequency; (○) 1 cycle; (×) 2 cycle; (●) 3 cycle; (△) 4 cycle; (▲) 5 cycle.

When these results were compared with the energy analysis of the unfiltered composite, an interesting disparity was noted in both the amount of energy produced and the concentration of anesthetic gas at which it peaked (Fig. 3). This would suggest that our frequency analysis technique has failed to detect a large energy curve probably in the low frequency band and somewhat approaching the hypothetical curve shown in Fig. 4.

It therefore became apparent that the preliminary method of scanning relatively wide frequency bandwidths had not only failed to exclude the possibility of small frequency shifts, but had effectively excluded a large energy content in the frequency bands below about 3 cps.

Single bandwidth filters were then obtained and the magnetic tapes of originally recorded EEG data were reanalyzed through these filters. This revealed that the predicted low frequency energy was in fact present, predominantly in the 1 cps band (Fig. 5).

From qualitative EEG studies, it is known that drugs which are CNS stimulants tend to produce a decrease in wave amplitude and an increase in frequency, while natural sleep and drugs which are sedatives tend to produce large slow waves. Quantitative analysis of the latter pattern would necessarily reveal an increase in EEG energy content and a shift of the energy to the lower frequencies. The low frequency energy shown in the halothane series (Fig. 5) can probably be regarded as an extreme example of this recognized effect of depressant drugs upon cortical electrical activity.

This paper has emphasized the effects of a general anesthetic agent, not an inert gas used in diving. It should be regarded as a progress report on evaluation of a method of providing a reproducible quantitative technique for analysis of EEG's in many situations where narcosis

is present. This method is now being employed to determine the narcotic effects of various inert gas–O_2 mixtures on nonhuman primates under extreme hyperbaric conditions.

ACKNOWLEDGMENTS

This work was supported in part by grants (HE–08184 and HE–08899) from the National Institutes of Health and by a contract (NONR 551) with the Office of Naval Research.

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Part XI. Influence of Inert Gases and Pressure upon Central Nervous Functions*

DISCUSSION

J. W. Miller, Chairman

Dr. Doebbler: Dr. Bennett, it would seem to me that the tremendous changes in cation concentration you showed as occurring under relatively low pressure conditions of 300 ft breathing N_2 are significant in terms of certain narcotic effects. You showed 30 or 40% decrease in urinary calcium and sodium. Do you have an explanation why one should get such a profound change under what otherwise should be not such a terribly stressful condition?

Dr. Bennett: It is not quite as great as that, but I have no explanation for the change. It is an effect we have only just discovered and we were surprised to find it in the very deep oxyhelium dives as well. At first we thought it was possibly due to hypercapnia, but in deep oxyhelium diving this is not likely.

Dr. Doebbler: Does Dr. Brauer have any comment regarding the basis of the effect of using very low pressures of N_2O to reduce the convulsions he sees in animals breathing He at high pressure? Does he consider that N_2 normally plays a role in the body and the absence of N_2 is somehow related to the general convulsions he finds? Does an anticonvulsant action also occur in animals with H_2 or with Ne?

Dr. Brauer: The answer is that the anticonvulsant effects are quantitatively related exactly as their anesthetic potency, as determined by loss of righting reflexes. In other words, if 1 mole of N_2O produces a 10% elevation of convulsion threshold, then 28 molecules of N_2 and approximately 100 molecules of H_2 would be needed to produce the same effect and do, in fact, exactly as predicted.

Dr. Doebbler: Then it would seem that you are seeing a separate kind of effect with He, that is, a convulsive response, which then is eliminated by small amounts of narcotic contribution from a second gas in the mixture. This is curious. It would seem that if this is a general phenomenon, then any very small gas molecule ought to be expected to behave in about the same way in producing convulsions at high pressures.

Dr. Brauer: The concentrations which produce the protection are not small. Moreover, this type of convulsion can be counteracted by central nervous system depressants such as barbiturates.

The convulsions themselves probably have nothing to do with He itself because they can be produced at exactly the same pressure on exactly the same compression rate in liquid-breathing mice. I would explain the rate effects we see in terms of adaptation to certain ionic shifts that I think we have reason to suspect begin at these pressures.

Dr. Hesser: I would like to make some comments on the role of CO_2 in compressed air narcosis. Many years ago, in 1941, Case and Haldane reported that whereas 3–6% CO_2 at normal atmospheric pressure had no measurable effects on manual dexterity and arithmetical performance, at 10 atm of air breathing the same inspired CO_2 pressure caused a marked deterioration in performance and a state of confusion developed. Within 5 min eight of the subjects became unconscious.

Case and Haldane concluded that the combined effects of N_2 excess and CO_2 excess are much more severe than either alone. It is obvious, however, that when dealing with psychological effects of different gases these effects should be related to the gas pressures at the site of action rather than to the inspired gas pressures.

We have found that due to the rises in O_2 pressure and gas density in breathing air at high pressure, the ventilatory response to CO_2 is much less at high atmospheric pressures than at sea level. Therefore, with the same elevated inspired CO_2 tension the alveolar P_{CO_2} will increase much more in hyperbaric conditions than at sea level.

* *Panelists:* J. G. Dickson, R. W. Brauer, P. B. Bennett, C. J. Knight, P. M. Criscuoli, C. M. Hesser, and R. Biersner.

We have studied the ventilatory response to CO_2 in three different conditions, namely, when breathing air at 1.3 and at 8 atm and O_2 at 1.7 atm. The inspired O_2 pressure was the same in the two last conditions. The slope of the ventilatory response curve was found to be only about one-third at 8 atm what it was at 1.3 atm, and about half of this decrement in slope is due to the increment in O_2 pressure.

We have also recently studied the effects of CO_2 on performance at high ambient pressures. Two tests were used, one on manual dexterity and the other on arithmetical performance. Again the experiments were done on subjects breathing air at 1.3 and 8 atm and breathing O_2 at 1.7 atm. Carbon dioxide was added to the inspired gas in concentrations equivalent to 2, 4, and 6% at sea level pressure. With no CO_2 added to the inspired gas, the number of problems attempted was reduced by about 25% when the ambient pressure was raised to 8 atm. Part of this reduction was apparently due to the rise in O_2 pressure. When the alveolar P_{CO_2} was increased by raising the inspired P_{CO_2}, performance remained unaffected in all three conditions until the alveolar P_{CO_2} exceeded about 40 mmHg. With a further increase in alveolar P_{CO_2}, performance deteriorated in all three conditions to the same extent.

From these observations it may be concluded that high N_2 pressures and high CO_2 pressures act synergistically in a simple additive manner rather than having a potentiating effect on each other.

Chairman Miller: Professor Criscuoli, how do you abolish muscular artifacts in EEG recordings during convulsive seizures?

Dr. Criscuoli: In our early experimental work the electroencephalograph records had many of the muscular artifacts. We have preferred to use rats in which the artifacts are few, or not present.

Dr. Smith: Dr. Bennett described an effect of anesthetics in perturbing cation transport in biological membranes. This is widely discussed, with some investigators considering that the membranes are perturbed so as to increase transport, others believing transport is reduced. We have actually studied the red blood cell membrane and have shown that neither active transport nor the passive leak of sodium and potassium are perturbed by anesthetics until concentrations between five and ten times the normal anesthetic dose are reached. Though the theory is widely held and certainly the effect occurs in certain membranes, change in permeability does not appear to be a universal action of anesthetics.

Chairman Miller: Throughout these sessions the EEG has been cited as a means of measuring convulsions and as a means of measuring various states of narcosis. Different techniques have been used, ranging from implanted electrodes, needle electrodes, electrode skull caps, and the standard electrodes pasted on. If we are attempting to interpret effects of various parameters on performance or on narcosis and then make recommendations for divers, we must adopt standard methods for recording critical data. Any of us who have worked with EEG recognize the tremendous difference in recordings with these various techniques.

Dr. Schreiner: Dr. Biersner, tests such as you have devised are very important in determining what a man can do under pressure. However, we also likely have problems with motivation. I refer in particular to the long, protracted decrement that persisted throughout the decompression phase of your deep He dive. We too, have had subjects get tired of doing tests and consequently we obtain results interpretable as decrements but which may merely tell us that the men are tired of the game.

What is your professional opinion about making these tests more realistically a measure of capability by tying them into an incentive of a sort that would motivate a man to do his best on a test?

Dr. Biersner: Of all the tests in our particular study the last one, which showed the prolonged and constant effect, was probably the one least liable to lack of motivation, largely because the divers competed against one another on this particular task. Their scores were read into the entire chamber and they compared scores and actually challenged each other to improvement.

You have cited a real problem. We had a control group in this study simply to take boredom and habituation into account. In an upcoming test we have designed a means of study of differential risk taken at various stages of decompression. This test involves blatant gambling by the subjects and should be especially attractive to divers.

Dr. Godfrey: Would either Dr. Biersner or Dr. Dickson comment on how we standardize tests for sensitivity to narcosis and for boredom, and relate this to general performance during compression and decompression?

Dr. Biersner: The tests themselves are not devised to detect sensitivity to narcosis. They are standard tests applied generally to the population. There is a problem in narcosis that is not generally noted. This is the basis for some of the tests being more sensitive than others. There is a difference in effect upon permanent and short-term memory. There is considerable literature available in neurophysiology and neuropsychology

on the effect of narcosis on the consolidation phenomenon in processing of information in various organisms, including man.

The reason that short-term memory is more sensitive than arithmetic ability or tests of orientation is that there is a disruption of the consolidation of permanent memory; the short-term memory is used as a device for measuring this effect. This alludes to an interesting application of narcosis other than in diving and studies of behavior at various depths. Nitrogen at high pressures or N_2O should be used and pursued by more people to study the actual basic consolidation processes and phenomena that are present in all organisms and by which all organisms code information into permanent storage. These are very useful techniques and I think they should also be used more extensively in the diving area.

Dr. Brauer: I must comment on the differences between the deep and essentially trouble-free dives Dr. Bühlmann has performed at Alverstoke and experiences I have had with prominent electroencephalographic and other indices of CNS effect. I am confident that the phenomena that we have seen are reproducible. They were reproduced in seven separate dives and can be reproduced on demand. It has been rather well agreed that as the compression rate is slowed the nervous effects become less marked.

One of the differences between our groups is that our subjects were at rest and the one or two individuals that were not at rest felt conspicuously more comfortable than the ones whom we had seated in a chair so that we could get good EEG's on them. I wonder to what extent in fact activity is a pertinent factor in reducing the intensity of the neurological effects. In the case of animals there is no question that at compression rates of the order of 20 atm/hr, which is slower than those used here, I have yet to see an animal that does not develop tremors.

It is unfortunate that none of us knows the neurological basis for the tremors which we can produce. There is no knowledge at the moment whether this is a peripheral or a central problem.

Dr. Bennett: Although there were no gross tremors in the studies with Dr. Bühlmann at the Royal Naval Physiological Laboratory at Alverstoke, certainly there were changes in the ballbearing test, which was a pretty sensitive test of tremors, as used in the past, during the first hour or so when you would expect to find this. In further agreement with past research, one would expect this to improve with continued exposure. Also, a number of the other tests showed a small decrement during the first hour. However, there was no major impairment in the men's functional capacities really. They could have worked if they had wanted.

Part XII. UNDERSEA AND MANNED CHAMBER OPERATIONS

PERFORMANCE ASPECTS OF AN OPEN-SEA SATURATION EXPOSURE AT 615 FEET

Joseph B. MacInnis

In 1967, we undertook a qualitative study of two He–O_2 divers performing realistic work while saturated in the open sea at the deep edge of the continental shelf. Although this dive to 615 ft was the first human open-sea saturation exposure to this depth, it was the logical continuation of a long series of studies carried out in our laboratories. One of these exposures has been previously reported in the third symposium, but since its physiological findings were the keystone of our open-sea studies, it is relevant to summarize it here briefly.

In 1965, two men were exposed to He with O_2 for 2 days in a dry chamber to a pressure equivalent to that at a depth of 650 FSW (3). At maximum pressure, P_{O_2} was maintained at 0.35 atm abs. Detailed cardiorespiratory, hematological, biochemical, psychological, and related investigations did not reveal any significant changes in homeostasis as a result of this exposure (Table I). The success of this laboratory study generated the confidence that allowed us to proceed with the applied study in the open sea.

Our investigative focus was now logically shifted to encompass performance rather than physiological aspects of the exposure.

In concert with Esso Production Research of Houston, Texas, an underwater mission relevant to subsea oil production systems was devised. This mission evolved with the knowledge that current diving tasks include a complex sequence of motor activities ranging from fine digital manipulation of small items to gross manhandling of heavy objects. Accompanying this physical effort is a parallel sequence of mental functions related to recognizing and solving problems imposed by the task and the environment.

Throughout the physical and mental efforts required to complete any task, the diver must pay constant attention to the safety and performance aspects of the dive. These include the status of his breathing apparatus and thermal protective suit, as well as the function of supportive tools and equipment. All of the above entities must be coordinated with a standby diver and the topside crew.

The study of "real work" tasks poses substantial difficulties. Attempts have been made by Bowen (2), Badderley *et al.* (1), and others to develop structured test batteries to investigate specific skills. However worthwhile these studies are, it is frequently difficult to relate and

TABLE I

SUMMARY OF A FOUR MAN-DAY (DRY CHAMBER) EXPOSURE TO 650 FSW

Exposure factors		Studies conducted
Pressure:	650 FSW (20.7 atm abs)	General medical
		Electrocardiogram
O_2:	0.35 atm abs	Blood pressure
CO_2:	<1% sea level equivalent	Body temperature
Inert gas (He):	20.3 atm abs	$Paco_2$ respiratory frequency
Temperature:	85–88°F	CO_2 response
Relative humidity:	75–90%	Pulmonary function
Trace contaminants:	insignificant	Exercise tolerance
		Work of breathing
		Psychological
		Urine stress steroids
Implications		
No acute or latent detriment to health Well-being insignificant change in functionality		

integrate the findings to actual tasks deep beneath the sea. For these reasons we decided to formulate a comprehensive simulation which would incorporate the full range of diving skills.

Our mission objective was to obtain a series of comparative performances of the same task conducted under varying environmental conditions. We wanted a "real life" work simulation which would require considerable mental, psychomotor, and strength function from the diver. We selected a task that required the diver to dynamically place a large and heavy object into a series of specific configurations. A mockup was built in order to carry out the work in the multiple environments we had selected (Table II).

The specific task was subdivided and an attempt was made to standardize its execution. It was surrounded with a series of related but unstructured tasks which were performed by the divers only during the open-sea phase of the study. During a 4-week period the standardized task was carried out a total of 79 times on dry land, in a 10-ft deep tank and in the shallow and deep sea. Intense observations were made to determine the conditions which influenced performance effectiveness. Four divers began the task and the best two were selected to perform the open-sea work.

Although diver performance was our primary study goal, we also made efforts to gather physiological data (Table III). To this end, daily weights, nutrition and fluid intake, and general medical histories were obtained. In addition, EEG and EKG tracings were obtained on the working divers by Dr. C. W. Sem-Jacobsen with his portable VESLA recording device.

The divers remained saturated for 53.5 hr. Excursions to 625, 632, and 636 ft were required due to slight variations in bottom topography where the mockup was placed.

The dive profile commenced with a compression to 625 ft which took 15 min. An inspection dive revealed the mockup to be so angled into the mud that the performance task would have been impossible to carry out. Thus the divers returned to the submersible chamber and ½ hr later they were transferred to the surface deck chamber. For the next 35 hr heavy seas and rain squalls prevented any underwater work. However, after 40 hr the first diver was able to

TABLE II

DEEP SATURATION PERFORMANCE STUDY: TASK AND ENVIRONMENTAL ELEMENTS

Standardized task	Unstandardized tasks	Environments
Valve rigging and placement	*General rigging*	Sea level
Shackle eye-bolt		
Loosen 4 nuts and bolts	*Lubricator task*	10 ft
Winch up valve	Remove bull plug	Warm water
Remove clamp assemblies	Uncouple lubricator from S.D.C.	Good visibility
Slide post horizontally	Transport lubricator to mockup	
Replace ring seals	Align and stab lubricator into union	10 ft
Cover valve flanges		Cool water
Rotate valve 180°	Hand tighten knurled nut of union	Blindfolded
Lower and raise valve		
Reposition post horizontally	Reverse whole procedure	45, 55, 70 ft
Remove protective plates		Cool water
Final alignment	*Bolt tightness task*	Good visibility
Replace clamp assemblies		
Winch down valve		632 and 636 ft
Secure bolts to 150 ft lb		Cold water
		Good visibility

lock out into the sea; he worked for 2 hr 37 min at 636 ft. Nine hours later the second diver worked for 2 hr 30 min at 632 ft. Decompression to the surface was carried out at 15 min/ft without incident.

During the overall exposure a total of 5.5 hr was spent swimming and working at an average depth of 630 ft at a water temperature of 56–58°F.

TABLE III

SUMMARY OF A FOUR MAN-DAY (OPEN-SEA) EXPOSURE TO 615 FSW

Exposure factors		Studies conducted
		Performance
Pressure:	615 FSW (19.6 atm abs)	Valve rigging and placement
		General rigging
Excursions to:	625, 632 and 636 ft	Lubricator task
O_2:	0.35 atm abs	Bolt tightness
CO_2:	<1% sea level equivalent	*Physiology*
Inert gas (He):	19.3 atm abs	General medical
Temperature:	86–88°F	Daily weights
Relative humidity:	80–90%	Nutrition and fluid intake
Trace contaminants:	not measured	EEG and EKG
		Body temperature
Implications		
No acute or latent detriment to health Well-being task performance slower than under terrestrial or shallow water conditions.		

The two divers selected for the open-sea performance were experienced young men who had worked for several hundred hours at depths greater than 200 ft and together had accumulated almost 20 years of diving experience. They were kept warm by hot-water immersion suits and both reported feeling thermally stable during their 5.5 hr at maximum depth. Heated water from the surface was pumped through an insulated hose to a matrix of small tubes and apertures that bathed their skin with hot water during their free-swimming period.

The breathing systems used were semiclosed devices which were new to the divers at the beginning of their training for the saturation dive. Although one man had previous U. S. Navy Underwater Demolition Unit experience with this rebreathing system, his total number of hours of practice was relatively small. This inexperience did not appear to subjectively affect task performance although breathing rates were in the order of 20–24 breaths/min.

As can be seen in Table II, the essential task involved the positioning and alignment of a 480-lb valve in order to replace the gasket seals. Mechanical assistance was provided by a hydraulic winch, but several times brute physical force was required.

It is extremely difficult to obtain relevant performance or physiological information from divers working in the deep sea. The multiple-stress environmental framework almost precludes study of discrete variables. However, we did make serial observations of the same divers performing the same task under progressively changing conditions. Study of our video tapes has revealed that at maximum depth we were watching divers who, although they were working effectively, proceeded with slower, more measured movements than were evident at shallower depths.

Several factors made valid comparison with the preparatory, shallow dives difficult. First, only two trials were made of the performance task at maximum depth compared with 47 at sea level, 24 at 10 ft, and 6 at the 45–70 ft range. Second, during the initial trial at maximum depth, the mockup landed at a sharp angle on the sea floor and made the performance mission significantly harder.

The time for each of the two divers to complete the standardized portion of his task was recorded (Table IV). Several observations are especially interesting. At sea level on dry land the completion times indicate that, at least in the terrestrial environment, the learning curve began to level off. It took the divers appreciably longer to carry out the tasks at maximum depth than under any other set of conditions. This increase in time can be attributed to many factors, such as the difficult angle of the mockup, situational anxiety regarding the breathing apparatus, and being the first to be so deep for so long in the open sea. Also important in slowing the time was the concern of the topside crew who repeatedly stressed caution and carefulness of movement to the divers. Although the divers said that they were warm and breathing easily, their performance times were almost four times longer than on dry land. It is highly likely that this figure would be revised downward following repeated exposures to this depth according to learning and adaptation factors.

Whatever the origins of the time delay, we know that a complex, multiple sequence of factors sufficiently stresses today's working diver so that he is unable to work with the same ease deep beneath the sea as he can on land.

Weltman *et al.* (4) in a recent report have concluded that "for well-defined complex tasks of moderate workload, the use of experienced divers in a cooled tank will provide a close approximation to actual ocean operations at nominal depths." While this may be true, it is imperative that we ascertain how man functions as a diver at great ocean depths. We know with

TABLE IV

DEEP SATURATION PERFORMANCE STUDY: TIME OF STANDARDIZED TASK COMPLETION

Divers and time in min		Environments
A.P.	G.T.	
4:45	4:15	(Task lengthened, made more complex and standardized)
8:50	8:12	
8:25	10:10	
8:40	7:32	Sea level
8:06	6:52	
6:57	6:15	
6:25	6:50	
6:05	5:20	
5:35	5:40	
7:00	5:53	
5:10		
4:50	5:15	
6:25	5:34	
8:18	8:10	10 ft
8:05	7:15	Warm water
6:45	7:20	Good visibility
11:37	15:20	10 ft
11:23	13:45	Cool water
12:05	15:52	Blindfolded
11:00		45 ft Cool water, good visibility
	19:30	70 ft Cool water, poor visibility
13:35	13:22	55 ft Cool water, good visibility
23:40		636 ft Cold water, good visibility
	21:38	632 ft Cold water, good visibility

some confidence that today's diver can complete gross and uncomplicated tasks at depths in the order of 200 m. We are aware that he works slower and with less efficiency, but we are not quite sure why. More important perhaps is that we really do not know how the diver will perform complex mental and physical tasks, particularly under the stress of an emergency. Prediction of future performance capability requires the obtaining of new and reliable data such as these symposia have been concerned with.

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1000-FOOT HELIUM SATURATION EXPOSURE

J. K. Summitt, J. S. Kelley, J. M. Herron, and H. A. Saltzman

At the time of the Third Symposium on Underwater Physiology in 1966, the efforts of the U.S. Navy to formulate safe decompression procedures for saturation diving were summarized (1). Since that time, 23 deep saturation–excursion dives have been conducted at the U.S. Navy Experimental Diving Unit (6), including the then record-breaking exposure of two men to a simulated depth of 1025 FSW in February, 1968. More recently, a number of nonsaturation dives and excursion dives have been conducted in other chamber facilities to simulated depths well beyond 1000 ft (R. W. Brauer, A. A. Bühlmann, and A. Galerne, personal communication). Animal studies have been conducted to depths in excess of 3000 ft (3, 5). These exposures have demonstrated that man and animals can survive and continue to be physically active at tremendous pressures but, for the most part, they have not provided objective, quantitative data concerning man's physiological limitations and physical capabilities even at pressures equivalent to 1000 ft.

In December, 1968, the U.S. Navy and Duke University effectively combined their medical and scientific resources to conduct a study of five trained men during compression to a simulated depth of 1000 FSW, during subsequent saturation at this pressure for 77 hr and 30 min, and during decompression back to sea level pressure. Tables I and II outline the studies that were completed in each phase of the dive sequence.

Dive Procedure

General Approach

Since the primary purpose of the dive was the successful completion of a series of biomedical and equipment tests, it was important that the subjects remain in the best possible physical condition throughout the exposure. Therefore, a dive profile was chosen which would assure, insofar as possible, unimpaired subject performance during all phases. The decompression schedule used was designed to minimize the risk of serious decompression sickness rather than to test new decompression procedures.

TABLE I

PREDIVE AND POSTDIVE MEDICAL EVALUATION

1. Medical history	8. Hemogram Hematocrit White cell count Differential
2. Physical examination	
3. ENT examination	9. Urinalysis
4. Audiogram	10. EKG
5. Ophthalmology examination Refraction Intraocular tension Color vision Polaroid stero test Maddox wing test Maddox rod test Slit lamp examination Funduscopic	11. EEG 12. Pulmonary function studies 13. Blood chemistries Blood urea nitrogen Electrolytes Uric acid Glucose Enzymes Complement
6. Chest X-ray	
7. Long bone X-rays	

ATMOSPHERE CONTROL

The P_{O_2} in the chamber (Fig. 1) remained between 0.26 and 0.35 atm except for two short periods during decompression. Approaching the 450-ft decompression stop, the O_2 pressure was allowed to drop to 0.19–0.20 atm in order to meet the special requirements of an experiment scheduled at that depth. During the decompression sickness treatment schedule used at 170 ft, O_2 pressure was allowed to rise to 0.5 atm.

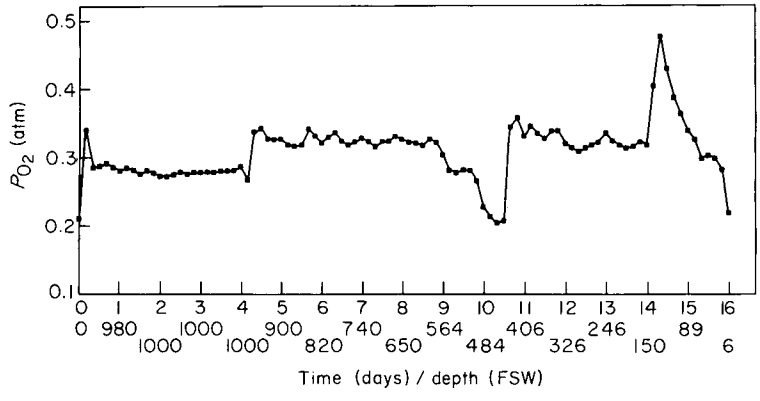

FIG. 1. P_{O_2} in the chamber environment during the dive.

TABLE II

EXPERIMENTAL STUDIES COMPLETED DURING THE DIVE

A. During descent to 1000 ft

1. Volume/flow respiratory studies
2. Helium unscrambler recordings
3. Human performance studies
   - Psychological stress studies
   - Manual dexterity tests
   - Learning and memory tests
   - Odd–even reaction time tests

B. At 1000 ft

1. Volume/flow respiratory studies
2. Helium unscrambler recordings
3. Human performance studies
   - Psychological stress studies
   - Manual dexterity tests
   - Learning and memory tests
   - Odd–even reaction time tests
4. Respiratory gas exchange study
5. Exercise–respiratory study
6. Visual studies
7. Metabolic studies
8. Neurophysiological studies
   - Sleep EEG's
   - Evoked potentials
9. Equipment tests
   - Cold water swims
   - Warm water swims
10. Miscellaneous
    - Physical examinations
    - Blood chemistries
    - EKG's
    - Unscheduled exercise

C. During ascent to the surface

1. Volume/flow respiratory studies
2. Helium unscrambler recordings
3. Human performance studies
   - Psychological stress studies
   - Manual dexterity tests
   - Learning and memory tests
   - Odd–even reaction time tests
4. Sound level measurements
5. Hearing threshold measurements
6. Equipment tests
   - Warm water swims at 850 ft
   - Warm water swims at 650 ft
7. Miscellaneous
   - Physical examinations
   - Blood chemistries
   - EKG's

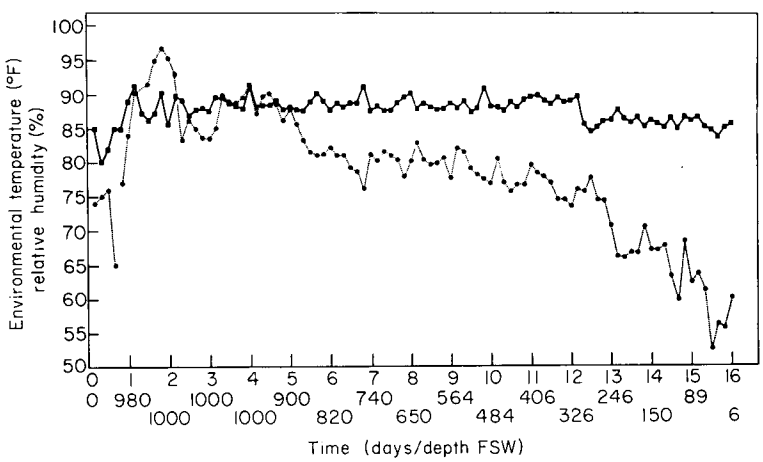

FIG. 2. Environmental temperature (■) and relative humidity (●) in the chamber during the dive.

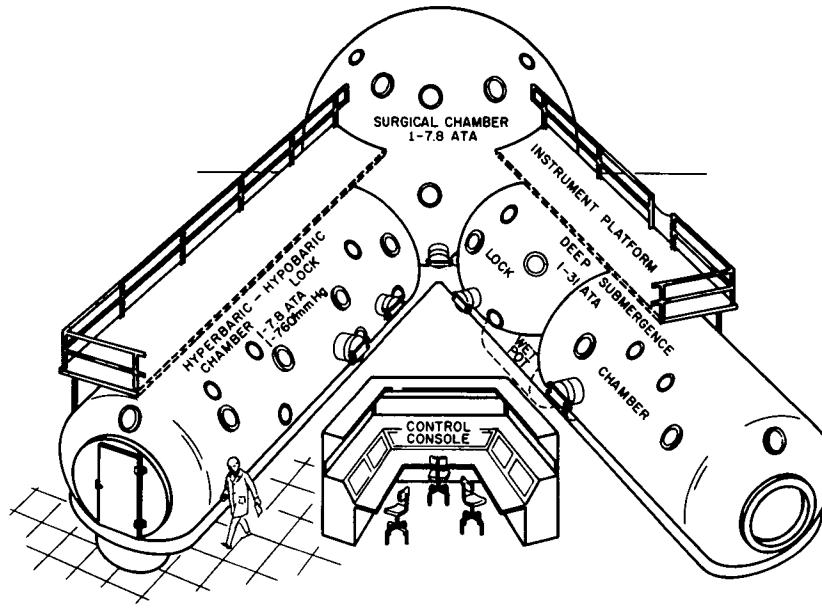

FIG. 3. Hyperbaric and hypobaric environmental facility at Duke University Medical Center.

The CO_2 pressure remained below the equivalent of a 0.25% concentration at sea level. Temperature and relative humidity varied over a wide range (Fig. 2) and were the source of some discomfort for the subjects.

COMPRESSION PHASE

At 1621, 2 December 1968, five subjects entered the hyperbaric chamber complex at Duke University (Fig. 3) and began compression. An initial phase of compression to 14 FSW was made with compressed air to establish the desired 0.3 atm of O_2 pressure. Thereafter, compression was accomplished using pure He and an average compression rate equivalent to 40 FSW/hr. This consisted of a 20-min compression period and a 40-min "compression stop" each hour, as indicated by the heavy dots on the compression line in Fig. 4. Oxygen was added as necessary to maintain the 0.3 atm P_{O_2}, and 24 hr and 22 min after leaving the surface, a pressure equivalent to 1000 FSW was reached. This pressure was maintained for a period of 77 hr and 30 min.

DECOMPRESSION PHASE

At 2213, 6 December 1968, decompression was begun from 1000 to 970 FSW at a continuous rate of 2 min/ft. Thereafter, ascent was scheduled to be continuous at a rate of 15 min/ft except for the addition of 4-hr stops at 10 staging depths as indicated in Fig. 4. This procedure was slightly modified, as follows: (*1*) The 450-ft stop was extended 14 min to allow completion of an experiment scheduled at that depth; (*2*) the 150-ft stop was interrupted to treat a mild case of decompression sickness in one diver.

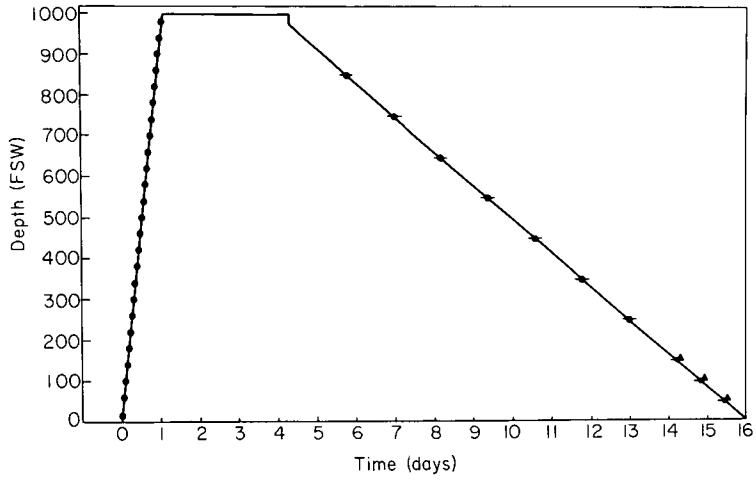

FIG. 4. 1000-ft saturation dive profile. Compression phase: 24 hr 22 min; saturation phase: 77 hr 30 min; decompression phase: 284 hr 50 min. (●) Compression stop; (-●-) decompression stop; (-▲-) recompression treatment cycle.

General Medical Observations

COMPRESSION ARTHRALGIA

All of the subjects experienced the sensation of mild joint stiffness during compression to and the stay at the 1000-ft chamber pressure. In addition, four subjects complained of occasional transient joint pains associated with exercise or sudden movements. Only one subject complained of constant joint discomfort throughout the bottom time and this was not disabling. In general, these symptoms gradually decreased with time at depth and disappeared completely early in the decompression phase.

Compression arthralgia has been relatively common during deep saturation dives at the Experimental Diving Unit. The knee, shoulder, elbow, and hand joints have been most commonly affected, but the ankle, hip, and sacroiliac joints have also been involved. Subjects have usually described a vague sensation of joint discomfort, such as tightness or stiffness, associated with increased pain and cracking of the affected joints during exercise. While the symptoms are usually mild, they may occasionally be severe enough to prevent normal activity on the bottom and, rarely, may be disabling enough to require termination of the dive and immediate decompression.

The basis for joint pain or discomfort on compression is not known. In the past, it has been associated with rapid rates of compression to depths greater than 300 ft. Indeed, slow compression does seem to reduce the severity, though not necessarily the incidence of the symptoms. Kylstra has suggested that osmotic gradients created by the inert gases dissolved during compression might result in a small fluid shift from the joint tissues and produce symptoms. It is not known whether this is a valid explanation.

From a practical point of view, compression arthralgia annoys the divers and complicates the diagnosis of decompression sickness during the subsequent decompression to the surface.

One must speculate as to whether the symptoms indicate significant tissue injury which might compromise decompression procedures or might manifest itself as a residual effect of deep diving operations.

Helium Tremors

Gross tremors could not be demonstrated in any of the subjects at 1000 ft using the standard neurological techniques. However, two subjects subjectively noted fine tremors while performing tasks which required precise motor function, i.e., inserting flexible arterial cannulas or changing membranes on the CO_2 and O_2 electrodes. One of these subjects also described momentary difficulty with muscular control while trying to lift a heavy deck plate. The remaining three subjects thought that their motor performance on the bottom was essentially the same as at the surface. It would appear that the so-called "helium tremors" did not present a serious problem under the conditions of this dive. However, other investigators have observed gross tremors and other motor dysfunctions during $He–O_2$ dives to depths shallower than 1000 ft (R. W. Brauer, H. V. Hempleman—personal communication). This would suggest procedural differences as a possible etiological factor and, as with compression arthralgia, He tremors have been subjectively associated with rapid compression. Unfortunately, rapid compression involves a situation in which precise environmental control is extremely difficult. Thus, temperature, humidity, and gas concentrations in the breathing mixture may also be important factors in producing this neuromuscular dysfunction.

Ambient Noise

During the first day of decompression, a malfunction of the main ventilation fan resulted in increasing ambient noise inside the chamber. By the second day of decompression, two of the subjects were beginning to complain of symptoms related to the noise level. Recordings taken with a General Radio Sound Level Meter (Type 1551–C) inside the chamber revealed a background sound level of approximately 80 dB. This reading compared reasonably well with the 83 dB recorded outside the chamber near the main ventilation shaft seal. The reported symptoms promptly disappeared when the main ventilation system was inactivated. The noise level inside the chamber then dropped to 63 dB, even with the auxiliary ventilation system operating. The auxiliary system was utilized throughout the remainder of the decompression, with operation of the main ventilation system restricted to brief periods when O_2 was being added to the chamber atmosphere. This was necessary to prevent pocketing of the O_2 under the deck plates in the main chamber.

Middle Ear Squeeze

All of the subjects experienced some difficulty keeping the middle ear pressure equalized with the ambient pressure during compression. No doubt, small but significant pressure differentials did exist part of this time, particularly during periods of sleep. Fortunately, only one subject developed a serous otitis media, and this resolved during the first day at 1000 ft.

External Otitis

All but one of the subjects developed moderately severe external otitis during the dive, and treatment with analgesic–antibiotic drops was only partially successful. One can specu-

late as to whether the particular circumstances of a closed environment, high ambient temperature, and high relative humidity are conducive to resistant superficial infections. Bacteriological studies performed in other saturation dives at the Experimental Diving Unit have demonstrated a marked increase in the bacterial flora of the skin and external ear canals following excursion dives. However, the bacterial counts progressively decreased toward the control levels following the last water exposure and were not associated with a higher incidence of infections or resistant infections (2). One additional factor may have been repetitive trauma to the external ear canals secondary to repeated otoscopic examinations.

All of these infections markedly improved within the first 24 hr after reaching the surface and resolved almost completely within 72 hr.

Abdominal Pain

Shortly after reaching 1000 ft, one subject (the medical officer) developed severe, colicky abdominal pain associated with increased flatus and intestinal motility. Partial relief was obtained following two normal bowel movements. After approximately 3 hr, the symptoms subsided spontaneously and did not recur. Fortunately, this incident did not develop into a serious problem requiring intensive medical support, but the possibility seemed very real at the time. With the large number of saturation dives being performed today, it is not unlikely that medical support will have to be provided at high pressure. More attention needs to be given to the selection of drugs, equipment, and procedures applicable to the care of an acutely ill patient on a deep dive.

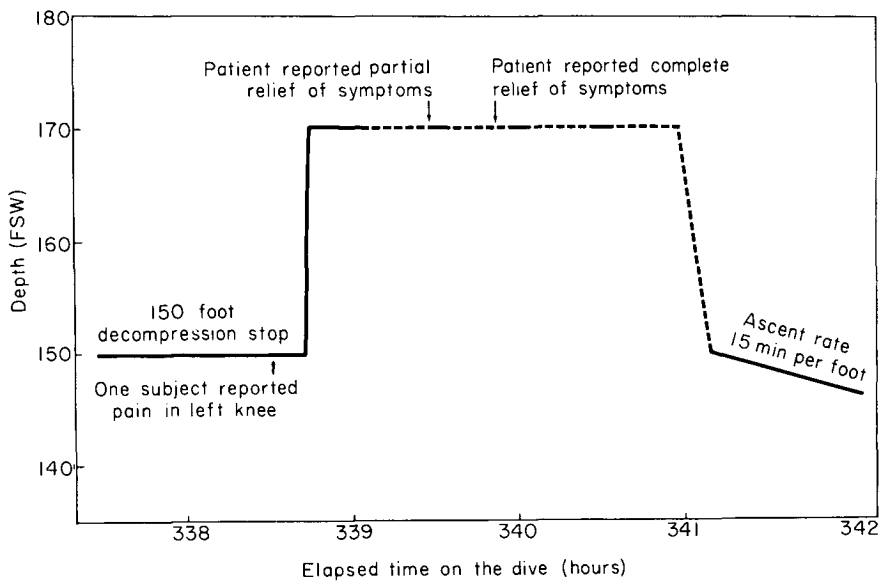

Fig. 5. Recompression treatment schedule. (——) Subjects breathing chamber atmosphere (P_{O_2}, 0.3–0.5 atm); (– – –) subjects breathing He–O_2 by mask (P_{O_2}, 2.46 atm).

Decompression Sickness

Approximately 2 hr after reaching the 150-ft decompression stop, one subject began complaining of a mild, aching pain in the left knee. A diagnosis of decompression sickness was made and immediate treatment was instituted, as follows (Fig. 5): The entire chamber complex was recompressed to 170 ft with pure He. All subjects were started on a therapeutic regimen consisting of intermittent high O_2 (2.46 atm) by mask. The patient obtained partial relief during the first high O_2 exposure period and complete relief during the second. Treatment was continued for 1 hr thereafter and was followed by decompression directly to 150 ft at a rate of 2 ft/min. At 150 ft, the regular decompression schedule was resumed. In an effort to decrease the probability of recurrent decompression problems, the 100-ft and 50-ft decompression stops were modified to include a 2-hr recompression cycle similar to the treatment schedule described above. No further difficulties were encountered.

Miscellaneous Problems

All of the subjects reported substantial dissatisfaction with the environmental control of temperature and humidity. External control frequently seemed inappropriate and a desire for internal control of the environmental conditions was expressed. The subjects also reported sensitivity to small changes in the temperature in the He atmosphere. A typical observation was that, when the subjects were lying down, the skin adjacent to the mattress had a sensation of excessive warmth and perspiration, while the areas covered only with clothing or a blanket had a sensation of being very cold. In general, the gas circulating system appeared to work adequately and the problem was not one of thermal stratification within the chamber.

There was difficulty keeping the communications and entertainment loud speaker systems at an appropriate sound level. Again, there was an expression of interest in internal control of these units.

With the intense level of activity and continuous environmental discomfort, fatigue was cumulative during compression and the time on the bottom. General observations after only 2 days reflected the effects of sleep deprivation. Another factor which was extremely irritating to the subjects was the essentially complete unintelligibility of He speech at 1000 ft.

Conclusions

Divers can perform well at pressures equivalent to 1000 FSW under the conditions of this dive, provided the life support systems provide other conditions equivalent to those at the surface. The medical problems encountered during the dive were relatively minor, but the potential for serious illness on deep saturation dives needs to be emphasized.

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HELIUM–OXYGEN SATURATION–EXCURSION DIVING FOR U.S. NAVY

Robert C. Bornmann

The SeaLab III operation was suspended shortly after it began in February 1969, due to the death of Aquanaut Berry Cannon and to other, unrelated, problems. In the preparation for and the short interval of this operation, considerable valuable information about deep saturation diving was acquired, and this information is being used to improve operations now with the Mark 2 Deep Diving System. It will be used as well to improve the SeaLab ocean floor habitat experiment as it is rescheduled. Unfortunately, this information chiefly concerns engineering and equipment. Medical scientists will have to await another opportunity to determine the total effects of the physiological and psychological stresses on man living and working out of a sea floor habitat at 100 fathoms.

Although only a sea floor operation can tie together all the interacting stresses in this deep, hostile environment, one should not overlook the solid accomplishments of more than six years of medical research and chamber dives which have been carried out under the direction of the U.S. Navy Deep Submergence Systems Project (DSSP) and its predecessors for the U.S. Navy SeaLab and the Man-in-the-Sea programs. The coming months will be utilized to full advantage; the requirements and the challenges for future open-sea operations are being investigated. This report concerns the first phase of a DSSP program which was begun in February 1969.

The diving procedure developed originally in preparation for SeaLab III is now a U.S. Navy standard and is the basis for all Navy saturation diving. The portions significant for saturation–excursion diving include control of the P_{O_2} in the habitat area (this term will refer either to a sea floor habitat or to a deck chamber of Deep Diving Systems) at 0.3 atm abs or 10 FSW. The saturated diver therefore will equilibrate at an inert gas tension which is 10 ft less than the absolute pressure inside the habitat. Excursion diving will ordinarily be done with a Navy semiclosed-circuit underwater breathing apparatus. The constant mass–flow regulator will be adjusted to maintain the inhaled P_{O_2} ("bag level") near 1.0 atm abs, but in practice, with changes in depth and in the diver's O_2 consumption, the bag level will vary between 0.5 and 1.5 atm abs. For decompression calculations it has been assumed that this level will never be less than 0.4 atm abs or 13 FSW. In an excursion the He pressure to which the diver is exposed is taken as 13 ft less than the absolute pressure of the excursion depth.

TABLE 1-A

NO-DECOMPRESSION LIMIT TABLE, REPETITIVE GROUP DESIGNATION TABLE, AND REPETITIVE EXCURSION TIMETABLE FOR EXCURSIONS FROM SATURATION EXPOSURE AT A DEPTH BETWEEN 150 AND 300 FSW GAUGE DEPTH

Depth of excursion from saturation exposure (ft)	No-decompression limits (min)	Repetitive group designation					
		A	B	C	D	E	F
+ 25	—	60	150	300	600	—	—
+ 50	270	30	60	100	150	210	270
+ 75	150	20	40	65	90	120	150
+ 100	60	10	20	30	40	50	60

When the diver makes an excursion to greater depths, his tissue inert gas tensions will increase. When he returns to the habitat they will return to the equilibrium level. This is the same situation as in diving from the surface and can be analyzed with the modified Haldane method used in the U.S. Navy (1, 6). However, the limiting elements for no-decompression excursion dives from increasing saturation depths reside in slower and slower half-time tissues, due to the splay of M values as depth increases. Therefore the magnitude of the dives permitted under these circumstances is much in excess of that for air excursion dives from the surface. Larsen and Mazzone (4) discussed this situation in 1966 when they reported on excursions from air saturation dives at 35 ft. Other work has been reported by Krasberg (3) and by Hamilton, Fructus, and Fructus (2). DSSP is developing tables for repetitive saturation—excursion diving for use with the Navy's new Deep Diving Systems.

The tables are limited to no-decompression dives and to excursion depths at which the bottom time is 60 min or more. Inert gas uptake in the excursions was calculated for a 200-min half-time rate, and gas elimination in the habitat was calculated at a 240-min half-time rate. The M value for both the 200- and the 240-min half-saturation-time tissue was accepted as 20 ft above the absolute depth of the saturation dive (6).

TABLE 1-B

NO-DECOMPRESSION LIMIT TABLE, REPETITIVE GROUP DESIGNATION TABLE, AND REPETITIVE EXCURSION TIMETABLE FOR EXCURSIONS FROM SATURATION EXPOSURE AT A DEPTH BETWEEN 300 AND 600 FSW GAUGE DEPTH

Depth of excursion from saturation exposure (ft)	No-decompression limits (min)	Repetitive group designation					
		A	B	C	D	E	F
+ 25	—	60	150	300	600	—	—
+ 50	270	30	60	100	150	210	270
+ 75	150	20	40	65	90	120	150
+ 100	100	15	30	45	60	80	100
+ 125	75	10	20	30	45	60	75
+ 150	60	10	20	30	40	50	60

All excursions are to depths greater than the saturation depth. The question of a "negative" excursion dive is an important one. In a sea floor habitat with a bottom hatch, the saturation pressure throughout the habitat is necessarily that of the water pressure on the bottom of the structure. Habitats such as the Navy SeaLab III can rise as much as 35 ft above the depth of this hatch. It might be extremely important sometime for an aquanaut to reach an item mounted high on this structure, and there is at present no good information about the risk of decompression sickness which he might face in doing so. This is a vexing problem, but it was not considered part of the present project.

In the particular case of a 350-ft saturation dive, tissues with half-saturation times less than 200 min play no part in limiting no-decompression dives. The inert gas tensions of the diver will equilibrate at 373 ft (383 abs − 10). For a no-decompression return to 350 ft, the tension in the diver's 200-min half-time tissue must not have increased to more than 403 ft (M = 383 + 20), or to more than 30 ft above the equilibration value (403 − 373). In a 150-ft excursion to 500 ft (533 abs), the He pressure of the diver's breathing mixture would increase to 520 ft (533 − 13) and this will develop an initial driving force or ΔP of 147 ft (520 − 373). In an excursion 25 ft shallower, the initial ΔP would also be 25 ft less.

Figure 1 graphs the time course of He uptake in the diver's 220-min half-time tissue in a 150-ft excursion dive and in a 125-ft excursion dive. The calculated increases are nearly linear. The 30-ft scale is divided into six 5-ft increments labeled A through F. In a 150-ft excursion the total pressure increase is less than 30 ft in 60 min, and the increase in each 10-min interval is also less than each 5-ft increment. In a 125-ft excursion the initial ΔP is smaller and the uptake curve lies below each 5-ft increment at the 10, 20, 30, 45, 60, and 75-min intervals. Six intervals have accordingly been used in Table I-B to tabulate the diver's debit against his no-decompression limits. Similar graphings have been used to devise figures for excursions 25,

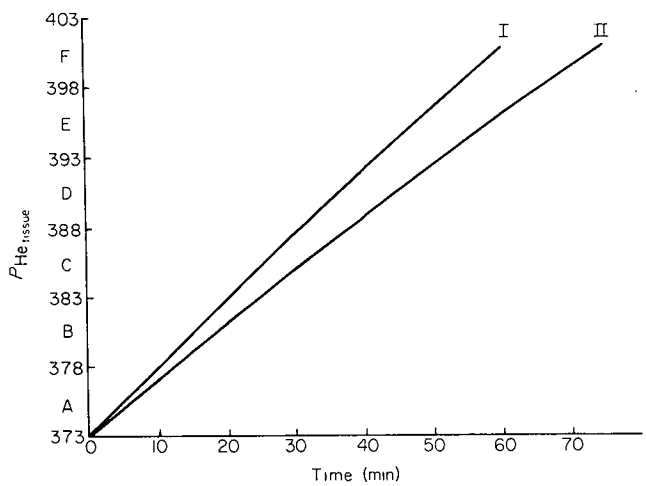

FIG. 1. Increase with time of the inert gas (P_{He}) in the 200-min half-saturation-time tissue of a diver in dives from a habitat at 350 FSW gauge depth to excursion depths of 500 and 475 ft. Tissue tension expressed in FSW abs. With P_{O_2} of habitat controlled at 0.3 atm abs, the diver has been previously equilibrated to inert gas pressure of 373 FSW absolute. Increase to 403 ft is maximum permitting no-decompression return to habitat. (I) Excursion to 500 ft, initial ΔP = + 147 ft; (II) excursion to 475 ft, initial ΔP = + 122 ft.

50, 75, and 100 ft deeper than 350 ft. The format for these tables follows as closely as possible U.S. Navy standard tables for repetitive air diving (5) and repetitive He–O_2 scuba diving (7). However, since these new tables are limited to no-decompression dives, it has been possible to eliminate the third "Repetitive Dive Timetable," as its figures in this case are identical with the "Repetitive Group Designation Table." In the system of designations used for the repetitive tables of the U.S. Navy Diving Manual, a 75-ft excursion for 90 min would put the diver in a "D" group at the end of his dive. A diver in a D group at the beginning of a 75-ft excursion would have to subtract 90 min from the no-decompression bottom time permitted to him. His "residual helium time" is 90 min in the latter situation.

The inert gas tension in the diver's slowest tissues will never be more than 403 ft if he stays within the limits of this table. His ΔP for inert gas elimination in the habitat during intervals at habitat pressure will always be -30 ft or less. Figure 2 graphs the fall of tissue gas tension at a 240-min half-time rate. This curve also can be blocked off into six intervals of 5-ft decrements and the results are as shown in Table II. The use of this table is exactly the same as the two other standard Navy Surface Interval Credit Tables (5, 7). Subtract the absolute depth from the above calculations for a 350-ft saturation–excursion dive and the figures will remain the same in similar excursion dives no matter how the saturation depth changes.

Figure 3 is a graphic display of the changes in M values with increasing depth, where M is the maximum inert gas tension which will permit ascent to the depth indicated. This is a slight modification of Workman's original table (6). The abscissa is gauge depth; the ordinate is the quantity M minus the absolute depth, or the excess of tissue inert gas tension over the hydrostatic pressure at each depth. The limits for the 200- and 240-min half-time tissues are identical and maintain a constant 20-ft distance above the saturation depth (zero slope). The lines for the 80- and 120-min half-time tissue limits are actually parallel and separated by a vertical distance of 2 ft but appear here as one line. The right-hand scale for the ordinate is

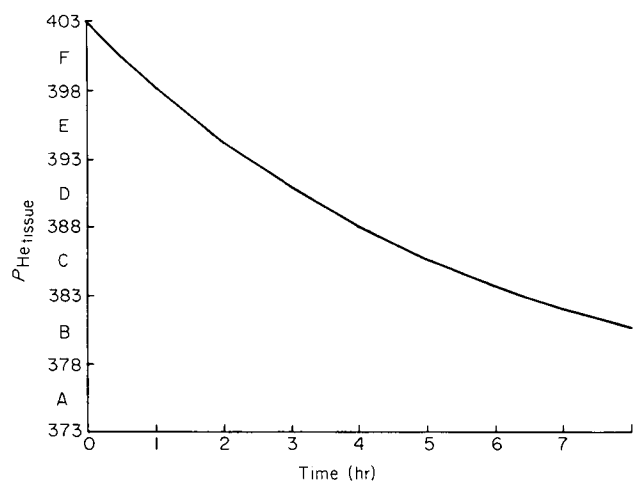

FIG. 2. Decrease with time of the inert gas (P_{He}) in the 240-min half-saturation time tissue of a diver on return to habitat at 350 ft gauge depth after excursion dive which has increased inert gas tension as high as 403 FSW absolute. With P_{O_2} of habitat controlled at 0.3 atm abs, the diver will eventually equilibrate again with inert gas pressure of 373 FSW abs.

TABLE II

HABITAT INTERVAL CREDIT TABLE FOR SATURATION EXPOSURE AT A DEPTH BETWEEN 150 AND 600 FSW GAUGE DEPTH

REPETITIVE GROUP AT THE BEGINNING OF THE HABITAT INTERVAL (from previous excursion)	REPETITIVE GROUP AT THE END OF THE HABITAT INTERVAL (before repetitive excursion)					
	F	E	D	C	B	A
F	To 1:00	2:30	4:00	6:30	12:00	24:00
E		1:30	3:00	5:30	10:00	24:00
D			2:00	4:00	8:00	24:00
C				2:30	6:30	24:00
B					4:00	24:00
A						24:00

consistently 10 ft greater than that on the left. It represents the tissue gas increase in an excursion from a saturation situation where the O_2 window is 0.3 atm abs.

The zero slope of the M value line for the 200-min half-time tissue means that if a 150-ft excursion for 60 min can be made safely from a 350-ft saturation depth, it can be made equally as well from any deeper saturation depth. How much shallower it can safely be made will depend upon other limiting considerations. The bar diagram on the right in Fig. 3 represents the inert gas tensions in the nine representative half-time tissues at the end of this excursion. The 5- and 10-min tissues have equilibrated at 147 ft. The 20-min tissue has increased 128 ft and the 40-min tissue has increased 96 ft. Both these latter increases intersect with their respective M value line at about 290 ft. Thus if a diver were saturated at 290 ft and made this excursion, he would be just at the limit (by criteria for the 20- and 40-min half-saturation-time tissues) for a safe return to 290 ft. If he made it from a saturation depth of 300 ft, he should be safe. If he attempted it from a saturation depth of 280 ft, he would run the risk of decompression sickness on his return to that depth. Our initial tests of this table have therefore been staged from a saturation depth of 350 ft.

A similar analysis of the 125/75 and the 100/100 excursions of Table I-B show a low limit at increasingly shallow saturation depths. On the left side of Fig. 3 is another bar diagram for the tissue inert gas tension increases during a 100 ft/60 min excursion. The increases of 85 ft in the 20-min tissue tension and of 63 ft in the 40-min tissue tension permit this only as an excursion from saturation depths of 140 ft or deeper. A proposed table for this shallower saturation depth range (200–350 ft) is shown as Table I-A.

Table I-B has recently been evaluated at the U.S. Navy Experimental Diving Unit in a 10-week test program of saturation dives to 350 ft. Five 12-day dive cycles included 1 day for compression and equilibration, 6 days for excursion dive testing, and 5 days for decompres-

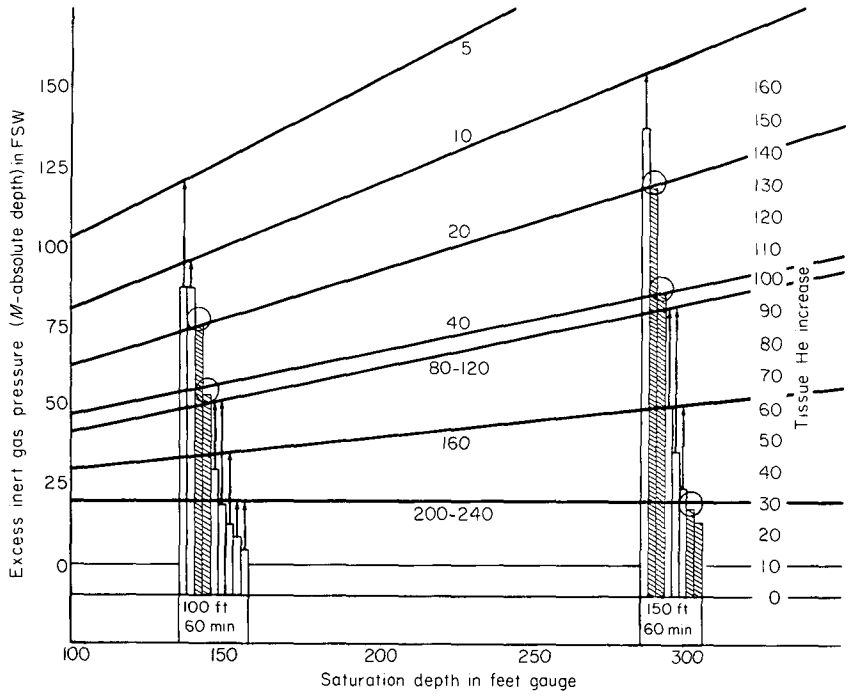

FIG. 3. Minimum saturation depths for no-decompression return following excursions 100 ft deeper for 60 min and 150 ft deeper for 60 min.

sion. Compression to saturation depth was staged at a rate of 40 ft/hr (8 hr 17 min total). Decompression was linear at 15 min/ft, but included four 4-hr stages of no pressure change added at 250, 150, 100, and 50 ft. Descent in the excursion dives was no faster than 60 ft/min. Bottom time was measured from the time of leaving saturation depth to the instant of leaving excursion depth in ascent. Ascent back to 350 ft was at a rate of 60 ft/min.

Two pairs of diver subjects made the saturation dive together but ran two separate excursion dive test patterns throughout the 6 days of testing. Each pair acted as habitat tenders while the other pair dived, and dives and surface intervals of the two pairs were alternated. A 12-hr overnight surface (and observation) interval was provided in both test patterns on each of the 6 days, and this also included an 8-hr night rest period. The schedule called for three excursion dives per day for each pair of divers. Decompression from the 350-ft saturation dive was not started until 12 hr after completion of the last excursion dive.

All the dive schedules used were subjected to computer analysis and proofing for decompression adequacy and safety prior to actual human testing. This analysis was carried out with a digital computer program which is a combined project of the Naval Ship Research and Development Center (formerly David Taylor Model Basin) and the Naval Medical Research Institute.

In the last three dive cycles (involving a total of 6 weeks of saturation diving) it was possible to test 208 excursion dives as shown in Table III. Each number in the table represents the total man-dives tested at each excursion depth with a repetitive group designation for the

diver (at the beginning of the excursion) as given in the column heading. The bottom time for each dive listed was the maximum time for a no-decompression excursion for the combinations presented. The 0 column includes the first 12 man-dives made on the first morning of each saturation cycle. The 60 dives under column A are the first dives of each of the other 5 mornings, when the 12-hr overnight surface interval has brought the diver back to Group A of the Repetitive Diving Tables. Note that the significant surface interval for repetitive diving with Table II extends to 24 hr, whereas that for the other standard Navy tables (5, 7) extends only to 12 hr. The remaining dives listed in Table III include variations of prior surface interval, excursion depth, and bottom time tested in subsequent dives each day.

It was hoped to obtain 8 to 12 test dives of each practicable combination in Table III as an adequate test of the decompression possibilities of Table I-B. The 25-ft excursion dives, the longer 50-ft dives, and the dives to 125 and 150 ft for less than 20-min bottom time (125E, 150D, and 150E) were excluded from testing. Generally the test schedule met this criterion with the exception of 150B. No signs or complaints of decompression sickness were recorded in any of the dive cycles at the Experimental Diving Unit during the 6 days of excursion dive testing, during the 12-hr observation period after each day of testing, or during the first 200 ft of saturation decompression (approximately 2 days). It is felt that this constitutes an acceptable test of Table I-B for excursion diving from saturation dives at 350 ft.

Theoretically the same table should serve equally well for excursions from greater saturation depths, but it is intended to run a deeper series of test dive cycles to demonstrate this. However, the task of validating a table for use with shallower saturation dives is a more attractive, difficult, challenging, and potentially more informative one. The next series of such test dives at the Experimental Diving Unit will demonstrate the adequacy of Table I-A in excursions from saturation dives at 200 ft.

Excursion diving permits a fuller utilization of the saturation diving potential. Operations will be made more flexible for those diving jobs spread over an area where water depth is variable. It is a matter of considerable interest that there are marked terraces on the Cobb Seamount off Seattle, Washington, at depths of 300, 450, and 600 ft. This is a common finding in oceanic geology. The exploration and exploitation of these important ocean structures by saturation divers will be enhanced through the use of excursion dive tables.

The present DSSP program is concerned with He–O_2 excursions from He–O_2 saturation dives. The Tektite operation points up a future requirement for the development of tables for He–O_2 excursions from shallow N_2–O_2 saturation dives and from deeper N_2–He–O_2 saturation dives. The experience gained in such projects must surely increase our understanding of the mechanisms by which the human body deals with inert gases under pressure.

It must be remembered also that a very slight change in the slope of the line of M values for the 200-min half-time tissues will permit no-decompression excursions on the order of 150 ft for 2 hr and 200 ft for 1 hr out of a saturation dive at 1000 ft. It is becoming evident that other physiological considerations will limit saturation exposures to lesser depths than those attainable for short bottom stays. Development of saturation–excursion tables for those extreme pressures will be necessary to permit man to work at the maximum possible depth.

Acknowledgments

No new concepts have been put forward in this paper. The excursion tables are a straightforward development from basic principles outlined by Workman in 1965 (6). Sincere ap-

preciation is extended to Captain Workman as well for his comments and advice in the development of these tables, to Lt. R. Buckles of the Naval Medical Research Institute, and D. Greenberg of the Naval Ship Research and Development Center (Carderock) for computer proofing of the test schedules, and to LCdr J. Summitt of the Experimental Diving Unit for actual conduct of the saturation test dive cycles.

TABLE III

NUMBER OF MAN-DIVE TESTS OF MAXIMUM BOTTOM TIME PERMITTED FOR LISTED COMBINATIONS OF EXCURSION DEPTH AND REPETITIVE GROUP DESIGNATION IN NO-DECOMPRESSION EXCURSIONS FROM SATURATION DIVES AT 350 FEET.

	O	A	B	C	D	E
PLUS 50 FEET					4	10
75	2	14	8	16	8	12
100	6	18	8	12	8	12
125	0	16	8	10	8	—
150	4	12	4	8	—	—

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SATURATION-EXCURSION DIVING: OPERATION LUDION II

R. W. Hamilton, Jr., and X. R. Fructus

In the normal course of conducting underwater operations at depths greater than about 10 m, the overwhelming limitation is the time required for decompression. As requirements occur for work at greater and greater depths, this problem assumes greater significance. While an hour of work at a depth of 30 m requires only about 38 min of decompression, the same period spent at 100 m requires several hours.

Saturation diving permits underwater crews to work for extended periods of time, alternating work and rest for several days or longer, and to pay the decompression penalty only once at the end of the period. Divers may be housed in an undersea habitat as in the initial experiences (1, 7), but such saturation–diving work is now generally done by the "commuter" technique whereby workers are housed in a deck chamber and shuttled back and forth to the work site without change in pressure.

Saturation–excursion diving is a combination of the two techniques, in that daily compressions to and decompressions from the work site are used, but the crew is housed at an intermediate undersea pressure between work periods. This method has the advantages that the habitat is at a more accessible pressure, simplifying the logistics, and that the divers can make substantially greater descent forays without complicating the decompression. Additionally, the final phase of decompression from saturation may be appreciably reduced.

Ludion II was a laboratory experiment designed to assess how well divers could tolerate the pressure and work of a 6-day work week using the saturation–excursion technique (2, 4). The name "Ludion" is the French word for the familiar Cartesian diver. The experiment was conducted in the pressure chamber complex located at the Comex facility in Marseille, as a joint venture between COMEX and Ocean Systems, Inc.

The working depth (D–2) was arbitrarily chosen as 400 feet of seawater, equivalent to 13.1 atmospheres or 122 meters depth. The plan called for two daily 2-hr work periods at this depth. The holding depth, designated D–1, was chosen so that stage decompression from each work excursion would not exceed two hours. This depth, 85 m, equivalent to 9.5 atm abs pressure, was computed using Ocean Systems' current decompression model and conservative ascent-limiting values of inert gas tensions. Details of the decompression calculation method have been reported elsewhere (8).

Two experienced divers, CW, age 38, and JD, age 30, served as subjects. The habitat con-

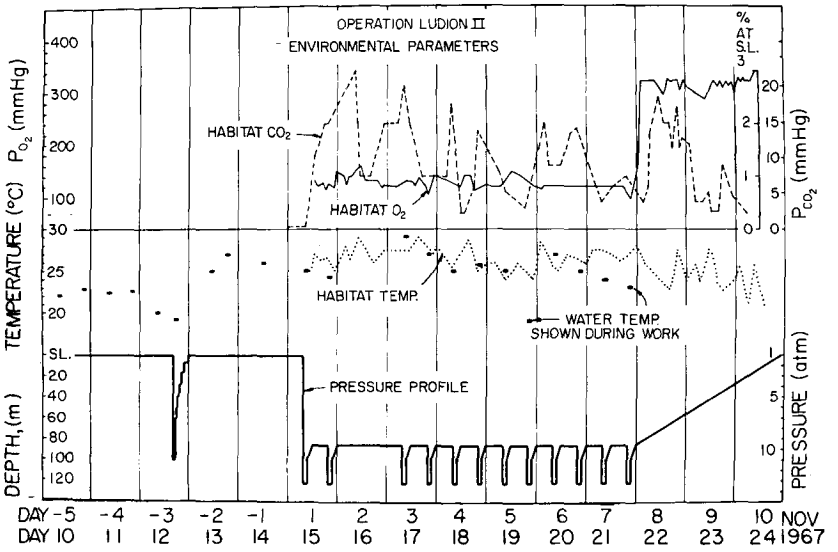

FIG. 1. Dive profile of Operation Ludion II.

sisted of a cylindrical chamber 2 m in diameter and 4 m long, equipped with a bunk and a hammock and with an external CO_2 scrubbing system. It was attached to a "wet pot," a vertically oriented cylinder 2.7 m in diameter and 4 m tall, half full of water over which was located a working platform. Extra locks were available for entry into either of these chambers from outside.

This experiment was basically an environmental exposure and observation of the response of man to the exposure. Accordingly, both the time course and the parameters of the environment are important to the interpretation of the results. Figure 1 shows a calendar of the experiment. The gaseous environment of the habitat was maintained at 2.6% oxygen with 15% nitrogen and the balance helium. During the five days just preceding the dive, the subjects lived in the habitat at sea level pressure and performed their daily work routines just as they would during the compressed phase. The pressure profile shows a practice dive performed on 12 November to test the operation of the complete system. The work routines were omitted on the second day because of problems with the CO_2 removal system, and an extra day was added on at the end.

The environment at the deeper, working pressure was slightly different from that of the habitat. A rubber membrane was used to separate the gaseous environments of the two chambers, and the divers climbed through a slit to go to and from "the work site." The work chamber was maintained at about 1.0 to 1.2 atm of oxygen partial pressure, and this was the atmosphere breathed by the divers when resting out of the water. The divers alternated as working divers; only one worked during each excursion while the other served as tender and administered tests. During periods of actual work and performance testing, the working diver wore a semiclosed breathing apparatus, the Mixgers unit, developed by the French Navy and marketed by the Fenzy Society of Paris. Both divers wore neoprene wet suits.

Certain items in the daily routine were firmly fixed. Work periods began at eight each morning and evening, and ended with the beginning of a 2-hr decompression. The remainder of the

day was devoted to general functions such as eating, sleeping, relaxing, personal hygiene, and medical monitoring.

During each excursion to D–2 the working diver performed several periods of work on a makeshift exercise device consisting of a 27-kg weight and an arrangement of pulleys. Work levels were set by timing the pulls to either 80 or 160 kg-M/min; this was continuous over periods nominally 20 minutes in length. On each excursion the subject diver was monitored for 20 min of rest, 20 min of work and over a half-hour recovery period. After rest the exercise period was repeated once or twice without monitoring. Monitoring consisted of ECG recording, the obtaining of Haldane-type end-expiratory alveolar gas samples, and estimation of ventilation and oxygen consumption. During exercise the P_{O_2} of the Mixgers breathing system, which was supplied with 10% O_2–90% He, was between 0.35 and 1.0 atm.

Carbon dioxide was determined with an infrared analyzer on samples passed out through a small lock in 50-ml nylon syringes. End-expiratory P_{CO_2} values taken from the breathing apparatus were close to the anticipated range most of the time (typically 36 to 48 mmHg), but on several occasions toward the end of a 20-min exercise period rose to a $P_{A_{CO_2}}$ as great as 60 to 80 mmHg. These high values were not seen during control experiments at sea level. Syringes left to stand and analyzed repeatedly showed no appreciable change even after 1 hr, and the delay was never that long in handling the samples. This indicated that there was probably no effect from differential diffusion. There was no indication of subjective stress to correlate with the high values. The Mixgers is a partial recirculating breathing system in which the diver breathes in and out of a 5-liter bag, while a smaller bag which moves with it is filled on expiration and then exhausts into the surrounding medium each time the diver inspires. As the volume of the large bag is reduced, extra mixed gas is added from the supply. Expired gas is passed through a Baralyme canister on the way to the large bag. On some occasions wet Baralyme was known to be responsible for an elevated CO_2, but this was not a satisfactory explanation for all high values.

Polarographic measurements of oxygen tensions supplied to the Mixgers and exhausted from it and pressure drop in the supply cylinder were used to calculate oxygen consumption values. These values, though perhaps low, were unremarkable and correlated fairly well with the level of exercise. Respiratory minute volume was estimated by multiplying the volume of gas supplied to the system by the ratio of the volume of the two bags. Using the value of 11:1 supplied with the unit, values were found which seemed to be about twice as large as expected for the exercise levels. The cause of this discrepancy was not discovered. Nevertheless, for a given level of oxygen consumption, the pulmonary ventilation was somewhat lower in measurements made at 13 atm than in control measurements made at sea level. This is in agreement with measurements made earlier at 20 atm (3, 5), and is consistent with increased breathing resistance.

Another phenomenon noted in the earlier exposure to 20 atm was bradycardia during rest and a relative bradycardia during exercise. Both subjects in the present experiment had significantly lower average heart rates during exercise at 13 atm than they did during approximately the same exercise at sea level. However, pulse rate was not significantly different during the recovery or rest periods at pressure and sea level. Since during "rest" the Ludion divers were often busy adjusting equipment and were not recumbent, subtle differences were impossible to detect. Possibly because a reproducible resting state did not exist in the Ludion exposure, no diurnal changes were evident, whereas these were observed in the earlier study.

TABLE I

BLOOD BIOCHEMISTRY

Date	Depth	NPN (g/liter)	Total protein (g/liter)	Uric acid (mg/liter)	Total lipids (g/liter)	Red blood cells (10^6/mm^3)	White blood cells (10^3/mm^3)	Hematocrit (%)
Normal values	(m)	0.28–0.40	62–85	20–69	3.5–8	4.5–6.5	4–11	40–54
				Diver CW				
8 Nov 67	0	0.35	72	56	8.9	4.51	6.1	46
11 Nov 67	0	0.36	70	60				
14 Nov 67								
Pre-dive	0	0.35	67	57				
+20 min	0	0.40	70			4.67	7.2	46
+ 2 hr	0	0.20	73.5			4.78	5.3	48
17 Nov 67	85	0.38	73.5			4.46	6.7	45
20 Nov 67								
Pre-dive	85					4.51	8.1	46
+20 min	97	0.35	85			4.5	7.8	46
+ 2 hr	85	0.33	81			4.46	7.7	45
21 Nov 67								
Pre-dive	85	0.40	70			4.37	8.2	44
+20 min	97	0.40	70					
+ 2 hr	85	0.38	70			4.47	8.7	44
27 Nov 67	0	0.30	87.5	55	5.8	3.96	5.7	41
1 Dec 67	0	0.30	79	49	5.5	4.1	8.2	44
				Diver JD				
8 Nov 67	0	0.45	72	65	6.1	4.37	8	45
11 Nov 67	0	0.30	75	50				
14 Nov 67								
Pre-dive	0	0.30	70	62		4.56	7.3	46
+20 min	0	0.20	70			4.54	8.7	45
+ 2 hr	0	0.18	78			4.65	8.1	47
17 Nov 67	85	0.36	84			4.79	7.3	48
21 Nov 67								
Pre-dive	85	0.35	77			4.67	7.8	45
+20 min	97	0.34	82			4.54	7.9	45
+ 2 hr	85	0.35	82			4.59	8.2	45
27 Nov 67	0	0.35	84	57	5	3.97	5.5	40
1 Dec 67	0	0.45	85.5	55	4.5	3.96	10	42

It was intended to learn whether the work at 13 atm would impose enough additional physical stress over similar work done at sea level to produce a difference in blood lactic acid. On two occasions (3 for CW) blood samples were taken just before the excursion, during the first decompression stop after work, and at the end of decompression to D–1 2 hr later. In addition, blood samples were taken on numerous other occasions throughout the experiment. The results of analyses made on these blood samples are shown in Tables I and II. In no case was there a change that could be related to the extra pressure, although the effect of exercise was reflected in several parameters. It should be emphasized that the work load was light and

TABLE II

BLOOD BIOCHEMISTRY

Date	Depth	Chlorine (mEq/liter)	Sodium (mEq/liter)	Potassium (mEq/liter)	Magnesium (mg/liter)	Glucose (g/liter)	Lactic acid (mg/100 ml)	Pyruvic acid (mg/100 ml)
Normal values	(m)	95–110	135–155	3.6–5.5	1.5–2.5	0.90–1.20	5–15	0.4–1
				Diver CW				
8 Nov 67	0	107	144	4.8	2.4	0.78		
11 Nov 67	0					0.80		
14 Nov 67								
Pre-dive	0	98	142	5.5	2.6	0.92	7.5	0.68
+20 min	0			4.9	2.5	1.04	12	1.44
+ 2 hr	0			5.1	2.5	0.90	17	1.16
17 Nov 67	85			4.8	2.4	0.80		
20 Nov 67								
Pre-dive	85					0.80	7.1	0.68
+20 min	97			4.5	2.4	0.82	21.3	1.37
+ 2 hr	85			5.9	2.6	0.82	14.2	0.61
21 Nov 67								
Pre-dive	85			5.2	2.6	0.92		
+20 min	97			4.9	2.6	0.76	10.6	1.16
+ 2 hr	85			5.5	2.6	0.72	17.8	1.03
27 Nov 67	0	105.7	145	5.9	2.1	1		
1 Dec 67	0	104	141	5.1	2.4	0.92		
				Diver JD				
8 Nov 67	0	113	143	4.9	2.5	0.80		
11 Nov 67	0					0.92		
14 Nov 67								
Pre-dive	0	102	143	5.2	2.5	0.96	5.7	0.68
+20 min	0			5	2.4	0.84	10.5	0.68
+ 2 hr	0			4.9	2.4	1.16	18.9	1.03
17 Nov 67	85			5.1	2.5	0.94		
21 Nov 67								
Pre-dive	85			5.9	2.6	1	8.3	0.84
+20 min	97			5.6	2.3	1.04	11.9	0.75
+ 2 hr	85			6.4	2.0	0.60	16.5	
27 Nov 67	0	110	140	5.8	2.0	0.96		
1 Dec 67	0	107	142	5.1	2.3	0.96		

there was an extended period of time for rest between the exercise periods. The work tasks and the entire exposure were well tolerated.

Four simple tests were used for assessing the effect of both pressure and work under water on human performance. These included:

Reaction Time: Time to make a *Yes* or *No* response to about 40 auditory and visual signals was measured each evening. Average time for response showed continual improvement throughout the experiment for one subject, and the other subject reached a plateau on the third

predive day and stayed level after that, with only a slight increase toward the end of the experiment. Errors were few, and the error rate did not change with time. On two occasions the test was given before and after a work session, and showed no difference as a result of the work. This test was performed with the diver out of the water, breathing the chamber atmosphere.

Washer Threading: This was a simple manual dexterity test which was given several times, just before and immediately after a 20-minute session of light work. The results showed no important changes from day to day, at sea level or at pressure. The usual result was that the time required to thread the washers was less in the test given immediately after work, except for one occasion (19 Nov) when the water was colder than usual. The test was given with the diver fully suited and under water.

Weight Estimation: This test and the following one are based on the observation in earlier laboratory dives (unpublished) that just after rapid compression to pressures of 15 or 20 atm a diver has an uncertain feeling about his muscular strength. Some divers feel stronger than usual, others seem to overexert themselves rather easily, as if they are not aware of how much work they are doing. In this test the subject was asked to guess, under water, the weight of a container weighing between 5 and 12 kg. The equipment was arranged according to a predetermined schedule, and the test was presented by the other diver. After each guess the correct answer was given. This test was performed several times, before and after work sessions at both sea level and D–2. The results are shown in Fig. 2. No prominent differences occurred either as a result of work or pressure; the accuracy of the guesses at 13 atm is a bit better, in that proportionately more answers are correct, but this might be as a result of slightly more experience.

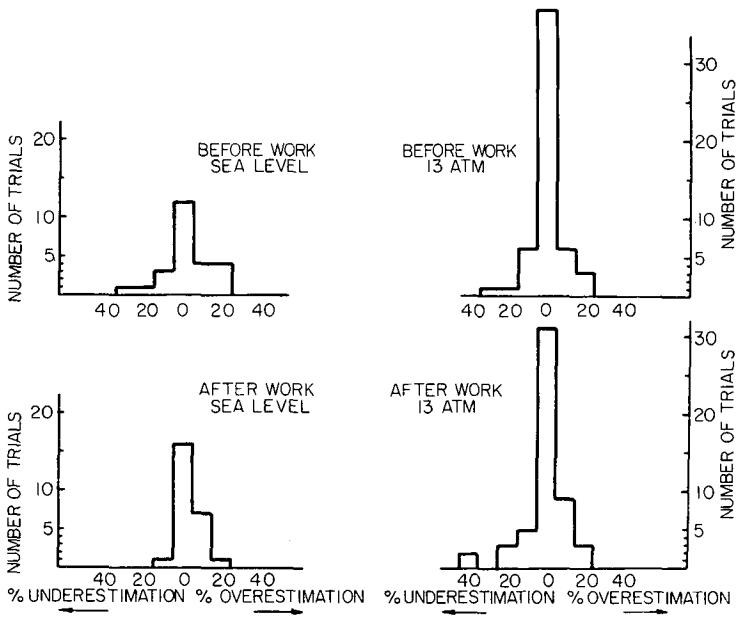

FIG. 2. Weight estimation test for Operation Ludion II, at sea level and at 13 atm.

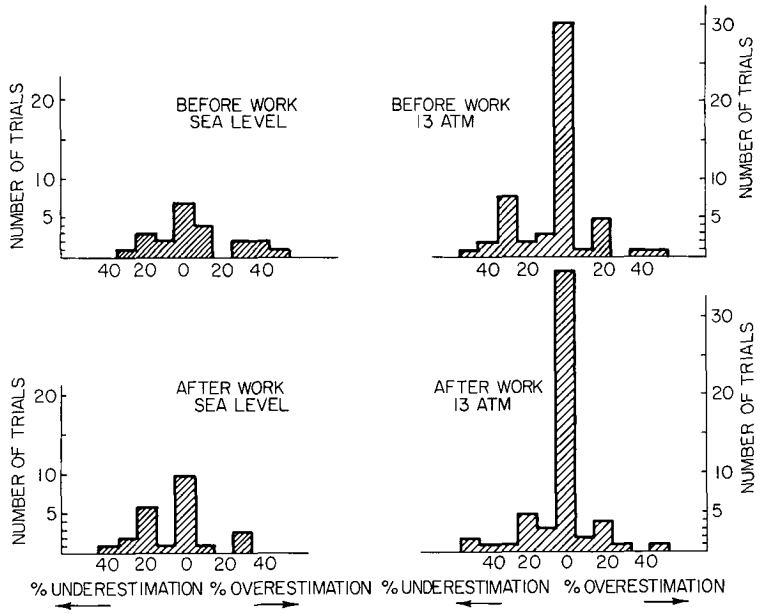

FIG. 3. Tension estimation test for Operation Ludion II, at sea level and at 13 atm.

Tension Test: For this test a spring balance was attached to the chamber wall, and the diver was asked to exert several specific tensions without looking at the scale. The results, shown in Fig. 3, indicate that at sea level following work there was a slight tendency to underestimate tension. This does not seem to be true at increased ambient pressures.

Both the weight estimation and the tension tests have the advantage that they are more or less free of the influence of motivation. Accuracy might suffer if the subject becomes bored, but it would be difficult for him to improve his score by trying harder. These tests support the notion that the conditions of this dive, with its particular pressures, gas mixtures, temperatures, and work levels, did not cause important performance decrements.

Electroencephalograms were recorded during sleep throughout the night, one time before the dive, once during the dive, and once during decompression, on each diver. Results, which have been reported elsewhere (9), show that sleep was not as sound in these divers during the pressure phase of the experiment as it was before the dive and during decompression. One diver (JD) showed a slight reduction and the other a substantial reduction in the relative amounts of deep sleep (Stages III and IV); there were no appreciable changes in the REM phase.

Throughout the study, including the predive and decompression periods, extensive medical, biochemical, and spirometric monitoring was carried out. In many of these parameters there were small changes associated with the exercise performed by the divers or with the stressful regime in general. In no case, however, were there important deleterious effects which could be attributed to the effects of pressure itself or to the special situation of performing physical work while at pressure. The divers consumed 3,500 to 4,000 calories per day and 1.5 to 2 liters of fluid. Each had a slight weight loss toward the end of the dive during decompression. Daily urine volumes showed normal fluctuations.

During and immediately after the 15-min initial compression, both divers experienced the familiar arthralgic condition in which joints of the body ache and feel as if they lack sufficient lubricant. There were no decompression problems at all, either during the twice daily returns to D–1 or during the final return to 1 atm. In all, the experience was well tolerated.

The work schedule was arbitrarily selected early in the planning stage of the experiment and the decompression and other routines were designed to conform to the work schedule plan. Considering both the divers and the support crew, a schedule exactly like the one used should be avoided in the future, even at the expense of a prolonged decompression time. The concept of two working excursions per day was good. However, spacing them at extreme ends of the day was not, since neither crew had time for a good night's sleep.

That the Ludion concept is a good one was further established by two occurrences that have taken place since the completion of the experiment. Operation "Contre–Ludion" (2) involved a work schedule such as the one here described but with total decompression to the surface each time, and it was clearly shown that such a procedure was not physiologically tolerable. "Operation Janus" (6) showed that a regime of saturation–excursion diving such as Ludion II could be put to work successfully at sea.

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PHYSIOLOGICAL EFFECTS OBSERVED IN THE COURSE OF SIMULATED DEEP CHAMBER DIVES TO A MAXIMUM OF 36.5 ATM IN HELIUM-OXYGEN ATM

X. R. Fructus, R. W. Brauer, and R. Naquet

During the first months of 1968, a series of simulated dives were conducted collaboratively in the hyperbaric complex of the Compagnie Maritime d'Expertises, Marseilles, France, partly to develop decompression schedules for proposed deep dives, and partly to determine whether the particular compression rates employed would produce physiological effects in man which could be related to events observed in other primates and rodents at the Wrightsville Marine Bio-Medical Laboratory in Wilmington, North Carolina. A total of seven dives using two subjects for each dive constituted this series. Four of these dives went to depths equivalent to or exceeding 1100 ft of seawater. The maximum depth, attained on June 27, 1968, was 1190 ft. Eight individual subjects participated in these experiments, and two of these were each involved in three dives exceeding 1100 ft.

Figure 1 shows the profile of three of the deepest dives. Sojourn at pressures in excess of 990 ft of seawater ranged from 24 to 111 min. Sojourns at the maximum pressures attained were relatively short and can be seen in Fig. 1. Table I shows other characteristics of these dives, including the fact that pressurization rates were such that maximum depths were attained in each of the deep dives within a little over two hours. The table also shows the total time employed during decompression in each of the dives. The gas mixtures used were monitored continuously.

Of the three deep dives the last one, Physalie III, is the one most completely documented and will serve as the major basis for this presentation. The oxygen partial pressure (P_{O_2}) was allowed to rise initially from 0.2 atm at the surface to between 0.58 and 0.62 atm and held there from the time that a total pressure of 20 atm had been reached until the early phase of decompression had been completed. Through use of an internal carbon dioxide absorption

system, the P_{CO_2} of respired gas was kept at 0.54 to 0.56 $\times$ 10^{-2} atm throughout the exposure period. Nitrogen partial pressures rose slightly from an initial 0.8 atm to 1.2 atm and were maintained at this pressure throughout the dive. Helium made up the balance of the chamber atmosphere.

High temperatures of the chamber atmosphere had existed in the earlier dives; in Physalie III, temperature was monitored in the immediate vicinity of the divers and controlled so that subjectively comfortable temperatures were maintained throughout. Chamber temperature rose from 28°C at the outset to a maximum of 35°C, reached at chamber pressure of 22 atm. From that stage in the exposure it decreased to between 30° and 32°C and was maintained in that range throughout the rest of the deep portion of the dive.

During the first two pressure exposures in the series, the divers had also experienced some inconvenience due to high noise levels and had suffered some evidence of vestibular disturbances during decompression at pressures between 10 to 18 atm. The noise problem was relieved in Physalie III by the use of appropriate ear muffs. Compression rate was decreased greatly as maximum pressure was approached, so that effects of noise, high-temperature and pressure fluctuations as such became minimal. In Physalie III, none of these influences was significant and the divers felt comfortable even when 30 atm had been reached.

Physiological effects noted in the three deep dives which involved the same two subjects were entirely comparable and paralleled observations made with other subjects to the limit of depth attained by each. "Helium tremors" were noted in each subject each time he was exposed, the onset pressure ranging from 22 to 28 atm. The effect increased in severity as pressure was increased, even though compression rates were progressively decreased. There

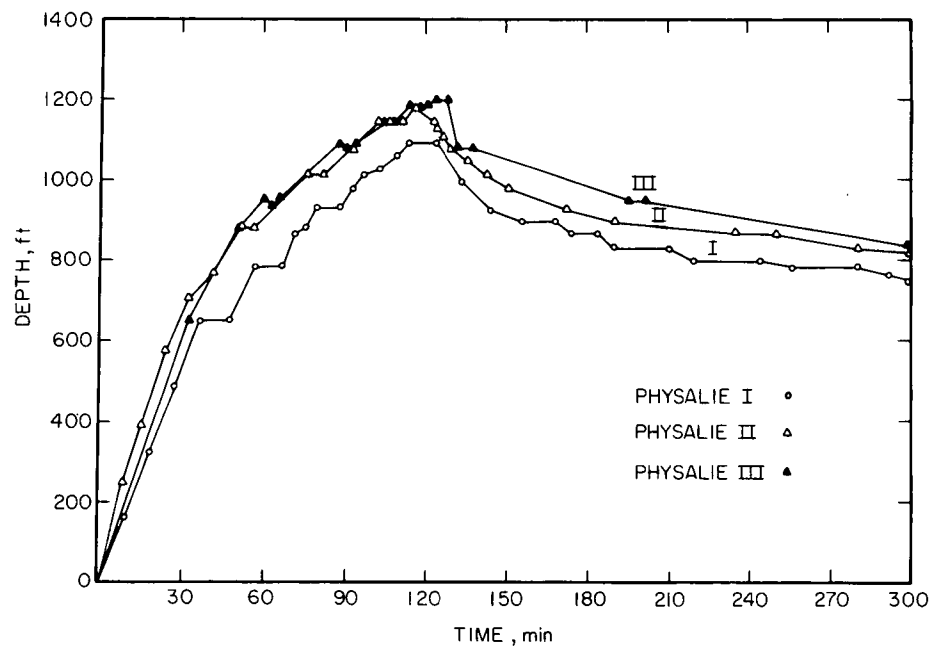


FIG. 1. Time course of compression and early phases of decompression in the three experiments of the Physalie series.

TABLE I

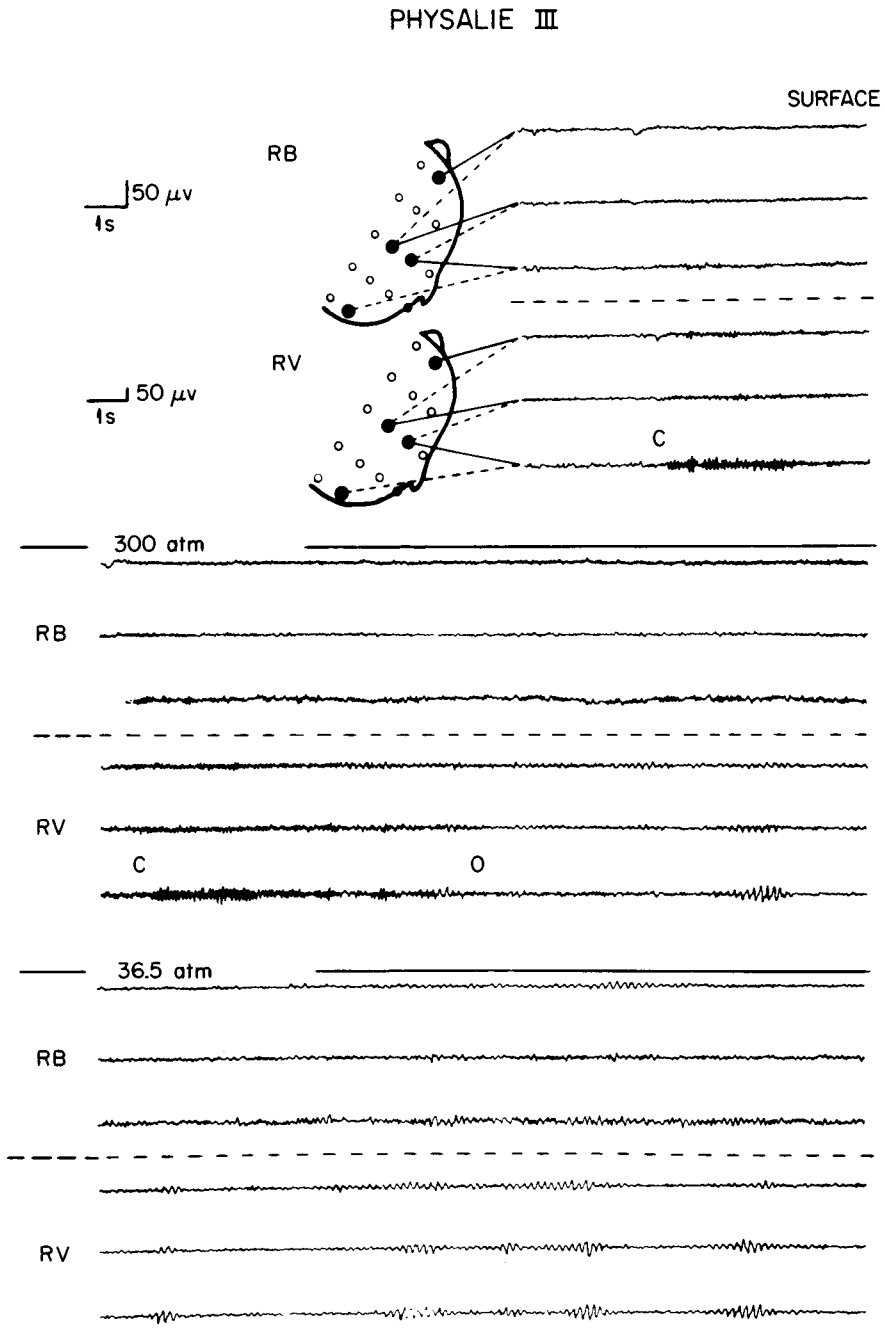
SIMULATED DIVES ASSOCIATED WITH SERIES PHYSALIE, 1968

Dive	PLC I	PLC II	PLC III	Phys. I	Phys. II	Phys. III	Phys. IV
Date	2/24/68	5/2/68	5/14/68	5/21/68	6/11/68	6/27/68	9/24/68
Subjects	RV/AJ	JD/HR	PF/AJ	RB/HD	HV/RB	RB/HV	JD/FF
Depth (feet seawater)	1100	900	990	1100	1180	1200	990
Time to beginning of decompression (minutes)	133	110	85	123	120	127	190
Time spent beyond 990 ft (minutes)	41	0	20	24	77	111	10
Duration of decompression (hours)	94^{37}	97^{15}	90^{50}	97^{33}	116^{31}	138^{34}	106^{50}

was some suggestion that tremors might have been most severe in experiment Physalie II in which the divers spent a large part of the compression respiring a pure helium–oxygen atmosphere from masks. The divers all agree that such tremors as they experienced tended to disappear rapidly with the beginning of decompression. Psychomotor tests, in particular dotting of circles of decreasing diameter, showed little change, but handwriting revealed the tremor. The syndrome is characterized by some degree of ratchet movements during flexion, and by the association of marked tremors especially with unconscious, rather than with intentional, movements.

EEG changes began to appear at pressures close to 30 atm, the threshold being consistently lower for one subject (RV) than for the second one (RB) in the three exposures to maximum pressure performed by each (Fig. 2). The EEG changes are characterized by the appearance of slow waves in the theta range, with a frequency approximately 5 to 6 cps. These increasingly displaced the alpha waves in the EEG pattern of the resting subject. Such signs increased in severity as the pressure was increased, and above about 33 atm were associated with behavioral effects including intermittent bouts of somnolence occupying an increasing portion of the time, associated with intermittent state 1, later state 2 sleep patterns in the EEG. Once again, these changes were noted sooner and more severely in RV than in RB. Associated with this was some loss of vigilance. During Physalie III, RB performed two hyperventilation tests of 60 seconds each. The first at 25 atm, before the onset of EEG signs in this subject, failed to produce any alteration in EEG or in behavior. The second, at 33 atm, produced no overt deterioration of the EEG. It was noted, however, that shortly afterwards there was evidence of confusion in this subject. Both subjects could be easily aroused from their intermittent bouts of somnolence, even at maximum depth. EEG changes persisted during decompression for approximately 10 hr in the one subject monitored during Physalie II, and for more than 12 hr in both subjects during Physalie III. These early phases of decompression were associated subjectively with fatigue and a degree of depression.

Among other parameters studied, heart rate did not change markedly, ranging between 55 and 65 beats/min in both subjects throughout the period of compression from 25 to 35 atm in Physalie III. Respiratory frequency did not change to an important degree in subject RV, while in RB there was a persistent, though slight, increase in respiratory rate after the second period of hyperventilation at 33 atm. Among psychomotor tests employed, an index of complex reaction time revealed no change in either subject until a pressure of 33 atm was reached, but


PHYSALIE III
SURFACE
RB
50 μv
1s
RV
50 μv
1s
C
300 atm
RB
RV
C
O
36.5 atm
RB
RV

increased by approximately 15% between 33 and 36 atm. This effect persisted during the early phases of decompression, and then disappeared.

This series of exposures to high ambient pressures breathing helium and oxygen indicates that both peripheral and CNS effects were associated with the particular compression schedule employed. Helium tremors appear to be highly susceptible to compression rate, as revealed by the observation that the two subjects who were compressed more slowly to 30 atm in Physalie IV failed to show observable helium tremors at depths where during the more rapid earlier dives of the series, one of the two subjects of this dive, as well as all other subjects, had revealed helium tremors. The EEG changes and the changes in wakefulness and alertness of the subjects likewise are consistent with a CNS effect. They have been shown to be reproducible in the same subject under similar compression conditions; they occur at an oxygen partial pressure between 0.5 and 0.6 atm and are not associated with any evidence of respiratory distress. They persist for some time after decompression has been initiated.

The etiology of this syndrome in man and its relation to the "high pressure hyperexcitability syndrome" (HPHS) observed in other primates remains to be determined. As pointed out in the chapter by Brauer *et al.*, page 487, this volume, susceptibility to HPHS seems to increase markedly as one proceeds from lower species to those with more highly developed central nervous systems. With this trend in mind, it is worth noting that at compression rates comparable to the ones employed in these human studies, squirrel monkeys show helium tremors beginning at about 20 to 25 atm and EEG changes such as spiking at 45 to 50 atm, and subsequently develop frank electrical and motor convulsive seizures at pressures which for helium–oxygen atmospheres average 62 atm. Considering the fact that the EEG effects in man were seen to begin near 30 atm and were fairly pronounced at 36.5 atm, it is possible that the two syndromes may be related and due to similar causes.

FIG. 2. Electroencephalographical tracings from Physalie III experiments showing a development of the patterns in two subjects at surface, at 30.0 atm, and at 36.5 atm. The tracings for subject RB at the surface show a basic alpha rhythm, 9–10 cps, of moderate amplitude, elicited in brief bursts when the eyes are closed (C). A few instances of beta frequencies are noted in the Rolandic areas. At 30.0 atm, alpha activity is more continuous, of greater amplitude, and interrupted by rare sporadic theta waves. The Rolandic beta frequencies persist. At 36.5 atm, the alpha activity is interrupted by theta activity, 5 cps in the temporo-occipital region. The anterior leads show independently bursts of 5 to 6 cps. The subject at this point, and during these periods, shows evidence of somnolence. The tracings for subject RV at the surface show activity at 10 to 11 cps, in prolonged bursts which come on when the eyes are closed (C). At 30.0 atm, alpha activity persists at the beginning, but subsequently decreases in intensity. Bursts of 6 cps waves in the frontal region and independent others of the same frequency in the temporo-occipital region become increasingly evident. This period corresponds to a decreasing level of vigilance. At 36.5 atm (and indeed from 33.5 atm on) the tracings are dominated by 5 to 6 cps activity which comes on in generalized bursts over the entire hemisphere, but which may now and again predominate independently in either the anterior or the temporo-occipital region. The subject shows evidence of somnolence. It is to be noted that while vigilance of RB persists undiminished at 30.0 atm, that of RV begins to decrease. At 36.5 atm, the two subjects at rest show markedly decreased vigilance. Arousing the subjects, as by asking them to perform a set task, reverses these changes, but as soon as the subjects are allowed to rest, the tracings revert to the patterns shown above. At the high pressures between 34.0 and 36.5 atm, the slow wave patterns fail to disappear upon opening the eyes, and even on arousal of the subject. In none of the tracings obtained in the three dives of this series have any clearly identifiable paroxysmal changes been observed in either one of the two divers.

It is also to be noted that in the primates tested, slowing the compression rate produces a perceptible increase in the effective pressures required to produce tremor or convulsions. In the squirrel monkey at a compression rate of 5 atm per hour, convulsions occur at pressures 15% to 20% higher than at a compression rate of 22.4 atm per hour. In the baboon compression rates of 2 atm per hour elicit convulsions at pressures between 70 and 80 atm. It seems reasonable to infer that if the human syndrome does indeed bear a relation to the one observed in the several lower primates, compression rates very much below those employed in the Physalie series should allow placing human divers at pressures in excess of 45 atm before changes in CNS activity comparable to those reported above might be expected.

PROJECT TEKTITE: AN OPEN-SEA STUDY OF PROLONGED EXPOSURES TO A NITROGEN–OXYGEN ENVIRONMENT AT INCREASED AMBIENT PRESSURE*

J. W. Miller and C. J. Lambertsen

Project Tektite† is a composite program of saturation diving which attempts to blend the medical, psychological, general biological, engineering, and operational aspects of shallow manned undersea activity. While its applications are ultimately to marine sciences, the Tektite project had its origins in human bioscience. Office of Naval Research psychologists suggested to the National Aeronautics and Space Administration that undersea habitats could serve more realistically in study of behavioral influences of prolonged isolation and confinement than do sealed chambers in dry land laboratories. This suggestion was extended by physiologists of the University of Pennsylvania's Institute for Environmental Medicine, who recommended the use of saturation with nitrogen with natural oxygen partial pressure, at 4 atm abs (about 100 ft of seawater), to effect what they designated as "physiological entrapment." It was considered that such exposure would provide for nearly normal vocal communication, but would absolutely require such slow and programmed decompression that any subject who wished to leave the high pressure environment of the submerged compartment would not be able to do so at will (i.e., physiological entrapment). This restriction would serve the interests of both the space and undersea psychologists concerned with study of men in truly hazardous circumstances.

The physiologists, in recommending saturation with nitrogen, had the ulterior motive of calling attention to the present need for detailed and extensive studies of nitrogen as a respiratory vehicle for oxygen in the important shallow and moderate diving depths which have been overstepped by recent attention to very deep helium diving. To provide a purposeful daily

* Project Tektite represented the cooperative efforts of individuals from a number of organizations, with the largest contributions being made by the Office of Naval Research, which coordinated the overall operation, the National Aeronautics and Space Agency (which sponsored the behavioral studies), the Department of the Interior (which provided the marine scientist–subjects), the University of Pennsylvania (which coordinated the biomedical and safety program), and the General Electric Corporation (which designed and constructed the habitat).

† The project title "Tektite" comes from the name for small particles of space-born matter which survive the fiery plunge through the earth's atmosphere and come to rest on the ocean floor.

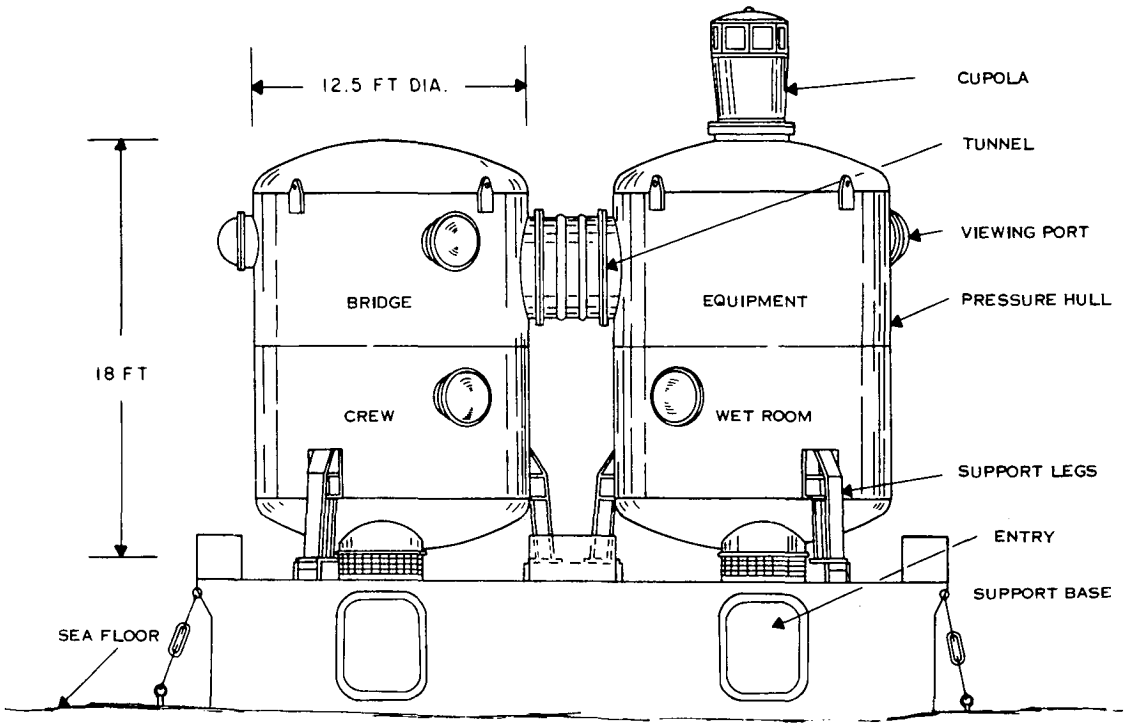


FIG. 1. Side view of the four-man Tektite habitat.

working basis for prolonged human residence in the open sea, marine scientists of the Department of the Interior were invited to join the program, and the General Electric Corporation proposed and constructed a habitat specially designed for application of saturation diving to marine sciences activities. Primarily to facilitate study of reef life and structure, but also to provide for a stepwise approach to future, deeper exposures, the first of the Tektite series of open sea saturation dives was limited to slightly less than 50 feet of seawater. The duration was maintained extreme, at 60 days of submerged work and observation.

The Habitat and Working Environment

The undersea habitat was a four-compartment structure well-designed for living and as a true laboratory base for conduct of marine science activities at the undersea site. The compartments (Figs. 1 and 2) were arranged in two tiers with one lower compartment having direct, continually open access to the sea. Pressure within the entire gas-filled habitat complex was thus maintained equal to the hydrostatic pressure at the level of the water at this open entry–exit hatch. The compartment opening to the sea served versatile functions as a diving staging room and laboratory, as well as for logistics activities. An upper compartment contained the mechanical support systems for ventilation, power, carbon dioxide removal, and the power and communications links to the dock and shore-based stations. One compartment

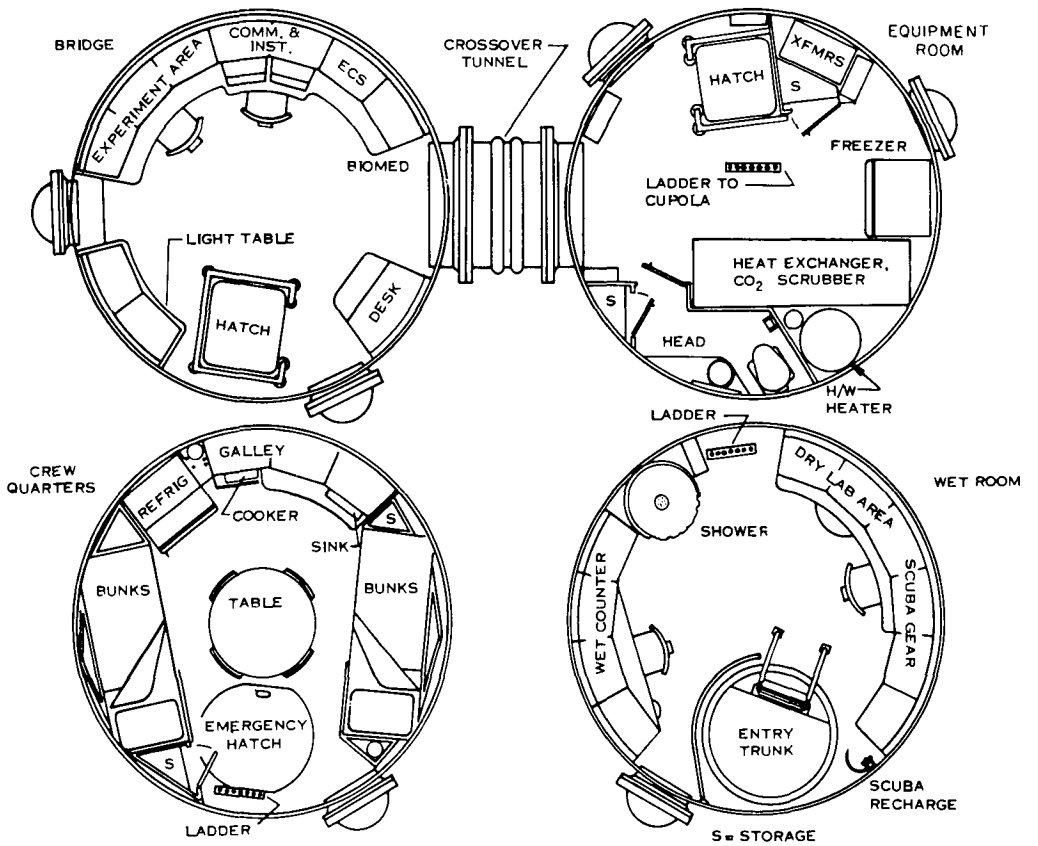


FIG. 2. Overhead view of individual habitat compartments.

served as a living area and the final compartment for specific physiological and marine sciences laboratory functions.

The gas respired in the habitat was 90.2% nitrogen with 9.8% oxygen which, at the physiological saturation depth of 38 ft of seawater, provided a nitrogen pressure equivalent to 48 ft breathing air. This depth was chosen to facilitate exploration of a particular reef structure at the selected site in Great Lameshur Bay, St. John island, in the Virgin Islands. The diver-subjects were permitted to dive 60 ft below and to ascend 20 ft above this saturation depth throughout the 60 days of undersea exposure.

The Scope of the Scientific Program and the Subject Group

The overall scientific program was comprised of three principal parts including the biomedical, the behavioral, and the marine sciences studies. To serve as subjects and investigators, four marine scientists were selected. The marine sciences program, encompassing marine biology, geology, and aspects of oceanography, was designed and conducted by these in-

TABLE I

STUDIES OF RESPIRATORY CONTROL AND PULMONARY FUNCTION[a,b]

Study/Measurement		Pre-exposure	Days after start of underwater exposure								Post-exposure
			5	12	19	26	33	40	47	54	
Respiratory control											
Air breathing at rest											
Alveolar P_{CO_2}	mmHg	37.5									37.2
Respiratory minute volume	L/min	7.30									7.51
Tidal volume	L	0.65									0.62
Respiratory frequency	breaths/min	12.7									12.6
CO_2 breathing at rest											
Slope of response to CO_2	L/min/mmHg	2.86									3.20
Pulmonary diffusion											
Diffusing capacity	ml/min/mmHg	30[c]									33[d]
Total lung capacity	L	7.5									8.0
Mixing efficiency	%	90									94
Pulmonary mechanics											
Compliance	L/cm H_2O	0.30	0.24	0.22	0.30	0.25	0.24	0.28	0.24	0.24	0.26
Resistance	cm H_2O/L/sec	2.0	2.5	2.8	2.8	2.2	2.0	1.8	1.9	1.8	1.9
Pulmonary ventilation											
Vital capacity	L, BTPS	5.73	5.76	5.72	5.76	5.74	5.74	5.81	5.92	5.94	5.96
Expiratory reserve volume	L, BTPS	1.71	1.98	2.06	2.33	1.83	1.85	2.23	2.07	2.30	2.16
Inspiratory capacity	L, BTPS	4.03	3.77	3.67	3.42	3.92	3.89	3.58	3.84	3.64	3.80
Forced expired volume	%, 1 sec	81.0	73.0	74.7	71.9	72.0	73.7	73.8	72.5	70.3	79.0
	%, 3 sec	96.4	96.5	97.0	95.4	95.6	95.5	95.3	96.4	95.4	96.0
Maximal midexpiratory flow rate	L/sec	4.82	3.55	3.63	3.50	3.46	3.60	3.62	3.66	3.63	4.46
Maximal midinspiratory flow rate	L/sec	7.35	5.52	5.40	5.70	5.77	5.62	5.54	5.91	5.76	8.92
Maximum voluntary ventilation	L/min	176.6	129.4	151.4	151.2	142.1	147.0	145.4	152.3	154.7	216.4

[a] This composite summary of average findings is derived from pulmonary function studies by A. B. Fisher, A. B. DuBois, R. W. Hyde, C. J. Knight, and C. J. Lambertsen (5) and a respiratory control study by J. G. Dickson, Jr., R. Gelfand, and C. J. Lambertsen (3).

[b] Mean values in four subjects.

[c] Measurements made 1 month prior to start of exposure.

[d] Measurements made 1 day after end of exposure.

dividuals at the same time that they served as subjects for the behavioral studies and for the biomedical studies which will be emphasized in this report.

The Biomedical Program

The original plan for detailed physiological study at 4 atm abs had emphasized the importance of appraising adaptations or deteriorations occurring over many days of sustained exposure to the combined stresses of nitrogen narcosis and the increased work of breathing the high density atmosphere. It was considered that even at a pressure of 4 atm abs, the maintenance of a natural oxygen pressure would completely eliminate the oxygen toxicity inevitable with breathing air itself. However, at 4 atm abs the composite of narcosis and limitation of pulmonary ventilation might produce hazardous reduction of intellectual performance and limitation of exercise capability in open water work.

At the shallower 38 foot depth selected for the open sea operations and the marine sciences program, it was considered

1. Unlikely that inert gas narcosis would be detectable. Its measurement therefore was not attempted
2. Unlikely that major circulatory derangements would result from either the increased nitrogen pressure or the increased ambient pressure. Therefore no major circulatory studies were included
3. Likely that the primary physiological stress would be the increase in airway resistance and work of breathing due to the greater density of respired gas. Therefore respiratory control and pulmonary function were selected as the measures most likely to provide sensitive indices of such stresses upon the respiratory–pulmonary system
4. Unlikely that changes would be induced in hematological or blood chemical characteristics. Nevertheless, extensive study of the blood was planned

Respiratory–Pulmonary Studies

Specific studies of respiratory function included those summarized in Table I.

Hematological Studies

It has been known from studies conducted in support of the manned space flight program that sustained multiday exposure to small elevations of inspired P_{O_2} leads to decrease in circulating red cell mass (4). By the intentional maintenance of a natural level of inspired P_{O_2}, it was considered that all forms of oxygen toxicity could be obviated. Complete hematological studies were nevertheless carried out and are reported elsewhere (8). The influences of sustained, high nitrogen pressure upon white blood cell formation are not at all well known and, even though nitrogen is usually considered a physiologically "inert" gas, it is not biochemically inert. Since multiday exposure to increased tensions of nitrous oxide has produced suppression of white blood cell formation (7), it was deemed essential to include measurement of white cell concentration in the subjects exposed to the extremely prolonged periods of increased tissue P_{O_2}.

For purposes of general survey the same extensive measures of blood chemical composition were performed as are conducted on the U.S. manned space flight teams (4).

Microbiological Studies

In men living in a state of close proximity to each other and in relative isolation from surface support groups, modification of individual flora might be expected over a 60-day period of undersea existence. In the repeated exposures to the wetness of a marine microbiological environment, the possibilities of exchange of flora between the sea and the habitat with its contents and occupants were also considered. Extensive and repeated direct cultures of the skin, throat, external auditory canal, nose, and rectum of each subject were paralleled by cultures from the gas and special internal surfaces of the habitat, as well as from the surrounding sea (8).

Clinical Studies

To assure the selection of an appropriate aquanaut group and to provide baselines for subsequent comparison with postexposure measurements, each subject was appraised in a series of detailed medical evaluations by clinical specialists with particular qualifications in environmental medicine. These clinical evaluations, which are summarized in Table II, are recommended for any group of aquanauts entering into a previously unexplored laboratory or open sea exposure. Of particular importance are the audiometric–vestibular function evaluations, the detailed ophthalmological examinations, and the dermatological survey.

Decompression Studies

Saturation diving with nitrogen had previously been employed only to a depth of approximately 1.6 atm of N_2 (33 feet of seawater) (2). To assure ability to decompress safely, even from the only slightly higher pressure of 1.9 atm of nitrogen in Tektite I, required practical chamber trials in man of procedures for decompression from the nitrogen-saturated state (8). Bends developed in the subjects in these trials until a decompression pattern slow enough to permit clearance of nitrogen from a 500-min half-time tissue was employed. Further studies indicated that subjects saturated with nitrogen at this pressure could be abruptly decompressed to one atmosphere, then would not develop bends within a period of minutes after surfacing (8). Demonstration of this safe surface interval provided the basis for using the "surface decompression" approach as the method for bends prevention in the event of accidental surfacing.

The decompression sequence actually employed following the 60-day saturation used the

TABLE II

Clinical Studies

General medical examination	—
Audiometry	—
Vestibular function	Caloric stimulation
Dermatological examination	Including corneocytes and quantitative microbiology
Neurological examination	Skull X-ray, electroencephalography
Ophthalmological examination	Slit-lamp examination, retinal photography, visual fields, angiography, refraction
Long bone radiography	—

addition of repeated periods of oxygen breathing to accelerate nitrogen elimination as the subjects neared the end of the approximately 20-hour decompression period. In spite of this prolonged decompression, one subject detected minor pain in a knee joint prior to the oxygen breathing phase of decompression. This pain disappeared spontaneously during the oxygen exposure. A lesion, presumed to be a small gas bubble, was found in the lens of his eye during postdecompression ophthalmoscopy about 12 hr after reaching the surface. This lesion entirely disappeared over the several days subsequent to decompression without recompression treatment (8).

The results of the entire multidisciplinary program, covering psychological, physiological, medical, microbiological, hematological, and marine studies, are gathered in a single, coordinated report (8). Individual aspects are also being reported in the general literature. For the special purposes of this symposium, the effects upon the subjects themselves can be summarized as follows.

1. No effect of the 2-month exposure to the 1.9 atm of nitrogen with natural oxygen tension appeared to be of a type or degree that would limit the ability of normal men to work for prolonged durations at the depth studied.
2. The slight induced increase in pulmonary resistance led not to a progressive deterioration of pulmonary ventilatory capability, but to a detectable increase in the strength of the respiratory musculature (5).
3. No suppression of white cell formation or other alteration of blood cellular or chemical characteristics was found.

The studies and practical trials related to decompression indicate that even the 480- to 500-min half-time postulated for rate of nitrogen exchange with the most slowly perfused body tissues (1, 8) may be too short for nonvascular tissues such as those of the eye (6).

In prolonged submersion with repeated actual immersion, the function of the skin as a barrier between the tissues and the external environment is challenged by repeated wetting. In the Tektite program, free opportunity was provided for cleaning and drying the skin, with the result that postexposure dermatological appraisal revealed no abnormalities of the general integument.

A special "dermatologic" problem related to the undersea exposure did occur. This was pseudomonas infection of the skin lining the external auditory canal in both ears of all subjects. After discovery and diagnosis the infection was completely overcome during the course of continued undersea exposure by drying the ears with alcohol acidified with boric acid and by instillation of coly-mycin S antibiotic drops.

The 2-month exposure produced no evident influences upon the ability of the subject–diver–investigators to perform their tasks.

Plans for Extension

Further studies of prolonged exposure to nitrogen–oxygen atmospheres will be carried out in laboratory chambers and then in the open sea. It is now planned to proceed with the originally planned exposures to nitrogen with natural oxygen pressures at an ambient pressure of 4 atm.

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AUTHOR INDEX

Numbers in parentheses are reference numbers and indicate that an author's work is referred to although his name is not cited in the text.
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